9. Recombinant bovine somatotrophins

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Addendum to the monographs prepared by the 40th and 50th Meetings of the Committee and published in *FAO Food & Nutrition Paper* 41/5 and *FAO Food & Nutrition Paper* 41/11, respectively[5].

Explanation

Somatotrophins are proteins secreted by the anterior pituitary gland that stimulate growth, cell regeneration and reproduction in humans and animals. Most anabolic and growth-promoting effects of somatotrophins are mediated through insulin-like growth factor-I (IGF-I). Bovine somatotrophins (bSTs) produced by recombinant deoxyribonucleic acid (DNA) techniques (rbSTs) are used in lactating dairy cows to increase milk production. Four bST analogues (somagrebove, sometribove, somavubove and somidobove) were previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 40th Meeting (JECFA, 1993 [TRS 832]) and further evaluated at its 50th Meeting (JECFA, 1999 [TRS 888]). Although the chemical properties of the recombinant products vary slightly from those of pituitary bST (for chemical structures, see FAO, 1993), the Committee considered the recombinant products to be biologically and toxicologically similar, as they all act by binding with high affinity to the bST receptor.

The Committee at its 40th Meeting established an acceptable daily intake (ADI) and maximum residue limits (MRLs) "not specified" for these four rbSTs. The term "not specified" was used because of the lack of bio-activity following oral intake of rbSTs and IGF-I, and the low concentrations and non-toxic nature of the residues of these compounds. The ADI and MRLs "not specified" were re-affirmed by the Committee at its 50th Meeting.

Draft Codex standards for rbSTs have been held at the final step (before adoption) for more than a decade. When considering the adoption of these standards, the Codex Alimentarius Commission at its Thirty-fifth Session (FAO/WHO, 2012) requested a re-evaluation of the four analogues of natural bST (somagrebove, sometribove, somavubove and somidobove) by JECFA, noting that the scientific assessment of bST dated back to the 1990s. In particular, the Commission requested that JECFA (i) update the toxicological evaluation, (ii) update the exposure assessment based on any new occurrence data in food, (iii) evaluate potential adverse health effects, and (iv) consider the need to revise or maintain the ADI and MRLs for rbSTs. The Commission further requested that JECFA 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, (ii) the possibility of increased levels of IGF-I in the milk of cows treated with rbSTs, (iii) the potential effects of rbSTs on the expression of certain viruses in cattle, and (iv) the possibility that exposure of human neonates and young children to milk from rbST-treated cows increases health risks (e.g. the development of insulin-dependent diabetes mellitus). JECFA was also asked to

[5] The text of this monograph also appears in *Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series*, No. 69 (2014).

consider aspects of antimicrobial resistance associated with the use of rbSTs in relation to human health.

rbSTs are registered in 21 countries in the world (Bolivia (Plurinational State of), Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Jamaica, Lebanon, Mexico, Pakistan, Panama, Peru, Republic of Korea, South Africa, Uruguay, Venezuela, the USA and Puerto Rico) for use in dairy cows and in Pakistan for use in buffaloes. Sometribove, marketed as Lactotropin, Posilac, Somatech or Lactotropina, is authorized for use at a dosage of 500 mg subcutaneously every 14 days in all cases. A dose of 375 mg is also authorized for use in Mexico. Treatment commences approximately 50–90 days postpartum until the end of lactation. Somavubove, marketed as Boostin or Hilac, is also registered for use in the Republic of Korea, and is exported to Mexico, Brazil, Colombia, Pakistan and South Africa. A zero withdrawal period exists in all cases. bST is administered to cattle either subcutaneously or intramuscularly.

In response to JECFA's call for data, data were submitted to the Committee by a sponsor and two Member countries. Additionally, the Committee undertook a systematic review 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 among 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 among 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?

Details of the search strategy and databases used are available on the WHO website as supplementary information to the meeting report, at http://www.who.int/foodsafety/chem/jecfa/publications/reports/en/index.html.

In addition, PubMed and Web of Knowledge databases were searched for toxicity studies of rbSTs in laboratory animals; bio-availability and bio-activity of oral IGF-I; and analytical methods.

Biological data

Biochemical aspects

The Committee at its 40th and 50th Meetings concluded that human and bovine somatotrophins are structurally different and have species-specific receptor binding activity. Furthermore, the total concentration of bSTs detected in tissues and milk of rbST-treated cattle is similar to that from untreated cattle, and rbSTs are denatured by high temperatures (e.g. by cooking and pasteurization) and biodegradation processes in the gut.

Laboratory animals

No new studies on biochemical aspects of rbSTs were submitted with the recent call for data, and none was available in the literature. Since the assessment of rbSTs by the 50th Meeting, a Health Canada expert panel has suggested, based on the detection of anti-rbST antibodies in

rats, that some rbSTs administered orally could potentially be absorbed (Health Canada, 1999). The study that reported this finding was a 90-day study in rats (Richard, Odaglia and Deslex, 1989). This study also included a satellite investigation on anti-rbST antibodies in sera of rats administered an rbST by gavage. The 40th Meeting had reviewed the toxicity data from this study; however, the results of the satellite study on the anti-rbST antibodies were not discussed in the toxicological monograph from the 40th Meeting and are summarized below.

Rats

Sometribove was administered daily by gavage at a dose of 0, 0.1, 0.5, 5 or 50 mg/kg bodyweight (bw) per day or subcutaneously at 1 mg/kg bw per day (positive control) to Charles River CD VAF rats (30 rats of each sex per group) for 13 consecutive weeks. Of these 30 rats of each sex per group, 15 rats were considered part of a satellite study to investigate the development of anti-rbST antibodies. Ten rats of each sex per group from the satellite study were euthanized at week 14, and five rats of each sex per group were maintained without dosing for an additional 14 weeks of recovery. Blood samples were collected from all rats pre-treatment and at week 14 (i.e. at the end of the treatment period), at week 7 from 10 rats of each sex per group that were euthanized at week 14 and at week 28 from the remaining 5 rats of each sex per group.

Sera were analysed by radioimmunoprecipitation, and the radioactivity in the pellet was corrected for non-specific binding. The titre in the test sera was expressed as the percentage of the corrected counts per minute in the precipitate over the total counts per minute tested. Greater than 11% sometribove binding capacity, which was equivalent to the upper 75th percentile plus 1.5 times the inter-quartile range for negative control sera, was used as a cut-off to classify a sample as antibody positive.

All rats were seronegative at the start of the study. Animals in the negative control and 0.5 mg/kg bw per day groups remained seronegative for sometribove antibodies throughout the experiment. In contrast, 20% of the animals were seropositive on both week 7 (4/20) and week 14 (6/30) in the 5 mg/kg bw per day group. In the 50 mg/kg bw per day group, 15% (3/20) and 30% (9/30) of the animals were seropositive at weeks 7 and 14, respectively. One animal only (3%) was seropositive on week 14 in the lowest-dose group (0.1 mg/kg bw per day). All but one positive control animal administered sometribove subcutaneously were seropositive (Richard, Odaglia and Deslex, 1989). Antibody levels in orally dosed animals were generally lower than those observed in the positive controls. Oral doses of rbST did not increase bodyweight or feed consumption, although a concomitant marked increase in bodyweight and feed consumption was recorded in the positive control group from week 2 of the experiment.

The study did not measure rbST in sera and cannot confirm whether intact rbST was absorbed into the systemic circulation. Also, there was no effect on bodyweight or feed intake, suggesting that a sufficient quantity of bio-active sometribove was not absorbed into the systemic circulation. Consequently, it is not possible to confirm whether the anti-rbST antibody response was a result of absorption of intact rbST or only an immunologically active peptide fragment (epitope or antigenic determinant) of rbST into the systemic circulation or due to mucosal immunity in the gut. It is known that exposure to ingested foreign proteins could stimulate a mucosal immune response in the gut, and activated antibody-producing cells could enter and produce antibodies in the systemic circulation (McCluskie and Davis, 1999; Valdes-Ramos *et al.*, 2010; Shin *et al.*, 2013). The findings of this study therefore do not confirm the systemic bio-availability of orally administered rbSTs.

Considering the similar levels of total bST detected in milk or tissues of animals treated with rbSTs (see section below on *Bovine somatotrophin in tissues and milk*), the expected level of human exposure to rbSTs would be much lower than the dose used in anti-rbST antibody-positive rats. Furthermore, because of the structural dissimilarities between human and

bovine somatotrophins, species-specific receptor binding, destruction of rbSTs by high temperatures (e.g. cooking or pasteurization) and biochemical degradation by gastro-intestinal enzymes, small quantities of rbSTs in milk or tissues of treated animals, if present, are not expected to have biological activity when administered orally.

Cattle

In a recent study (Le Breton *et al.*, 2009), the elimination kinetics of an rbST in serum was characterized in a cow in which the concentrations after treatment with a single subcutaneous injection of 500 mg sometribove (Lactotropin, Monsanto, Elanco Animal Health) were measured using liquid chromatography coupled to tandem mass spectrometry in positive electrospray ionization mode. This allowed for the unambiguous identification and quantification of rbST in serum. Detection of the rbST was possible from 4.5 hours to 4 days after administration, and concentrations up to 10 ng/ml were reported.

No other new biological or pharmacokinetic studies were available.

Toxicological studies

The Committee at its 40th Meeting evaluated the toxicity of different rbSTs (JECFA, 1993 [TRS 832]). Acute oral toxicity studies in rats with rbST doses up to 5 g/kg bw, two 2-week oral feeding studies in rats with doses of rbSTs up to 10 mg/kg bw per day and two 4-week oral feeding studies in rats with doses up to 50 mg/kg bw per day caused no effects up to the highest dose tested. Similarly, no treatment-related effects were observed at the highest dose tested in two 90-day oral feeding studies in rats with rbSTs at doses up to 100 mg/kg bw per day and a 90-day oral feeding study in dogs at doses up to 10 mg/kg bw per day.

No new toxicity studies on rbSTs were available since the previous evaluation of rbSTs by the Committee at the 50th Meeting (JECFA, 1999 [TRS 888]).

Long-term studies on toxicity and carcinogenicity of recombinant mouse and rat somatotrophins

A search of the published literature identified long-term (2-year) carcinogenicity studies in mice and rats for related, but distinct, compounds (i.e. mouse and rat growth hormones) (Farris *et al.*, 2007). These studies did not use the oral/gavage route for administration of the test articles and did not test rbSTs. The Committee therefore considered these data not directly relevant to the risk assessment of rbSTs, but relevant to understanding the carcinogenic potential of other related somatotrophins in respective mammalian species. The study findings are therefore summarized briefly in this monograph.

Mice

In a 2-year study compliant with good laboratory practice (GLP), groups of CD-1 mice 39 days of age and weighing 18.5–27.5 g (females) and 20.2–32.8 g (males) at the beginning of the study were allocated into five groups (50 mice of each sex per group). Mice received daily subcutaneous injections of vehicle (two groups) or recombinant mouse somatotrophin (rmST) at 0.1, 0.2 or 0.5 mg/kg bw. Animals were observed daily for mortality and weekly for clinical signs. Bodyweight measurements and ophthalmic examinations were conducted routinely. Dead mice and those euthanized at the end of the study were necropsied, and 58 tissues per mouse were examined for gross and histopathological lesions.

Daily subcutaneous injection of rmST over 2 years elicited no treatment-related mortality or physical or ocular signs in mice. No effects on bodyweight were seen in trend analysis in either sex. The final mean bodyweights were 36.8, 37.5, 37.1 and 38.2 g in females and 46.1, 48.3, 49.3 and 47.6 g in males in the control, 0.1, 0.2 and 0.5 mg/kg bw per day treatment groups, respectively. Examination of the pituitary gland at necropsy did not reveal treatment-related gross changes or changes in pituitary weight. When compared with concurrent or historical controls, there was no significant treatment-related increase in the

incidence of tumours in any tissue examined in both males and females (Farris *et al.*, 2007).

Rats

In a GLP-compliant 2-year study (Farris *et al.*, 2007), groups of Sprague-Dawley rats 37 days of age and weighing 102–149 g (females) and 129–195 g (males) at the start of the study were allocated into five groups (50 rats of each sex per group). Rats received daily subcutaneous injections of vehicle (two groups) or recombinant rat somatotrophin (rrST) at 0.2, 0.4 or 0.8 mg/kg bw. Animals were observed daily for mortality and weekly for clinical signs. Bodyweight measurements and ophthalmic examinations were conducted routinely. Dead rats and those euthanized at the end of the study were necropsied, and 57 tissues per rat were subjected to gross and histopathological examination.

Daily subcutaneous injection of rrST over 2 years elicited a treatment-related decrease in mortality in female rats, but there was no effect on mortality of male rats. Compared with 62–64% survival of the control groups, 82% of female rats treated with rrST at 0.4 mg/kg bw per day and 80% of female rats treated with rrST at 0.8 mg/kg bw per day survived to study termination. The increased survival was attributed in part to reduction in deaths due to pituitary tumours in females. No treatment-related physical or ocular signs were observed. Female rats treated with rrST had a higher average bodyweight ($P < 0.001$) at all doses. At the end of the study, mean bodyweights of female rats were 324, 343, 363 and 381 g in the control, 0.2, 0.4 and 0.8 mg/kg bw per day treatment groups, respectively. In male rats, the bodyweights in the 0.4 and 0.8 mg/kg bw per day groups were significantly higher than those in the control and 0.2 mg/kg bw per day dose groups. Bodyweights at the end of the study in male rats were 627, 630, 647 and 650 g at 0, 0.2, 0.4 and 0.8 mg/kg bw per day, respectively. Overall, when compared with concurrent or historical controls, no significant difference in tumour incidence was detected in the different treatment groups. However, after adjustment for multiplicity of statistical tests, the incidence of pituitary adenoma in female rats showed a decreasing trend when the treatment dose was increased.

Bovine somatotrophin in tissues and milk

Bovine somatotrophin is not readily transferred from blood or plasma to milk. At the 40th Meeting of the Committee (JECFA, 1993 [TRS 832]), it was concluded that studies of rbST residues in milk demonstrate that the proposed use of rbSTs, even at exaggerated doses, will not lead to any detectable concentrations of total bST in milk above those normally present in milk from untreated cows (0.9–1.6 µg/L). Similarly, cows treated with rbSTs have, at most, a 2-fold increase in residues in tissues, to total bST concentrations of 3.1–4.2 µg/kg in muscle and 16–25 µg/kg in liver, compared with 2.2–3.7 µg/kg in muscle and 9–13 µg/kg in liver of untreated cows.

The 50th Committee meeting evaluated a published study (Choi *et al.*, 1997) in which rbST was administered in two different dosage forms by subcutaneous injection to beef cattle every 2 weeks for 20–24 weeks. Treated cattle were slaughtered 2 weeks after the final dose. Tissue concentrations of total bST ranging from 1.45 ±0.86 to 4.94 ±1.47 µg/kg in muscle, 4.82 ±1.95 to 9.33 ±5.23 µg/kg in fat, 3.56 ±1.73 to 5.36 ±1.21 µg/kg in liver, and 3.58 ±1.14 to 4.49 ±1.83 µg/kg in kidney were reported. Total bST concentrations were measured using a radioimmunoassay procedure. There were no significant differences between treated animals and controls in the concentrations of total bST in muscle, fat, liver or kidney (FAO, 1999; JECFA, 1999 [TRS 888]).

A limited number of studies that provide new data on bST residues in tissues (Kweon *et al.*, 2000) and in milk of lactating cows and buffaloes were published since the 50th Committee meeting (Mishra *et al.*, 2005; Mishra, Mahapatra and Shukla, 2006; Vicini *et al.*, 2008). Also, the results of several studies published before the previous meeting were not discussed in the reports of the previous meetings (Torkelson and Miller, 1987; Groenewegen *et al.*, 1990).

Torkelson and Miller (1987) injected eight cows intramuscularly and another eight subcutaneously at 14-day intervals with 500 mg rbST in a sustained release formulation. Ten untreated animals served as controls. Milk and blood samples were collected 2 days prior to injection, on the day of injection and on Days 1, 2, 3, 4, 6, 8, 10, 12 and 14 after injection during the fourth treatment cycle. The concentration of total bST in milk was determined by radioimmunoassay. No further information on validation of the assay was provided. The results demonstrated no correlations between total bST concentrations in blood and milk, regardless of the route of administration. Total bST concentrations in most milk samples were below the limit of detection (<0.3 ng/ml).

Groenewegen *et al.* (1990) determined the concentrations of total bST in milk of untreated cows (n = 3) at 82.3 ±17 days postpartum and cows (n = 3) treated at 78 ±6 days postpartum. Cows in the treated groups received 10.6 mg rbST (American Cyanamid) daily starting at 28 days postpartum. This formed part of the study in which the bio-activity of milk from cows treated with rbST was examined in hypophysectomized rats. The concentration of total bST in milk was measured by radioimmunoassay, with a level of detection of 0.5 ng/ml and an average recovery of 96%, and was reported as 3.3 and 4.2 ng/ml in milk of control and treated cows, respectively.

Mishra *et al.* (2005) reported somatotrophin concentrations in milk of lactating buffaloes (n = 20) treated with rbST (Boostin-250, LG Chemicals India) subcutaneously at 250 mg on three occasions at 14-day intervals, compared with saline-treated controls (n = 10). Total somatotrophin concentrations were measured in six fortnightly milk samples starting from 15 days pre-treatment to 60 days post-injection using a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) that utilized (r)bST-specific antibodies. The assay was validated for sensitivity, specificity, precision and recovery. Parallelism was demonstrated between the standard curve using rbST (NHPP, California) and serially diluted serum, milk and pituitary-derived growth hormone. The sensitivity of the assay was 0.1 ng/ml. The specificity of the assay was determined by western blot using non-specific proteins such as bovine serum albumin, gelatine and bovine prolactin with rbST. Presence of a single band only on the rbST column indicated that the antibody used in the assay was specific to bST only. The intra-assay and inter-assay variations for serum and milk were 3.36–8.81% and 6.01–14.31%, respectively. Recovery of exogenous bST from serum and milk ranged from 90% to 102% and from 96% to 108%, respectively. Mean total somatotrophin concentrations pre-treatment and post-treatment in both rbST-treated and control animals at each fortnightly collection are summarized in Table 9.1. No significant difference in the total somatotrophin concentrations was observed between rbST-treated and control animals. These concentrations are similar to those reported for cattle at the 40th Committee meeting.

Table 9.1. Fortnightly changes in total somatotrophin concentrations in milk in lactating buffaloes treated with rbST (n = 20) compared with saline-treated buffaloes (n = 10)

Treatment group	Somatotrophin concentrations (ng/ml)						Overall mean	Significance
	Pre-treatment	1	2	3	4	5		
Saline	1.27 ±0.07	1.10 ±0.11	1.06 ±0.10	1.10 ±0.10	1.18 ±0.06	1.13 ±0.05	1.14 ±0.04	NS
rbST	1.39 ±0.03	1.16 ±0.08	1.17 ±0.07	1.22 ±0.07	1.19 ±0.04	1.25 ±0.06	1.23 ±0.03	

NOTES: NS = not significant; rbST = recombinant bovine somatotrophin.
SOURCE: Adapted from Mishra *et al.*, 2005.

Mishra, Mahapatra and Shukla (2006) performed a study similar in design to the one previously reported in buffaloes (Mishra *et al.*, 2005), in lactating crossbred (*Bos taurus* × *Bos indicus*) cows (n = 20) treated with rbST (Boostin-250, LG Chemicals India) subcutaneously at 250 mg on three occasions at 14-day intervals, compared with saline-treated control cows (n

= 10). No significant difference (P >0.05) was found in the mean total bST concentrations in milk from rbST-treated cows (1.16 ±0.08 ng/ml) compared with control cows (1.10 ±0.34 ng/ml) measured in fortnightly milk samples by indirect sandwich ELISA. No validation information on the assay used was provided in the publication.

In a cross-sectional study, total bST concentrations were determined in retail milk samples (n = 344) collected from retail outlets in 48 contiguous states within the United States of America where rbST is approved for use (Vicini *et al.*, 2008). Samples were obtained in blocks over a period of three weeks from purchased milk labelled as conventional (milk that did not contain any claims about supplementation with rbST or organic practices), rbST-free (milk that has a processor claim that cows were not supplemented with rbST) or organic (milk from farms that were certified as meeting United States Department of Agriculture (USDA) organic standards). A block consisted of a shipping container collected on one day by one sampler and in one city to minimize the effects of shipping conditions. At least two blocks of samples were collected from each state. More samples were collected from states with larger populations or larger quantities of milk production. The freshest (based on expiry date) pasteurized whole milk in plastic or paper containers of any retail brand was preferred. Ultra-high-temperature pasteurized milk was avoided. bST concentrations in milk were measured by electrochemiluminescent immunoassays (ECLIA) using a Sector Imager 6000. Assays were performed at Monsanto. No information on the validation of the assay was provided. The milk samples were also examined for quality (antimicrobials and bacterial counts), nutritional value (fat, protein and solid-not-fat) and additional hormonal composition. There were no significant differences (P >0.05) in concentration of total bST in milk, regardless of label type. Approximately 82% of milk samples had total bST levels below the limit of quantification (0.033 ng/ml), and 72% were less than the limit of detection (0.010 ng/ml) for the assay.

Another study was reported by Kweon *et al.* (2000), in which 32 Holstein bulls and steers were randomly assigned to one of four groups: (i) bull group, (ii) untreated steer group, (iii) steers treated with rbST when they were about 80 kg live weight ($rbST_1$), or (iv) steers treated with rbST when they were about 300 kg live weight ($rbST_2$). Treated steers were given rbST every 14 days at 0.03 mg/kg bw per day intramuscularly, alternately in the rump and shoulder. Concentrations of total bST were measured using an immunoradiometric assay. No details on the validation procedure of the analytical method were provided. The concentrations of total bST in tissue with or without rbST treatment are summarized in Table 9.2. There were no significant differences between rbST-treated and untreated steers. The tissue concentrations of total bST reported in this study in both control and rbST-treated animals are slightly higher than those reported at the 40th and 50th Committee Meetings.

Table 9.2. Concentrations of total bST in tissues of rbST-treated and untreated steers

Tissues	Total bST in tissues of untreated steers (ng/ml)	Total bST in tissues of treated steers (ng/ml)		
		$rbST_1$	$rbST_2$	SEM
Injection site	5.80	7.23	8.83	0.89
Muscle	6.18	6.85	7.63	0.91
Kidney	15.93	17.75	23.05	1.77
Liver	19.83	18.05	20.10	1.15

NOTES: bST = bovine somatotrophin; $rbST_1$ = steers treated with recombinant bovine somatotrophin when they were about 80 kg live weight; $rbST_2$ = steers treated with recombinant bovine somatotrophin when they were about 300 kg live weight; SEM = standard error of the mean.

SOURCE: Reproduced from: Kweon, U.G., Kim, H.S., Yun, S.K., Nam, K.T., Kim, J.B., Ahn, J.B. & Kim, J.S. 2000. Effects of rbST administration on the changes in the concentration of blood and carcass hormones in Holstein bulls and steers. *Journal of Animal Science & Technology (Korea)*, 42(4): 451–458. with permission of the Korean Society of Animal Science and Technology.

Insulin-like growth factor-I in tissues and milk

IGF-I concentrations in milk

The 40th Committee Meeting cited an average concentration of IGF-I in milk of 3.7 ng/ml for untreated cows (JECFA, 1993 [TRS 832]). An average concentration of 5.9 ng/ml was reported in cows treated with rbST; although this average concentration was significantly higher than that in milk from untreated cows, most of the concentrations were less than 10 ng/ml and within the normal physiological range observed in the milk of lactating cows. IGF-II concentrations in cows' milk were not affected by rbST treatment.

At the 50th Committee Meeting, it was noted that the IGF-I content in normal bovine milk was highly variable, depending on the state of lactation, nutritional status and age (JECFA, 1999 [TRS 888]). Over an entire lactation, IGF-I concentrations in milk ranged between 1 and 30 ng/ml, with the highest concentrations in colostrum and a constant decline thereafter. Multiparous animals were reported to have higher concentrations of IGF-I in milk compared with primiparous cows. Bulk milk from cows not given rbST had IGF-I concentrations of 1–9 ng/ml. In milk from rbST-treated cows, the concentrations of IGF-I ranged from 1 to 13 ng/ml in most studies.

Since the 50th Committee Meeting, there have been limited additional data published on IGF-I residues in milk from untreated lactating cows (Daxenberger, Sauerwein and Breier, 1998; Liebe and Schams, 1998; Taylor *et al.*, 2004) and from lactating cows treated with rbSTs (Daxenberger, Sauerwein and Breier, 1998; Pauletti *et al.*, 2005; Collier *et al.*, 2008). Additionally, concentrations of IGF-I in retail milk in the United States of America based on the label, e.g. rbST-free, organic or conventional, were reported (Vicini *et al.*, 2008). Changes in IGF-I concentrations in milk from lactating buffaloes and goats following treatment have also been reported (Faulkner, 1999; Prasad and Singh, 2010; Castigliego *et al.*, 2011). A summary of all new studies is provided in Tables 9.3a and 9.3b.

Daxenberger, Sauerwein and Breier (1998) determined naturally occurring IGF-I concentrations in 5777 random milk samples from dairy cows (not treated with rbST) collected over a 1-year period covering all regions of Bavaria. In samples from lactation weeks 7 through 33, the effect of somatic cell count, protein content and parity was quantified and corrected to obtain a normal distribution of the corrected logarithmic IGF-I concentrations. IGF-I concentrations in the milk were measured using a validated non-extraction radioimmunoassay following de-fattening. The method involved competitive displacement of IGF-I from IGF binding proteins by IGF-II and has an intra-assay variation of 5.1% and an inter-assay variation of 13.4%. IGF-I concentrations in milk from untreated animals ranged from 1 to 83 ng/ml. The distribution of the IGF-I was skewed to the right, with a median concentration of 4.4 ng/ml and 90th and 95th percentiles of 9.5 and 12.5 ng/ml, respectively. There was no detectable effect of region, season, the quantity of milk produced or the milk's fat content on IGF-I concentrations. Stage of lactation strongly influenced the concentration of IGF-I in milk (Figure 9.1).

Table 9.3a. Summary of the normal variation of IGF-I concentration in cow's milk and the effect of rbST treatment on IGF-I concentrations in milk. (a) Naturally occurring IGF-I

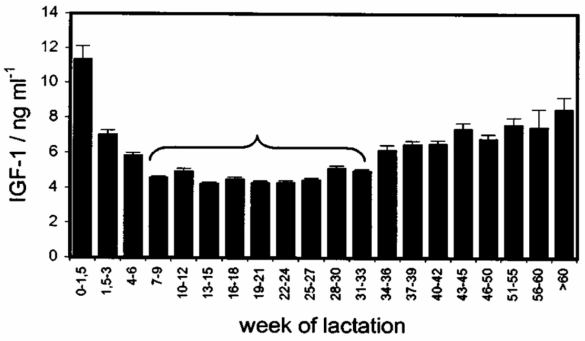
Study	No. of samples	IGF-I concentrations	Assay method
Daxenberger, Sauerwein and Breier, 1998	5 777	Range 1–83 ng/ml; median 4.4 ng/ml; 90th percentile 9.5 ng/ml; 95th percentile 12.5 ng/ml	Non-extraction radioimmuno-assay following de-fattening
Liebe and Schams, 1998	12 in barned study 12 with clinical mastitis 22 with subclinical mastitis	Healthy quarters: 8.3 ±1.7, 8.5 ±2.1, 14.1 ±1.7 and 15.1 ±1.8 ng/ml in loose housing, and 10.7 ±2.1 and 6.6 ±1.5 ng/ml in tied portion of barned study Clinical mastitis: 35.5 ±23.5 vs 21.2 ±6.8 ng/ml in healthy quarters Subclinical mastitis: 36.9 ±31.3 vs 17.7 ±11.3 ng/ml in healthy quarters	Extraction radioimmunoassay in skimmed milk
Taylor *et al.,* 2004	50 multiparous	>16 ng/ml 1st week of calving; 6–9 ng/ml 2–20 weeks postpartum	Ethanol–acetone–acetic acid radioimmunoassay in whole milk

Table 9.3b. Summary of the normal variation of IGF-I concentration in cow's milk and the effect of rbST treatment on IGF-I concentrations in milk. (b) rbST treatment studies

Study	Treatment	No. of animals	IGF-I concentrations		Assay method
			No rbST	rbST	
			Cows		
Daxenberger Sauerwein and Breier, 1998	1 × 500 mg sometribove (Posilac, Monsanto)	34 (33 for data analysis)	~4 ng/ml	Increase of 2.3 ng/ml for lactation 1; 1.6 ng/ml for lactation 2–6; and 1.9 ng/ml (48%) for all lactations	Non-extraction radioimmunoassay following de-fattening
Collier *et al.,* 2008	25 mg/day sometribove (winter)	6 per group	3.7 ng/ml	4.8 ng/ml	Radioimmunoassay
	25 mg/day sometribove (summer)	6 per group	3.4 ng/ml	3.8 ng/ml	
Pauletti *et al.,* 2005	3 × 500 mg (Boostin) at 14-day intervals from Day 35 prepartum until parturition	21 per group	Day 1 postpartum (colostrum): 674 ±270 ng/ml Day 7 no significant differences from treated animals	Day 1 post-partum (colostrum): 875 ±335 ng/ml Day 7 postpartum: 12.9 ng/ml	Immune radiometric assay
			Buffaloes		
Castigliego *et al.,* 2011	5 × 500 mg (Boostin) s.c. at 14-day intervals	8 per group	1.5–3.0 ng/ml	4.5–7.0 ng/ml	Sandwich ELISA
Prasad and Singh, 2010	5 mg rbST (Boostin) i.v. daily for 5 days	10	29.7 ±4.5 to 38.1 ±3.4 ng/ml	42.0 ±5.2 ng/ml (highest concentration measured on Day 1 after treatment)	Double-antibody radioimmunoassay
			Goats		
Faulkner, 1999	2 × 3 mg s.c. of ovine somatotrophin	5	~5 ng/ml	Maximum of ~15 ng/ml	Double-antibody radioimmunoassay
			Retail milk survey		
Vicini *et al.,* 2008	Conventional; rbST-free and organic labelled milk		"rbST free" 3.0 ±0.1 ng/ml; "organic" 2.7 ±0.1 ng/ml	"Conventional" 3.1 ±0.1 ng/ml	ECLIA

NOTES: ECLIA = electrochemiluminescent immunoassay; ELISA = enzyme-linked immunosorbent assay; IGF-I = insulin-like growth factor-I; i.v. = intravenously; rbST = recombinant bovine somatotrophin; s.c. = subcutaneously.

Figure 9.1. Mean IGF-I concentrations (±SEM) in milk from cows not treated with rbST during the entire lactation.

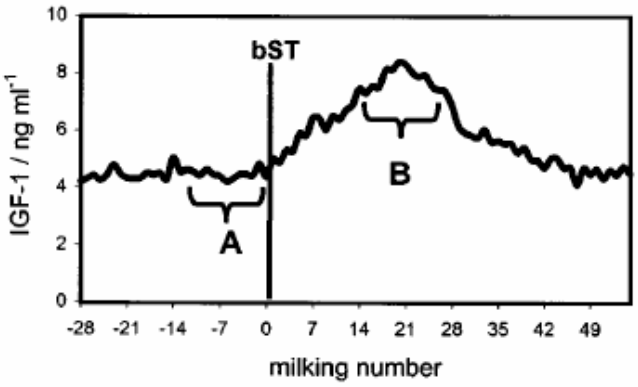


NOTES: IGF-I = insulin-like growth factor-I, given as IGF-1 in figure; SEM = standard error of the mean; rbST = recombinant bovine somatotrophin

SOURCE: Reproduced from Daxenberger, A., Sauerwein, H. & Breier, B.H. 1998. Increased milk levels of insulin-like growth factor 1 (IGF-1) for the identification of bovine somatotrophin (bST) treated cows. *Analyst*, 123: 2429–2435, with permission of the Royal Society of Chemistry.

IGF-I concentrations in milk varied 2- to 3-fold across lactation, with the average concentration being the highest in the first 1.5 weeks of lactation at approximately 11.5 ng/ml, then falling rapidly before levelling out between weeks 7 and 33 at approximately 5 ng/ml, before rising steadily again to reach a concentration of approximately 8 ng/ml in late lactation. Somatic cell count in milk and milk protein percentage had small but positive correlations with IGF-I concentrations in milk. The number of lactations (first, second or third to sixth) and breed also had some influence on IGF-I concentration in milk. High variability in IGF-I concentrations was observed in cows after six lactations. Samples of Holstein-Friesian cows showed slightly higher IGF-I concentrations compared with other breeds. The Daxenberger, Sauerwein and Breier (1998) study also included an animal phase in which 34 Brown Swiss cows were given a single treatment of rbST (Posilac, Monsanto) according to the label instructions (500 mg). Milk samples were taken twice daily from these animals for 2 weeks during the pre-treatment period and for 4 weeks in the post-treatment period. Statistical analysis was performed on the changes in IGF-I concentration in milk derived from 33 animals from days 7 to 13 after treatment (period B) compared with the 7 days before treatment (period A) (Figure 9.2). IGF-I concentration in milk pre-treatment was close to 4 ng/ml, which increased significantly after treatment, with the maximum concentration (approximately 8 ng/ml) detected 10 days after treatment. The mean increase in IGF-I compared with that of the contemporary control was 2.3 ng/ml for lactation 1, 1.6 ng/ml for lactations 2–6 and 1.9 ng/ml (48%) for all lactations combined.

Figure 9.2. Mean IGF-I* concentrations in milk after rbST** treatment. Statistical analysis was based on (A) the control period and (B) the main effect period



NOTES: IGF-I = insulin-like growth factor-I, given as IGF-1 in figure; rbST = recombinant bovine somatotrophin, given as bST in figure.

SOURCE: Reproduced from Daxenberger, A., Sauerwein, H. & Breier, B.H. 1998. Increased milk levels of insulin-like growth factor 1 (IGF-1) for the identification of bovine somatotrophin (bST) treated cows. *Analyst*, 123: 2429–2435, with permission of the Royal Society of Chemistry.

Liebe and Schams (1998) studied the interrelationship between concentrations of IGF-I, basic fibroblast growth factor and somatic cell count in normal milk and the presence of these growth factors in the milk from cows with clinical and subclinical mastitis. Twelve

Brown Swiss cows in their fourth lactation and in their 1st to 10th months of lactation were used. The study was performed in two periods, with four and eight cows in periods 1 and 2, respectively. Cows with chronically elevated somatic cell count in at least one quarter due to a history of mastitis or trauma were selected from their loose housing and moved to a separate stanchion barn for a period of 5 days and then transferred back to the original loose environment. The periods of 5 days before and after relocation were referred to as control. Four milk samples from each quarter were taken daily at the morning milking. In addition, quarter milk samples (n = 48) from 12 cows affected by clinical mastitis and quarter milk samples (n = 88) from 22 cows (German Fleckvieh) affected by subclinical mastitis obtained from four small Bavarian farms were investigated. IGF-I concentrations were measured in skimmed milk samples by using an extraction radioimmunoassay technique with 3.8% intra-assay and 16% inter-assay coefficients of variation. The concentrations of IGF-I in milk in the relocation portion of the study in the controls were 15.1 ±1.8 and 14.1 ±1.7 ng/ml before and after being barned in the first period of the study, and 8.3 ±1.7 and 8.5 ±2.1 ng/ml in the second period; concentrations of IGF-I were 10.7 ±2.1 and 6.6 ±1.5 ng/ml during the time barned during the first and second study periods, respectively. The concentrations of IGF-I in milk from quarters with clinical (35.5 ±23.5 ng/ml) and subclinical (36.9 ±31.3 ng/ml) mastitis were almost twice the concentrations detected in corresponding healthy quarters (21.2 ±6.8 ng/ml and 17.7 ±11.3 ng/ml, respectively).

Taylor *et al.*, (2004) reported the concentrations of IGF-I in blood from Holstein-Friesian cows not treated with rbST and the influence of stage of lactation from 142 primiparous and 177 multiparous (mean lactation number of 3, range 2–8) cows. Blood samples were collected from 1 week before to at least 12 weeks after calving in the multiparous cows and before calving and 3, 5 and 8 weeks after calving in the primiparous cows. The concentrations of IGF-I in milk were measured in 50 of the multiparous cows. Whole milk samples were collected weekly after calving until week 12 and at week 20, and frozen until assayed for IGF-I. The concentrations of IGF-I in plasma and milk were determined by radioimmunoassay after ethanol–acetone–acetic acid extraction of IGF-I binding proteins. The inter-assay and intra-assay coefficients of variation were 11.2% and 6.7%, respectively. Concentrations of IGF-I in plasma were significantly (P <0.001) higher in the primiparous cows (about 130 and 100 ng/ml) than in the multiparous cows (85 and 60 ng/ml) before and after calving, respectively. IGF-I concentrations in milk in the 1st week after calving were above 16 ng/ml, decreased rapidly in subsequent weeks and thereafter fluctuated between 6 and 9 ng/ml until 20 weeks post-calving (Figure 9.3). There was no direct correlation between concentrations of IGF-I in blood plasma and milk.

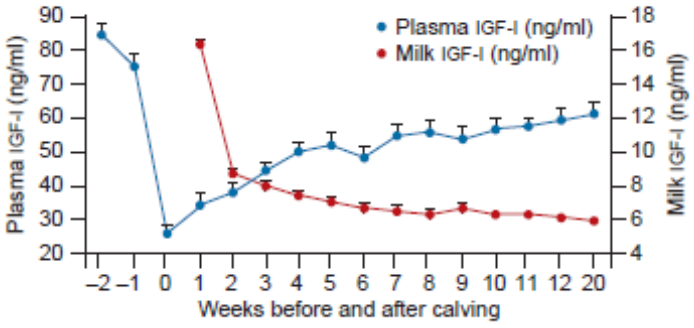


Figure 9.3. Insulin-like growth factor-I (IGF-I) concentrations in plasma and milk from 50 multiparous Holstein-Friesian cows (SOURCE: Reproduced from Taylor *et al.*, 2004. Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. *Veterinary Record*, 155: 583–588, with permission from BMJ Publishing Group Ltd.)

Collier *et al.,* (2008) investigated the effect of rbST on IGF-I concentrations in milk from lactating cows separately in summer and winter. Summer and winter each consisted of six treatment periods: (1) season farm management of all cows for the first 30 days; (2) 7 days' adjustment to conditions in the climate chambers; (3) exposure of one half of the animals to thermoneutral conditions and exposure of the other half to appropriate cold or hot conditions for 10 days; (4) cold or hot adjustment for 4 days; (5) reversed temperature exposure from period 3 for 10 days; and (6) 5 days post-treatment in a switchover design. Winter conditions were 5°C and climate chambers for cold set at -5 to +5°C and for thermoneutral conditions at 15–22°C. Summer conditions were 18–35°C and climate chambers set at 24–35°C for hot conditions and at 15–22°C for thermoneutral conditions. Cows were given daily injections of rbST (sometribove, USAN; 25 mg/day; six cows each study) or saline (control; six cows each study). During on-farm periods, blood and milk (morning and afternoon) samples were collected once weekly. During climate chamber periods, blood samples were collected every 2 days, and milk samples (morning and afternoon) were collected daily. Plasma and milk concentrations of IGF-I and IGF-II were determined by radioimmunoassay. IGF-I and IGF-II concentrations in plasma were increased in cows treated with rbST. Milk yields in experimental cows were higher in winter (31.3 kg/day) than in summer (27.0 kg/day), but the response to rbST in milk production was numerically greater in summer than in winter (7.5 kg/day versus 5.0 kg/day). A pronounced seasonal pattern in basal and rbST-stimulated IGF-I concentrations, but not IGF-II concentrations, was detected in plasma. Higher basal and rbST-stimulated IGF-I concentrations in plasma occurred in summer despite large decreases in feed intake and energy balance. IGF-I and IGF-II concentrations in milk were not affected by rbST treatment or season (Table 9.4). Although IGF-I and IGF-II concentrations in milk were unaffected by rbST treatment, total IGF output increased due to increased milk yield. It was concluded that the observed seasonal patterns in IGF-I concentrations in plasma (winter: 3.7 ng/ml versus 4.8 ng/ml; and summer: 3.4 ng/ml versus 3.8 ng/ml, in control and treated groups, respectively) may be indicative of seasonal differences in the coupling of the somatotrophin–IGF axis. The studies failed to detect an uncoupling of the somatotrophin–IGF-I axis in summer, despite an induced negative energy balance during thermal stress.

Table 9.4. The effect of treatment and season on milk yield, IGF-I and IGF-II concentrations in milk and total milk IGF-I and IGF-II output

	Milk yield (kg/day)	Milk IGF-I		Milk IGF-II	
		Concentration (ng/ml)	Output (µg/day)	Concentration (ng/ml)	Output (mg/day)
Treatment					
Control	26.0	3.91	101.6	45.7	1.2
rbST	32.3**	4.26	137.6**	51.2	1.7*
Season					
Winter	31.3***	4.67	146.2***	48.2	1.5
Summer	27.0	3.51**	94.8	48.7	1.3

NOTES: IGF-I = insulin-like growth factor-I: IGF-II = insulin-like growth factor-II; rbST = recombinant bovine somatotrophin; * = rbST different from control, $P < 0.05$; ** = rbST different from control, $P < 0.01$; *** = winter different from summer, $P < 0.01$

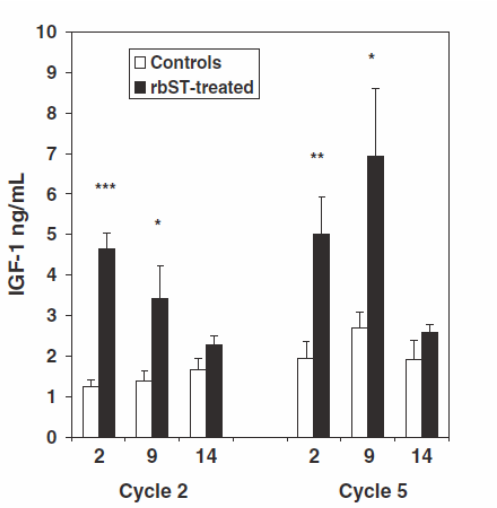
SOURCE: Reprinted from Collier, R.J., Miller, M.A., McLaughlin, C.L., Johnson, H.D. and Baile, C.A. 2008. Effects of recombinant bovine somatotrophin (rbST) and season on plasma and milk insulin-like growth factors I (IGF-I) and II (IGF-II) in lactating dairy cows. *Domestic Animal Endocrinology*, 35(1): 16–23, with permission from Elsevier.

Pauletti *et al.*, (2005) studied the changes in IGF-I concentrations in colostrum in 42 pregnant multiparous Holstein cows randomly assigned to equally sized groups treated with either 500 mg of rbST (Boostin, Cooper) or vitamin E, used as control. The treatments were initiated 35 days prepartum and repeated each 14 days until parturition. Colostrum and mammary secretions were collected daily for 7 days postpartum. IGF-I concentrations in serum, colostrum and milk were measured using an immunoradiometric assay. The mean IGF-I concentration in colostrum of rbST-treated cows was significantly (P <0.05) higher than that of the control cows (874.5 ±335.0 ng/ml versus 674.2 ±269.5 ng/ml) on day 1 after calving. No significant differences (P >0.05) in IGF-I concentrations in milk were subsequently observed between the two treatment groups; by day 7 postpartum, IGF-I concentrations in milk had decreased to 12.9 ng/ml. At days 6 and 8, concentrations of IGF-I in milk in the control group were higher than those in rbST-treated cows, but not significantly.

In the cross-sectional study on retail milk samples (Vicini *et al.*, 2008), described above, the mean concentrations of IGF-I in conventionally labelled milk and milk labelled as rbST-free and organic were 3.1 ±0.1, 3.0 ±0.1 and 2.7 ±0.1 ng/ml, respectively. The mean IGF-I concentration was not different (P >0.05) between conventional and rbST-free labelled milk, but was significantly lower (P <0.05) in organic labelled milk. IGF-I concentrations in milk were measured by ECLIA using a Sector Imager 6000. Assays were performed at Monsanto. No information on the validation of the assay was provided.

Castigliego *et al.*, (2011) determined hormone variations in serum and milk as potential indicators of treatment with an rbST in buffaloes. Eight lactating Italian buffaloes (*Bubalus bubalis*) were treated 5 times with a slow-release formulation of an rbST (Boostin®, LG Life Sciences) at 500 mg subcutaneously every 2 weeks over a period of 10 weeks. An additional eight buffaloes were administered physiological saline and used as controls. Blood samples were collected on the day before treatment and on Days 2, 5, 9 and 14 following each treatment. Milk samples were collected at the end of the mechanized morning milking on the day prior to the second and fifth treatment cycles and on Days 2, 9 and 14 following these two treatments. Concentrations of total somatotrophin in serum and concentrations of IGF-I in milk were measured using a sandwich ELISA validated for each compound and matrix. Total somatotrophin concentrations in serum increased on Day 2 after rbST treatment. The average total somatotrophin concentrations were approximately 20 times higher in treated relative to control buffaloes, and were significantly different (P <0.001) in all five treatment cycles. IGF-I concentrations in serum increased rapidly after rbST treatment and persisted at least until Day 9, with significant differences (P <0.001) in treated and

Figure 9.4. IGF-I variation in buffalo milk after rbST treatment. Comparisons between treated buffaloes (n = 8) and the controls (n = 8) are reported for Days 2, 9 and 14 of the cycles of injection 2 and 5. Data are reported as means ±SEM; * P <0.05; ** P <0.01; *** P <0.001.



NOTES: IGF-I = insulin-like growth factor-I (given as IGF-1 in figure); rbST = recombinant bovine somatotrophin; SEM = standard error of the mean.

SOURCE: Reproduced from Castigliego, L., Li, X.N., Armani, A., Grifoni, G., Boselli, C., Rosati, R., Gianfaldoni, D. and Guidi, A. 2011. Hormone variations in serum and milk of buffaloes (*Bubalus bubalis*) as potential indicators of treatment with recombinant bovine somatotrophin. *Journal of Dairy Research*, 78: 412–420, with permission of Cambridge University Press.

control animals. The IGF-I concentrations in milk were significantly (P <0.05 to <0.001) higher in treated animals compared with the control animals at each day after treatment on each treatment cycle (Figure 9.4). The IGF-I concentration in milk increased after treatment but returned to a concentration similar to that of controls by 14 days post-treatment. The highest IGF-I concentrations reported in milk from treated buffaloes were 4.5–7 ng/ml, compared with 1.5–3 ng/ml in untreated controls.

Prasad and Singh (2010) determined the influence of short-term treatment of rbST on plasma growth hormone, IGF-I, prolactin and milk production of Murrah buffaloes in early lactation. Ten Murrah buffaloes in early production were each infused with 5 mg of an intravenous solution of rbST (rbST, Monsanto; NHPP-NIDDK, lot M010-001) per day for 5 consecutive days (Days 21–25 postpartum). A mean IGF-I concentration in milk of 34.8 ±3.5 ng/ml (29.7 ±4.5 to 38.1 ±3.4 ng/ml) was observed pre-treatment. IGF-I concentrations were low at the start of the treatment on Days 1, 2 and 3 but increased on Day 4 onwards, reaching a maximum of 42.0 ±5.2 ng/ml on Day 1 after the last treatment and declining thereafter. No significant changes (P >0.05) in IGF-I concentration in milk were observed in pooled data of all three phases (before, during and after treatment) of the study.

Faulkner (1999) studied the changes in concentrations of glucose and IGF-I in plasma and milk in response to ovine somatotrophin in five British Saanen goats in their third to fifth lactations. Lactating goats were treated with 3 mg ovine somatotrophin subcutaneously on the 3rd and 4th days of the study. The concentrations of IGF-I were determined in milk after de-fattening using a double-antibody radioimmunoassay. Prior to determination of total IGF-I in fat-free milk, samples were extracted for 48 hours at pH 3.7 in glycylglycine to remove or inactivate binding proteins. The concentration of total IGF-I in milk increased significantly (P <0.04) immediately after ovine somatotrophin treatment (30-minute sample) from pre-treatment concentrations of about 5 ng/ml, reaching a peak of about 15 ng/ml, and preceded that in plasma by approximately 48 hours. This would indicate that the increased concentrations of IGF-I in milk are due to increased local production within the environment of the mammary gland or as a result of an efficient extraction of IGF-I from the circulation.

Although the species most commonly used for milk production is cattle, references to administration in goat (Faulkner, 1999) and buffaloes (Prasad and Singh, 2010; Castigliego *et al.*, 2011) showed that even using different dosages, the resulting effects and concentrations of rbST and IGF-I are constant, regardless of the species.

The Committee considered all new information on the normal variation in IGF-I concentrations in cow milk and the effect of rbST treatment on IGF-I concentrations in milk, as summarized in Table 9.3, and noted that the conclusions made at the 40th and 50th Meetings are not substantially changed. No new information provided by the sponsor or sourced from the literature was obtained from studies performed according to GLP. Analytical methods used for bST and IGF-I in the various biological matrices are all immunologically based and measure mostly total content. Nevertheless, the available data examined corroborate the Committee's previous conclusions that IGF-I concentrations in cow's milk are highly variable and are influenced by parity, stage of lactation, season, udder health and somatic cell counts of the milk. Treatment of cows with rbST increases the mean IGF-I concentration in milk, but such increases are within the normal physiological variations observed in lactating cows. The wide range of IGF-I concentrations and different conclusions about the increase after bST application might be due to different analytical methods used, including potential interference caused by IGF binding proteins.

IGF-I concentrations in tissues

The 40th Meeting reported that IGF-I concentrations in biopsied muscle and liver of rbST-treated cows increased at most 2-fold when compared with those of untreated cattle (JECFA, 1993 [TRS 832]). The concentrations of IGF-I in muscle and liver ranged from 91 to 312 µg/kg and from 72 to 162 µg/kg, respectively, in rbST-treated cattle, compared with 68–272 µg/kg

and 70–77 µg/kg, respectively, in untreated cattle. It was suggested that the elevated IGF-I concentrations in muscle could have been attributed to wound healing and not to rbST treatment. At the 50th Meeting, no significant differences were found between treated cows and untreated controls in the concentrations of IGF-I in muscle, fat, liver or kidney (JECFA, 1999 [TRS 888]). Concentrations of IGF-I measured by radioimmunoassay varied from 34.9 ±15.2 to 131.8 ±24.6 µg/kg in muscle, from 203.6 ±52.6 to 339.1 ±229.2 µg/kg in fat, from 294.4 ±88.4 to 389.6 ±132.3 µg/kg in liver, and from 821.1 ±124.0 to 997.0 ±140.2 µg/kg in kidney. Previous assessments of the Committee summarized that the residues of rbST or IGF-I in various tissues of rbST-treated cows did not significantly differ from those of controls or that the slight increase in tissue residues is unlikely to be of concern for human health. A literature search did not identify new information on tissue IGF-I concentrations in rbST-treated animals.

Methods of analysis

The analytical methods used to determine bST and IGF-l in milk and tissues evaluated at the 40th and 50th Meetings of the Committee were exclusively immunoassay procedures and could not distinguish between natural bST and rbST.

Methods for assaying IGF-I were considered by the present Committee. Although incomplete removal of IGF binding proteins or variation of standard source and extraction methods might influence reported values, these factors were not perceived to materially alter the conclusions that were taken. While some studies reported higher concentrations of IGF-I in milk, the Committee considered these studies to reflect differences in extraction procedures.

Some of the new methods that have been developed for detection of rbST/bST are summarized in Table 9.5. Most of the methods (e.g. immunoassays) do not differentiate between native bST and rbSTs. However, a few mass spectrometry methods allow the unambiguous identification of endogenous and recombinant forms (Pinel, André and Le Bizec, 2004; Bailly-Chouriberry *et al.,* 2008). These methods were developed to identify non-compliant use of rbSTs in countries where they are not authorized.

The Committee noted that a recent review described the state of the art in the detection of rbSTs in food-producing animals (Dervilly-Pinel *et al.,* 2014).

Table 9.5. Summary of recent bST analytical methods

Method	Species and tissues	Sensitivity	Reference
ECLIA	Bovine milk	<5 pg/ml	McGrath *et al.,* 2008
ELISA	Bovine milk	0.05 ng/ml	Castigliego *et al.,* 2007
ELISA	Buffalo serum and milk	0.1 ng/ml	Mishra *et al.,* 2005; Mishra, Goswani and Shukla, 2007
ELISA	Shrimp feed	10 µg/g	Munro and Boon, 2010
LC-MS/MS	Goat plasma	10 ng/ml	Le Breton *et al.,* 2008
LC-MS/MS	Bovine serum	10 ng/ml	Le Breton *et al.,* 2009
LC-MS/MS	Bovine milk	CCα ≤1.24 ng/ml; CCβ ≤1.92 ng/ml	Le Breton *et al.,* 2010a
LC-MS/MS	Bovine blood	CCα ≤2.5 ng/ml; CCβ ≤6.8 ng/ml	Le Breton *et al.,* 2010b
LC-MS/MS	Trout serum	0.5 µg/ml	Rochereau-Roulet *et al.,* 2013

NOTES: bST = bovine somatotrophin; CCα = decision limit; CCβ = detection capability; ECLIA = electrochemiluminescent immunoassay; ELISA = enzyme-linked immunosorbent assay; LC-MS/MS = liquid chromatography–tandem mass spectrometry.

Bio-availability and bio-activity of IGF-I

The 40th Committee Meeting concluded that many of the physiological effects of rbSTs are mediated by bovine IGF-I, which is structurally identical to human IGF-I and is likely to have similar effects in humans (JECFA, 1993 [TRS 832]). The Meeting further concluded that IGF-I had no bio-activity when administered orally to normal and hypophysectomized rats at doses up to 2 mg/kg bw per day.

The 50th Committee Meeting (JECFA, 1999 [TRS 888]) reported that IGF-I is found in abundance in a variety of body fluids (Table 9.6).

Table 9.6. IGF-I concentrations in milk and body fluids of humans

Fluid	IGF-I concentration (ng/ml)
Cow's milk (bulk milk)	
Untreated	1–9
Treated with rbSTs	1–13
Human milk	
Milk	5–10
Colostrum	8–28
Human plasma	
Children	17–250
Adolescents	182–780
Adults	123–460
Human gastrointestinal secretions	
Saliva	6.8
Gastric juice	26
Pancreatic juice	27
Bile	6.8
Jejunal chyme	180
Daily adult human production	10^7 ng/day

NOTES: IGF-I = insulin-like growth factor-I; rbSTs = recombinant bovine somatotrophins.
SOURCE: Adapted from Table 8 in WHO, 1998.

The 50th Meeting indicated that, for quantitative risk assessment, the slight increases in IGF-I concentrations in milk from rbST-treated cows have to be compared with the physiological variations in IGF-I during lactation as well as with the concentrations in human breast milk, in the secretions of the gastrointestinal tract and in serum. It estimated that the incremental human exposure to IGF-I through consumption of 1.5 L/day of rbST-treated cow milk represented 0.79% of the IGF-I secreted daily in the gastrointestinal tract and less than 0.09% of the daily production (10^7 ng/day) of IGF-I in adults. Whereas the 40th Meeting considered IGF-I to be completely and rapidly degraded in the gastrointestinal tract, the 50th Meeting considered that some milk-borne IGF-I may escape digestion by gastrointestinal enzymes and be bio-available, leading to some absorption. Nonetheless, the 50th Meeting concluded that even if IGF-I in milk were absorbed, the additional amount would be negligible and unlikely to have an adverse impact in humans. Limited additional data available on the bio-availability or bio-activity of IGF-I since then, and summarized below, do not substantially change the previous conclusions of the Committee.

Consistent with previous reports of the Committee, new *in vitro* digestion studies (Rao *et al.*, 1998; Shen and Xu, 2000; Fellah *et al.*, 2001; Anderle *et al.*, 2002; Nabil *et al.*, 2011) suggest that IGF-I is degraded by intestinal enzymes, but *in vivo* IGF-I degradation by gastrointestinal enzymes could be delayed by the components in milk/colostrum (Shen and Xu, 2000). Also, analytical methods used could influence the outcome of such measurements. For example, degradation of IGF-I measured by trichloroacetic acid precipitation often overestimated the amount of intact IGF-I when compared with the data from receptor binding assays (Rao *et al.*, 1998; Shen and Xu, 2000).

New data from *in vivo* studies in laboratory animals (Philipps *et al.*, 2000, 2002) demonstrate that a fraction of orally administered IGF-I is absorbed from the intestines. Suckling rats 10–12 days of age were administered ^{125}I-labelled recombinant human (rh) IGF-I (4×10^6 counts per minute) by gavage in milk, and the radioactivity in portal and cardiac blood was examined at 5, 10, 20 and 30 minutes post-treatment (Philipps *et al.*, 2000). Purified radioactive samples were tested by gel chromatography and receptor binding assays. Radioactivity was detected in both portal and cardiac blood (maximum levels detected at 20–30 minutes post-treatment), but it was lower in the latter. The radioactivity present in the cardiac blood co-migrating at the position of native IGF-I was highest at 5 minutes post-treatment, but decreased significantly thereafter. However, a statistically non-significant

numerical increase in radioactivity was observed in the portal blood from 5 to 30 minutes post-treatment. It was estimated that approximately 17–26% of the dose administered, as measured by radioactivity, reached the portal blood, but only a fraction of that reached the systemic circulation. Also, the radioactive peak found in hepatic blood from IGF-I-fed animals was receptor active, although its binding in the competitive assay was weaker when compared with native IGF-I binding. Owing to extremely low concentrations, the authors could not perform adequate competitive binding studies on purified radioactive material from cardiac blood. This study, while demonstrating that almost a quarter of IGF-I administered in milk is absorbed from the intestine, could not definitively determine what proportion of IGF-I absorbed into the portal circulation enters the systemic circulation. In a subsequent study, the intestinal transport of IGF-I in suckling rats was shown to be non-saturable up to 1 µg/ml of IGF-I, a concentration 200-fold in excess of that in colostrum (Philipps *et al.*, 2002).

Kim *et al.* (2006) demonstrated that weanling mice (n = 35) administered a single oral dose (1 µg/g) of IGF-I in phosphate-buffered saline (PBS) had a transient higher concentration of serum IGF-I between 4 and 8 hours after treatment, with the highest concentration at 4 hours, when compared with PBS-treated controls (n = 35). Serum concentrations of IGF-I and IGF-II did not differ in weanling mice (n = 20) administered five separate doses of IGF-I at 1 µg/g repeated every 3 days, compared with PBS-treated controls (n = 20) at Days 7 and 13 post-treatment. Although the authors concluded that increased serum concentrations of IGF-I in treated rats are evidence of its oral bio-availability, the experimental design cannot rule out whether such an increase was modulated by the local action of IGF-I in intestinal mucosa or due to its systemic availability. The dose of IGF-I administered to the mice, which is more than 150 times the amount that a person would consume per day in milk from rbST-treated cows (9 µg per 1.5 L of milk, as concluded at the 50th Meeting), may further have contributed to the systemic absorption.

Also, there is some evidence in the literature that orally administered IGF-I might have some local activity in the gut (e.g. increase in the weight of small intestine, increased enzyme activities) of laboratory animals (Burrin, 1997; Houle *et al.*, 2000; Alexander and Carey, 2001; Burrin *et al.*, 2001; Kim *et al.*, 2006).

Le Breton *et al.* (2010a) conducted a study on the effects of industrial processes on milk stability together with the detection of rbSTs. The study was conducted on commercial ultra-high-temperature (UHT) milk as well as on raw milk, condensed milk and milk powder. The milk treatments analysed were defatting, heating, freezing, pasteurization and spray-drying. The results demonstrated that the processes that did not involve heating allowed a recovery of the hormone up to 90%, whereas heating, pasteurization and spray-drying induced a significant loss. Regarding the concentration of IGF-I, it is known that higher temperatures, such as those associated with infant formula preparation, will denature it.

Studies in humans suggest that low nutrition level, including malnutrition, starvation, semi-starvation, fasting and caloric restrictions, lowers the IGF-I concentration in plasma (Livingstone, 2013). IGF-I concentrations in plasma are also affected by various physiological or pathological stages in humans (Livingstone, 2013). Several studies have indicated that IGF-I concentrations in human serum could be associated with nutritional status and milk intake. Milk consumption is particularly shown to be associated with an increase in concentrations of IGF-I in plasma in both the young and adults. In an intervention study, when men aged 55–85 years were instructed to drink three servings of non-fat or 1% milk per day as part of their normal diet, IGF-I concentrations in serum increased significantly (10%) in the intervention group by the end of the 12-week intervention period compared with concentrations in those who maintained their normal diet (Heaney *et al.*, 1999). In another intervention study in Mongolia, after a month of drinking whole milk, 10- to 11-year-old school children had higher mean levels of IGF-I, ratios of IGF-I to IGF binding protein 3

(IGFBP-3) and 75th percentiles of growth hormone levels in plasma. A similar, albeit smaller and non-significant, increase in IGF-I, IGF-I/IGFBP-3 and growth hormone levels in plasma was also observed after a week of drinking low-fat milk by girls aged 6–8 years in Boston, Massachusetts, United States of America (Rich-Edwards *et al.*, 2007). A Danish intervention study demonstrated that IGF-I concentrations in serum and serum IGF-I/IGFBP-3 ratio in 8-year-old boys (n = 12) increased from baseline after daily consumption of 1.5 L of milk for 7 days. However, in boys (n = 12) supplemented with similar levels of protein from 250 g of low-fat meat, these changes were not observed, suggesting that consumption of milk, but not animal protein alone, is associated with the increase in IGF-I level in plasma (Hoppe *et al.*, 2004). A case-control study in the United States of America also suggested that low-fat-milk intake, but neither red meat, poultry nor fish intake, was positively associated with IGF-I level in serum and IGF-I/IGFBP-3 ratio (Ma *et al.*, 2001). A European prospective investigational study (Crowe *et al.*, 2009) associated dairy protein and calcium intake with increased IGF-I concentrations in serum. A mean increase in IGF-I concentration in plasma of 13.8 ng/ml (95% confidence interval 6.1–21.5) in intervention groups consuming cow milk when compared with the controls was reported in a meta-analysis of published literature (Qin, He and Xu, 2009). Evidence therefore points to the fact that drinking milk is associated with an increase in IGF-I levels in plasma, which, however, could be modulated by the existing nutritional or health status of a person. The effect of nutrition or foods, especially milk, on IGF-I level in plasma is, however, short lived (i.e. with no long-term effect). In a British long-term study (Carnegie [Boyd Orr] Survey) involving 728 subjects followed up for 65 years, IGF-I level in adulthood was negatively correlated with childhood family diets (based on 7-day household food inventories) high in milk (Martin *et al.*, 2007).

Although the studies reviewed above demonstrated that consumption of milk could increase the IGF-I concentrations in blood, whether such increases were due to absorption of IGF-I from milk into the systemic circulation or stimulation of endogenous IGF-I production was not investigated.

Studies on the absorption of orally consumed IGF-I in humans were also available. In one study, the effect of enteral IGF-I supplementation on feeding tolerance, growth and gut permeability in premature infants during the 1st month of life in a prospective, double-blind, randomized study was examined (Corpeleijn *et al.*, 2008). The study was conducted according to European good clinical practice regulations. Neonates received either standard infant formula (n = 32) or standard formula supplemented with IGF-I, extracted from bovine whey, at 100 µg/L (n = 28) during the first 28 days of life. Enteral IGF-I supplementation had no statistical effects ($P > 0.05$) on concentrations of IGF-I, IGFBP-1 and IGFBP-3 or growth hormone in serum compared with the control group throughout the study. No statistical difference in the primary end-points of days to full enteral feeding, days to regain birth weight or rate of weight gain as well as a range of clinical and anthropometric measures was observed. The results of a lactulose/mannitol excretion test as a secondary end-point, performed at 7-day intervals as a measure of intestinal permeability, indicated no statistically significant differences ($P > 0.05$) between the two groups on Day 1, 7, 21 or 28. On Day 14, the ratio was significantly reduced ($P = 0.022$), indicating reduced gut permeability in the IGF-I-treated group. There were no differences in intestinal maturation expressed as lactase activity at the same time-points. This study, where the controls were supplemented with similar formula with lower levels of IGF-I, provided no evidence of oral absorption of IGF-I at a dose roughly 1–2 times the concentration found in human colostrum (Table 9.6) and at about 20 times that of milk from contemporary rbST-untreated cows.

The second study specifically examined the effect of bovine colostrum supplementation on IGF-I concentrations in serum in one portion of the study and the oral absorption of IGF-I in a second portion in adult athletes (Mero *et al.*, 2002). In the first portion of the study, adult male and female athletes were randomly assigned in a double-blind design to either a

colostrum-treated group (n = 19) or a placebo-treated control group (n = 11). The colostrum-treated group received an oral bovine colostrum supplement (20 g) that contained a total of 74 µg IGF-I, and the control group received maltodextrin (20 g), daily during a 2-week training period. A significant increase (17%; P <0.01) in IGF-I concentrations in serum was observed in the colostrum-treated group compared with the placebo-treated group. The concentration of circulating IGF-I steadily increased (0.38 nmol/L per day) over the 14-day treatment period, which was ascribed to either direct absorption of IGF-I from the colostrum supplementation or enhanced stimulation of human IGF-I synthesis. In the second portion of the study, the absorption of ^{123}I-labelled rhIGF-I orally administered to six male (mean age 29.1 years) and six female (mean age 23.9 years) athletes was examined. The study involved the preparation of ^{123}I-labelled IGF-I, validation of the biological activity of the radiolabelled IGF-I by receptor binding assay and blood sampling (n = 7) of subjects over the test day following oral administration of the ^{123}I-labelled rhIGF-I. IGF-I concentrations in serum measured using a two-site immuno-enzymometric assay showed no significant differences during the first 180 minutes after ^{123}I-labelled rhIGF-I treatment. At 7 hours after treatment, following a standard lunch, the concentrations were significantly increased (17%; P <0.01) compared with the pre-treatment concentration (20 nmol/L). Gel filtration of serum samples demonstrated radiolabel in low molecular weight substances, but no radioactivity at the elution positions of free IGF-I or the IGF-I binding proteins. The results provided no evidence for the absorption of orally consumed IGF-I in adult athletes; alternatively, the absorbed IGF-I was subject to an extensive first-pass effect.

Four separate randomized controlled studies investigated whether supplementing bovine colostrum with IGF-I (2 mg/kg) would increase the concentrations of IGF-I in plasma from human volunteers who were active athletes or participating in endurance training (Buckley *et al.*, 2002; Coombes *et al.*, 2002; Kuipers *et al.*, 2002; Buckley, Brinkworth and Abbott, 2003). Volunteers were supplemented with 60 g of bovine colostrum or 60 g of concentrated whey protein for 4 or 8 weeks. In all four studies, IGF-I concentrations in plasma from the intervention group did not differ either pre-treatment or during or at the end of the supplementation when compared with whey protein–fed controls. Data reviewed in the earlier section *Insulin-like growth factor-I in tissues and milk* and those reviewed by the 50th Meeting of the Committee (WHO, 1998) suggest that the mean IGF-I concentrations in milk from rbST-treated and control cows are approximately 6 ng/ml and 4 ng/ml, respectively. A person consuming 1.5 L of milk from rbST-treated cows would therefore be exposed to 9000 ng of IGF-I per day, and the incremental increased exposure coming from the rbST use would be only 3000 ng/day. In contrast, in the trials reviewed above, study participants were supplemented with 120 000 ng of IGF-I per day. However, the IGF-I concentrations in their plasma did not differ from those of whey protein-fed controls. These findings suggest that the circulating IGF-I concentrations in humans would increase by ingestion of milk (or its components), but would not be affected by the amount of IGF-I ingested in food.

Milk nutritional composition

The Committee at its 40th and 50th Meetings examined the effects of rbST on milk composition and concluded that nutritional components and further processing characteristics of milk are not altered by rbST treatment (JECFA, 1993 [TRS 832]; JECFA, 1999 [TRS 888]). Furthermore, the composition of milk from treated cows is well within the normal variation observed during the course of a lactation.

The composition of milk from cows treated with rbST in comparison with untreated controls that are available from recent publications is summarized in Table 9.7. In concurrence with the conclusions of the previous meetings, these data demonstrate that there is no impact of rbSTs on the nutritional qualities of milk.

Table 9.7. Milk yield and protein, fat and lactose contents among rbST-treated and control animals

Species	Group treatment	Milk yield (kg/day or L/day)	Protein (%)	Fat (%)	Lactose (%)	Reference
Cattle	Control	23.5	3.65	4.29	9.00	Kim and Kim, 2012
	rbST	27.7	3.30	3.84	8.89	
Cattle	Control	20.7	3.16	3.50	4.51	Campos *et al.,* 2011
	rbST	22.6	3.16	3.52	4.39	
Cattle	Control	15.6	3.27	3.67	–	Macrina, Tozer and Kensinger, 2011
	rbST	17.9	3.28	3.65	–	
Cattle	Control	41.9	2.86	3.65	–	Rivera *et al.,* 2010
	rbST	45.4	2.81	3.30	–	
Cattle	Control	36.1	2.90	3.82	–	Liboni *et al.,* 2008
	rbST	37.6	2.83	3.78	–	
Cattle	Control	12.9	3.45	3.94	4.90	Chaiyabutr *et al.,* 2007, 2008
	rbST	14.6	3.51	4.24	4.62	
Cattle	Control	33.5	3.08	3.53	–	Al-Seaf, Keown and van Vleck, 2007a, b
	rbST	36.8	3.06	3.55	–	
Cows	Control	22.3 (1)	3.0	3.6	4.8	Annen *et al.,* 2007
	rbST	22.4 (1)	3.1	3.5	4.9	
Cattle	Control	38.8	2.84	3.61	–	Blevins, Shirley and Stevenson, 2006
	rbST	39.6	2.78	3.54	–	
Cattle	Control	32.5	3.11	3.57	4.75	Rose, Weekes and Rowlinson, 2005
	rbST	36.6	3.03	4.33	4.79	
Cattle	Control	13.11	3.27	3.60	4.52	Maksiri, Chanpongsang and Chaiyabutr, 2005
	rbST	16.02	3.16	4.70	4.79	
Cattle	Control	16.2	3.22	3.65	–	Fike *et al.,* 2002
	rbST	17.7	3.23	3.80	–	
Cattle	Control	25.9	3.13	3.55	5.00	Capuco *et al.,* 2001
	rbST	29.3	2.84	3.80	4.98	
Cattle	Control	40.2	2.92	3.12	–	Moallem, Folman and Sklan, 2000
	rbST	45.4	2.94	3.19	–	
Cattle	Control	29.0	3.05	3.13	4.89	Tarazon Herrera *et al.,* 1999
	rbST	32.6	3.05	3.31	4.95	
Cattle	Control	30.5	3.3	4.2	4.8	Miller *et al.,* 1999
	rbST	25.2	3.3.	4.2	4.7	
Cattle	Control	28.8	3.15	3.64	–	Bauman *et al.,* 1999
	rbST	33.0	3.17	3.57	–	
Buffaloes	Control	7.17	3.78	4.69	4.75	Feckinghaus, 2009
	rbST	8.59	3.78	4.85	4.90	
Buffaloes	Control	5.67	4.75	6.96	–	Jorge, Gomes and Halt, 2002
	rbST	7.53	4.58	6.82	–	
Goats	Control	0.960	3.14	4.64	3.58	Qudus *et al.,* 2013
	rbST	1.473	3.28	4.76	3.92	
Goats	Control	8.9	3.31	4.39	4.34	Moraes e Amorim *et al.,* 2006
	rbST	9.0	3.30	4.44	4.47	
Sheep	Control	1.23	4.89	6.14	–	Andrade *et al.,* 2008
	rbST	2.51	4.88	5.92	–	
Sheep	Control	0.683	4.6	3.6	4.8	Sallam, Nasser and Yousef, 2005
	rbST	0.868	4.8	3.8	4.8	

NOTES: rbST = recombinant bovine somatotrophin; (1) Half udder milk yield.

Possible effects of rbSTs on the expression of certain viruses and prions in cattle

The 50th Meeting of the Committee evaluated whether the immunomodulatory effect of bST would affect expression of retroviruses or prion proteins in treated animals and concluded that (i) available studies provided no evidence that rbSTs affect the expression of retroviruses in cattle, and (ii) the possibility of a link between rbST treatment and bovine spongiform encephalopathy (BSE) was highly speculative, and there was no evidence for a direct link between rbST treatment and BSE (JECFA, 1999 [TRS 888]).

The literature search as described above for publications from 1998 to August 2013 retrieved 126 unique articles that included the term "virus" OR "lentivirus" OR "retrovirus" OR "prion". None of these articles, however, investigated the effects of rbSTs on the expression of viruses or prions in cattle or other ruminants. No new information on the role of rbSTs in the expression of retroviruses or prion proteins in ruminants was available from the literature.

Possible increased health risks to human neonates and young children

Diabetes

The published literature does not associate milk or dairy consumption with type 2 diabetes (Aune *et al.,* 2013; Gao *et al.,* 2013). However, the literature is inconsistent on an association between milk or dairy consumption and risk for development of type 1 diabetes. Some, but not all, published studies have indicated that in children genetically predisposed to type 1 diabetes, cow milk feeding in early infancy, when an infant's gastrointestinal tract is not fully developed, could stimulate the production of antibodies that can cross-react with pancreatic islet β-cell surface antigens (Knip, Virtanen and Akerblom, 2010; Norris, 2010). These auto-antibodies may be a risk factor for activation of autoreactive T cells and type 1 diabetes (Skyler, 2007). Stimulation of aberrant immune response in infancy, however, is not limited to milk components alone, as infants genetically predisposed to type 1 diabetes also have a generalized aberrant immune response to several other proteins, including those from cereals, fruits, berries, bacteria and viruses (Harrison and Honeyman, 1999; Vaarala, 2005, 2012; Simpson and Norris, 2008; Atkinson, 2012; Eringsmark Regnéll and Lernmark, 2013; Pugliese, 2013).

Studies reviewed by the 50th Meeting, as well as those published in the scientific literature since then (see Table 9.7), suggest that the composition of milk from rbST-treated cows does not differ from that of untreated controls. The only exception is a transient increase in the mean concentration of IGF-I in the milk from rbST-treated cows, which, however, falls within the normal physiological range observed in untreated animals (see earlier section on *Insulin-like growth factor-I in tissues and milk*).

Data primarily from knockout mice, but also from human studies, suggest that IGF-I is unlikely to have an adverse impact on the pathogenesis of diabetes in humans. When IGF-I was locally expressed in pancreatic islet β-cells, transgenic mice treated with streptozotocin had milder type 1 diabetes, and all transgenic mice survived, in contrast to control mice, which developed severe diabetes and died (George *et al.,* 2002). Similarly, transgenic CD-1 mice expressing IGF-I in β-cells were also able to counteract the effect of autoimmune destruction of β-cells (Casellas *et al.,* 2006). Results from other studies (Agudo *et al.,* 2008; Robertson *et al.,* 2008) also support that IGF-I produced locally in the islet of Langerhans promotes β-cell replication, reduces apoptosis and has antidiabetic effects by improving islet cell survival and/or providing insulin-like effects. Locally expressed IGF-I, however, did not cause the growth or mass increase of the islet itself. The parenteral administration of IGF-I or IGF-I/IGFBP-3 combinations reduced the severity of insulitis and reduced the onset of type 1 diabetes in non-obese diabetic transgenic mice (Chen *et al.,* 2004).

In general, circulating levels of IGF-I are lower in patients with diabetes (Capoluongo *et al.,* 2006), and case reports in humans have demonstrated that patients with severely insulin-

resistant type 1 diabetes could become insulin sensitive for a prolonged period after weekly intravenous bolus infusion of IGF-I at 500 µg/kg bw (Usala *et al.*, 1994). A clinical trial evaluated the efficacy of rhIGF-I in patients with type 1 diabetes in a randomized double-blind study (Thrailkill *et al.*, 1999). Treatment with rhIGF-I and insulin improved glycaemic control and significantly reduced the glycosylated haemoglobin level and daily insulin requirements. Other studies in humans have also demonstrated beneficial effects of IGF-I in the treatment of type 1 (Carroll *et al.*, 2000) or type 2 diabetes (Moses *et al.*, 1996; Murphy, 2006).

Available evidence suggests that IGF-I is unlikely to have an adverse impact on the pathogenesis of type 1 or type 2 diabetes in humans. As the milk composition did not materially differ between cows treated with rbSTs and untreated cows, the milk from rbST-treated cows would not pose an additional risk for the development of diabetes.

Cancer

The Committee considered the potential cancer risk to humans associated with the consumption of milk from rbST-treated cows. rbSTs are not absorbed from the gastro-intestinal tract, have species-specific receptor binding and are not bio-active in humans. Also, the orthologue (e.g. mouse and rat) somatotrophins did not cause cancer in mice and rats, respectively, when administered subcutaneously (see section above: *Long-term studies on toxicity and carcinogenicity of recombinant mouse and rat somatotrophins*). Therefore, the carcinogenicity risk of rbSTs themselves was considered negligible.

The normal physiological range of IGF-I in human plasma is very wide, ranging from 17 to 250 ng/ml in children, from 182 to 780 ng/ml in adolescents and from 123 to 460 ng/ml in adults (see Table 9.6). Several prospective and case-control epidemiological studies have shown that circulating IGF-I levels are higher, although within the normal physiological range, in some cancer patients (Clayton *et al.*, 2011). Moreover, these findings were inconsistent between studies and between different types of cancer. No significant difference was noted in the concentrations of IGF-II or IGF binding proteins in blood between cancer patients and their controls (Clayton *et al.*, 2011). Most of the observations on higher levels of circulating IGF-I in cancer patients were made in epidemiological studies in which the impact of reverse causation cannot be ruled out. Additionally, a recent review on possible carcinogenic hazard to consumers from IGF-I in the diet concluded that the available database is insufficient to link dietary IGF-I directly with breast cancer (Committee on Carcinogenicity, 2012).

Literature reviewed on the bio-availability of IGF-I (see the earlier section on *Bio-availability and bio-activity of IGF-I*) suggested that milk consumption could increase the concentrations of IGF-I in human serum. However, evidence was lacking that the increase was due to absorption of IGF-I in milk. The endogenous IGF-I production in humans will therefore be influenced by whether a person consumes milk at all, irrespective of whether the milk comes from rbST-treated or untreated cows. Further, when compared with the overall daily IGF-I production in human adults of 10 mg (see Table 9.6), the putative contribution of milk-borne IGF-I is considered negligible. For example, a person consuming 1.5 L of milk from rbST-treated cows on average will be exposed to 9000 ng of IGF-I per day, which is equal to 0.09% of the daily production of IGF-I in an adult.

Increased use of antimicrobial agents to treat mastitis in cows treated with rbSTs

The effect of rbST treatment on mastitis incidence and somatic cell count in milk from treated cows was not reviewed by the Committee at its 40th Meeting, as these effects were considered outside the Committee's terms of reference. At its 50th Meeting, the Committee reviewed published information and the results of a post-approval monitoring programme for sometribove (Posilac®) in the United States of America on the influence of rbSTs on mastitis and animal health. The Committee concluded that the effects of rbSTs on the

incidence of mastitis and general health as well as the resulting days of treatment per animal with any medication are an issue of animal health and outside the terms of reference of the Committee. However, the Committee did consider the results of the post-approval monitoring programme on the percentage of milk discarded due to non-compliant (violative) drug residue as a consequence of antimicrobial use after the market availability of Posilac®. It was concluded, based on the results of the programme, that the use of rbSTs will not result in a higher risk to human health due to the use of antimicrobial agents to treat mastitis and that the increased potential for drug residue in milk could be managed by practices currently in use by the dairy industry and by following label directions for use.

The present Committee updated the assessment performed at the 50th Meeting of the Committee. While acknowledging the issue of mastitis *per se* to be one of animal health and outside the terms of reference of the Committee, 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". 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 9.8).

Table 9.8. Studies investigating rbST use and mastitis or milk somatic cell counts in dairy animals.

Study	Study design	Test animal	No. per group	Treatment	Results
Bauman *et al.*, 1999	Epidemiological	Dairy herds of the north-eastern USA during years 1994–1998	Herd no. per group: 164–176	Herds that used Posilac during specified time period vs herds that did not use Posilac	Significant increase in SCC in rbST-treated herds vs control ($P < 0.01$)
Boutinaud *et al.*, 2003	Prospective clinical	Saanen goats (INRA Experimental Farm, Brouessy, France) in week 32 of lactation	3	5 mg rbST/day s.c. for 23 days vs control. Each goat milked 3×/day on right udder half and 1×/day on left udder half	Increased SCC with rbST from treatment days 5 to 17, after which no difference
Brozos *et al.*, 1998	Prospective clinical	Polytocous Chios ewes (Institute of Reproduction and AI, Ionia, Thessaloniki, Greece)	11	160 mg rbST sc every 14 days during lactation days 5–182 vs control (no injection)	Increase in mean SCC after lactation day 105; no significant differences in percentages of bacteriologically positive milk samples, distribution of bacterial isolates, or prevalence of subclinical mastitis
Campos *et al.*, 2011	Prospective clinical	Dairy cows	12–14	500 mg rbST every 14 days, starting on 63rd day of lactation; 500 mg rbST every 12 days, from the 63rd day of lactation, treatment continued until 280 days in milk; control	No effects on SCC or mastitis incidence
Chadio *et al.*, 2000	Prospective clinical, switch-back design with three 28-day periods	Multiparous crossbred alpine goats in lactation week 8	4	160 mg sustained release rbST sc every 14 days vs control	No significant difference in SCC
Chiofalo *et al.*, 1999	Prospective clinical	Multiparous Comisana lactating ewes	40	120 mg rbST sc every 21 days (total two treatments) vs control	No effect on SCC

Study	Study design	Test animal	No. per group	Treatment	Results
Collier *et al.,* 2001	Prospective clinical	Commercial dairy herds (Holstein or Jersey cows) in the north-eastern, south-eastern, upper Midwest and western USA	Primiparous: 209–210; multiparous: 352–355	500 mg sometribove zinc-oil formulation sc/14 days) or control (oil excipient sc), lactation week 9 or 10 to dry-off or lactation day 400	No effects on percentages of cows with mastitis, average mastitis cases/100 cow-days, mastitis case duration, use of mastitis therapies, mastitis ORs for primiparous or multiparous cows, and numbers of cows culled for mastitis
de Souza Paula and da Silva, 2011	Prospective clinical	Dairy cows in Santa Rosa, Brazil	12	rbST, 2 applications, 14 days apart vs saline control	Increased SCC values with rbST treatment
Dohoo *et al.,* 2003	Meta-analysis of prospective clinical trial data	Dairy cows	Unstated	Unstated	Significant increase in incidence rates and RRs (~25%) for clinical mastitis in rbST-treated cows; no significant effect on incidence rate or RR for subclinical mastitis (as increase in SCC)
Feckinghaus, 2009	Prospective clinical	Lactating Murrah water buffaloes	14	Single application 500 mg rbST vs no injection	No effect on SCC on 1st, 3rd, 5th, 7th, 10th and 14th days after application
Fitzgerald *et al.,* 2007	Prospective clinical	Healthy primi-parous Holstein cows (2nd gest-ation, 1st dry period; Univ. of Arizona) with SCC scores of <300 000	4	Control vs 500 mg rbST/14 days during the 60-day dry period through lactation day 30, with half-udder treat-ments of either 2×/day milking or 4×/day milking	No significant differences in SCC during the 1st 30 days postpartum
Gulay *et al.,* 2003	Prospective clinical	Multiparous Holstein cows (University of Florida), 4 weeks prior to calving	95–98	Control vs biweekly 142.9 mg rbST s.c. 21 ±3 days prior to calving through postpartum day 42; all cows received Posilac beginning 100 ±4 days postpartum	No significant differences in SCC, incidences of health problems (types unspecified) or culling rates
Gulay *et al.,* 2004	Prospective clinical	Multiparous Holstein cows (Univ. of Florida) were assigned to treatment groups in a 2 × 3 × 2 factorial arrangement 8–9 weeks pre calving	42	Control vs biweekly injections 0.4 ml (142.9 mg) Posilac per cow from 21 ±3 days prior to calving to 42 ±2 days postpartum; all cows treated with rbST after 56 ±2 days postpartum	Decreased SCC in treated cows through 42 ±2 days postpartum
Gulay *et al.,* 2007	Prospective clinical, also data from a retrospective study analysed separately	Holstein cows in the University of Florida Dairy Research herd	162–166 (prospective) 109 (retrospective cohort)	142.9 mg rbST/cow s.c. 2-week intervals, 19–24 days before calving until 39–45 days postpartum vs control	Decreased incidences of mastitis and total disease in rbST-treated versus controls
Judge *et al.,* 1999	Prospective clinical	Commercial dairy herds in Michigan Dairy Herd Improvement Association	261–277	500 mg rbST every 14 days between lactation days 63 and 301 vs control	No effect of rbST on incidence of mastitis
Kim, Chang and Kim, 2002	Prospective clinical	Holstein dairy cows	9	Group I: rbST alone; Groups II, III and IV: rbST treatment + retinyl palmitate and cholecalciferol; an untreated control group	No significant effect on mastitis incidence, but there was decreased SCC in rbST + retinyl palmitate and cholecalciferol–treated groups

Study	Study design	Test animal	No. per group	Treatment	Results
Kim and Kim, 2012	Prospective clinical	Lactating Holstein dairy cows in Kyunggi Province, Republic of Korea	25	Boostin-250 and vehicle (control), administered weekly; Boostin-S and Posilac every 14 days	No effect on incidence of clinical mastitis and SCC
Liboni *et al.,* 2008	Prospective clinical	Multiparous Holstein cows (University of Florida)	25–27	Group I: no rbST; Group II: postpartum rbST; Group III: prepartum rbST; Group IV: pre-partum and postpartum rbST; prepartum rbST every 2 weeks beginning 21 days before calving; postpartum rbST during the first 63 days of lactation every 2 weeks; all cows received rbST after 63 days in lactation	No changes in SCC between treatment groups
Lucci *et al.,* 1998	Prospective clinical	Crossbred Holstein first-lactation pregnant heifers	9	rbST 500 mg dose, Groups (A) control; (B) bST each 28 days; (C) bST each 21 days; (D) bST each 14 days for 112 days	No effect on SCC
Masoero *et al.,* 1998	Prospective clinical	Italian Friesian lactating cows	25 per trial × 2 trials: 1. winter–spring, 2. autumn–winter	rbST (500 mg, every 2 weeks for 10 times) vs control	No effect on SCC
Moraes e Amorim *et al.,* 2006	Prospective clinical	Toggenburg goats at farm in Água Limpa, Brazil	12	250 mg rbST, every 14 days (four injections) vs saline (control)	Decreased SCC in treated goats
Mukherjee, 2007	Prospective clinical	Lactating buffaloes	30	Boostin (250 mg/2 weeks); no control group	Increase in SCC on day 4, 18 and 32, bacterial plate count $<0.40 \times 10^3$ cfu/ml
Posada *et al.,* 2008	Prospective clinical	Holstein-Freisian cows, 1–4 parity, 60–180 days in milk (Antioquia, Colombia)	10	Group 1, rbST (500 mg) + vitamin E + lecithin, group 2, rbST (500 mg), and control group without treatment; nine injections every 2 weeks	No significant effect on mastitis incidence (measured by California Mastitis Test, and analysed for proportion affected by confidence intervals)
Requena *et al.,* 2010	Prospective clinical	Lactating Manchega dairy ewes (Polytechnic University of Valencia, Spain)	18	Control vs 40, 80 or 120 mg of bST every 14 days from 2 to 20 weeks of lactation	No effect on SCC
Ruegg, Fabellar and Hintz, 1998	Epidemio-logical	32 dairy herds in Indiana, Michigan and Ohio surveyed August 1994 – August 1995	Herd nos per group: 13–19	rbST used for ≥25% cow-days vs control	No effects on culling density, or rate or incidences of SCC-related or mastitis-related culling
Schneider *et al.,* 2012	Prospective clinical	Holstein heifers, southern Brazil, 35 days prior to expected calving	15–16	500 mg rbST/cow s.c. 35, 21 and (if relevant) 7 days before calving vs control	Significantly decreased SCC with rbST
Vallimont *et al.,* 2001	Prospective clinical	Multiparous Holstein dairy cows	13–15	500 mg sustained release Posilac, s.c. 28 and 14 days prior to calving vs control	No effects on mastitis incidence or SCC
VanBaale *et al.,* 2005	Prospective clinical	Multiparous Holstein cows at Arizona commercial dairy	60	rbST, 60–66 to 305 days in milk vs control (cows in both groups were milked 6×/day during the first 21 days in lactation, and 3×/day thereafter)	Increased SCC in cows treated with rbST

Study	Study design	Test animal	No. per group	Treatment	Results
Studies available in abstract form only					
Bayram *et al.*, 2006	Prospective clinical	Anatolian buffaloes in mid- and late lactation	10	500 mg rbST sc every 14 days vs control	No effect on SCC
Hassan *et al.*, 2007	Prospective clinical	Buffaloes in 2nd–3rd lactations, 70–80 days postpartum	6	Control vs biweekly low (250 mg/head) and high (500 mg/head) doses of rbST for 90 days	Significantly ($P < 0.01$) increased SCC

NOTES: cfu = colony-forming units; OR = odds ratio; rbST = recombinant bovine somatotrophin; RR = risk ratio; s.c. = subcutaneously; SCC = somatic cell count.

The meta-analysis publication by Dohoo *et al.*, (2003) was a re-analysis 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 40th and 50th 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, Fabellar and Hintz, 1998) published since then (see Table 9.8) 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 9.8 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, Fabellar and Hintz, 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 Committee at its 50th Meeting compared the non-compliant antimicrobial drug residues in bulk tank milk in the United States of America 2 years before approval of rbST (1992–1993) and 2 years after approval of rbST (1994–1995) as part of a post-approval monitoring programme. Results of the same programme were available for the years 1996–2012 (NMDRD, 2013) for the present Committee to review. The National Milk Drug Residue Database (NMDRD) is a voluntary industry-reporting programme, whereas mandatory reporting is required by state regulatory agencies under the National Conference on Interstate Milk Shipments (NCIMS). Data are reported on the extent of the national testing activities, the analytical methods used, the kind and extent of the animal drug residues identified and the amount of contaminated milk that was removed from the human food supply. The system includes all of the milk supply, of which approximately 95% is regulated through the NCIMS by state regulatory agencies. The trend in milk tankers positive for antimicrobial residues in the United States of America since 1995 is presented in Figure 9.5. As noted at the 50th Meeting, the United States of America switched to a more sensitive test for antimicrobial residues in 1995, corresponding to the highest level of residue non-compliance reported. The bulk milk tankers positive for antimicrobial residues increased slightly between 1995 and 1996. Since 1996, the percentage of bulk milk tankers positive for antimicrobial residues has steadily declined to 0.017% in 2012, compared with 0.10% in 1995 (Figure 9.5). These results provide no evidence of increased human risk for exposure to antimicrobial drug residues associated with the use of rbSTs in the dairy industry in the United States of America over the last 19 years.

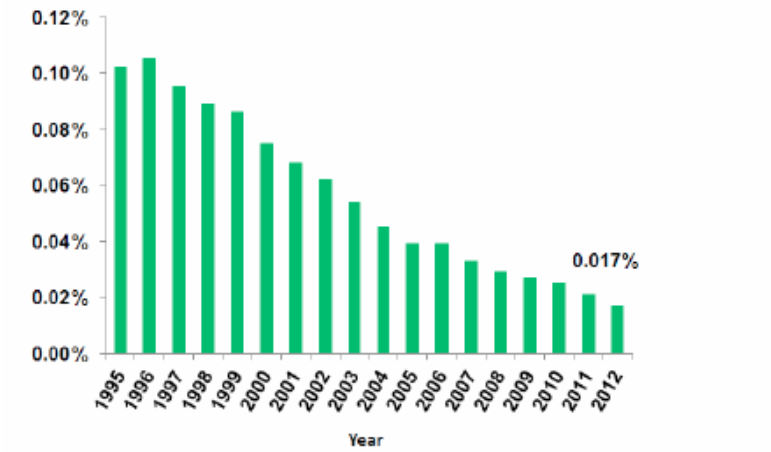


Figure 9.5. Percentage of bulk tankers positive for antimicrobial residues from 1995 to 2012 (Source: NMDRD, 2013)

Several factors could influence the observed decline in non-compliant drug residues, including adherence to good veterinary practice and improved animal husbandry practices. Moreover, the available data did not provide individual animal-level data to correlate with the use of rbSTs. Nonetheless, the available evidence suggests that in the United States of America, the approval of rbSTs was not associated with an increased incidence of non-compliant antimicrobial residues in bulk milk. However, no relevant monitoring data were available from other jurisdictions where rbSTs are authorized for use.

A survey of retail milk in the United States of America (Vicini *et al.*, 2008), which tested 334 retail milk samples labelled as conventional, rbST-free or organic milk from stores in 48 contiguous states within the United States of America, detected no antimicrobial residues.

The use of antimicrobial agents is an important tool in the management of clinical mastitis. However, the Committee could not analyse the potential association between the use of rbSTs and the use of antimicrobial agents. This was due to the unavailability of data on the use of antimicrobial agents to treat mastitis on farms using rbSTs when compared with farms not using rbSTs. The results of the systematic literature review of the studies published since the last Committee meeting and the antimicrobial residue monitoring data from the United States of America, however, provided an indirect indication that when antimicrobial agents are used in accordance with the label directions, human exposure to antimicrobial residues is unlikely to increase due to potential increased use of antimicrobial agents to treat mastitis in rbST-treated cows.

An excerpt from the USDA's Animal and Plant Health Inspection Service's Centers for Epidemiology and Animal Health fact sheet on bulk tank milk somatic cell counts (BTSCC) was also provided (Bauman and Collier, 2013). BTSCC refers to the number of white blood cells (primarily macrophages and leukocytes), secretory cells and squamous cells per millilitre of raw milk. The average BTSCC in milk in the United States of America was stable between 1998 and 2003 and has declined steadily since 2003. BTSCC declined from 319 000 cells/ml in 2003 to 233 000 cells/ml in 2009 (27% decline). An average BTSCC of 224 000 cells/ml in 2010 and 206 000 cells/ml in 2011 indicates that the pattern of decline continues. Operations with increased BTSCC are more likely to have milk that is non-compliant with antimicrobial residues (van Schaik, Lotem and Schukken, 2002). A continuous decrease in somatic cell count in milk in the United States of America is an additional indirect support for the lack of evidence linking the use of rbSTs with an increased risk for antimicrobial residues in milk.

Studies from the USDA's National Animal Health Monitoring System (NAHMS) reported that 10.1% of cows in the United States of America in 1996, 22.3% in 2002 and 17.1% in 2007 were treated with rbSTs (USDA, 2007). During those years, the percentages of cows with mastitis increased slightly, from 13.4% (1996) to 14.7% (2002) to 16.5% (2007). Although the slight increase in prevalence of mastitis from 1996 to 2002 could be linked with a more than doubling in the percentage of cows given rbSTs, mastitis prevalence continued a trend upwards in 2007, despite a 5% decrease in the percentage of cows administered rbSTs. The increase in mastitis prevalence was more closely related to the increased annual milk yield per cow of 1–3% per year since 1991 (USDA, 2007).

Ruzante *et al.* (2010) analysed data collected during the NAHMS Dairy 2007 study (USDA, 2007) from dairy farms in the United States of America to study factors associated with the presence of *Salmonella* in environmental samples in dairies in the United States of America. Environmental samples to test for *Salmonella* were collected from a subset of 260 dairy operations used in the overall study. The association of the presence of *Salmonella* in environmental samples with the use of rbSTs was examined as one of the factors. A higher presence of *Salmonella* in the environment was observed with the use of rbSTs. The biological significance of this finding is unclear, and the study was not designed to capture any related factors, such as management practices.

In its systematic review of the literature, the Committee did not find specific studies that investigated the associations between the use of rbSTs and the development of antimicrobial resistance in mastitis pathogens. Controlled studies have not determined whether the use of rbSTs may increase this risk or, for that matter, help to decrease it. 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 for concern (Oliver, Murinda and Jayarao, 2011; Oliver and Murinda, 2012), in the absence of properly designed studies, whether the use of rbSTs in cows or farms increases antimicrobial resistance remains speculative. It is concluded that there is a lack of evidence that the use of rbSTs in dairy herds contributes to antimicrobial resistance in dairy herds.

Available new information therefore does not change the conclusion of the 50th Committee Meeting in regards to the risk to human health due to the use of antimicrobial agents to treat mastitis.

Comments

Biochemical data

The Committee at its 40th and 50th Meetings concluded that human and bovine somatotrophins are structurally different and have species-specific receptor binding activity. Furthermore, the total concentration of bST detected in tissues and milk of rbST-treated cattle is similar to that from untreated cattle, and bST is denatured by high temperatures (e.g. by cooking or pasteurization) and biodegradation processes in the gut. No new biochemical data on rbSTs were available since the previous evaluation of the compound by the Committee at its 50th Meeting. The Committee evaluated a part of a study submitted to previous JECFA meetings, but not specifically discussed in the respective monographs. This study investigated the level of anti-rbST antibodies in serum as a surrogate measure for oral absorption/bio-availability in rats administered an rbST by gavage for 90 days. The results indicated increased levels of circulating anti-rbST antibodies in 20% and 30% of rats treated with the rbST at 5 and 50 mg/kg bw per day, respectively, and in one animal (3%) treated with the rbST at 0.1 mg/kg bw per day. The experimental design, however, did not allow an assessment as to whether the antibody response was a result of absorption of intact rbST or only an immunologically active peptide fragment (epitope or antigenic determinant) of the rbST into the systemic circulation or due to mucosal immunity in the gut. Also, there were no

systemic effects on growth or feed intake in orally treated rats. These data, together with the data evaluated at previous meetings of the Committee, confirm the absence of the biological activity of rbSTs following oral intake.

Toxicological data

The Committee at its 40th Meeting evaluated the toxicity of different rbSTs. Acute oral toxicity studies in rats with rbST doses up to 5 g/kg bw, two 2-week oral feeding studies in rats with rbST doses up to 10 mg/kg bw per day and two 4-week oral feeding studies in rats with rbST doses up to 50 mg/kg bw per day caused no effects up to the highest dose tested. Similarly, no treatment-related effects were observed in two 90-day oral feeding studies in rats at rbST doses up to 100 mg/kg bw per day and a 90-day oral feeding study in dogs at rbST doses up to 10 mg/kg bw per day, the highest doses tested. No new toxicity studies on rbSTs were available since the previous evaluation of rbSTs by the Committee at the 50th Meeting.

The present Committee evaluated long-term carcinogenicity studies in rats and mice using related, but distinct, compounds (i.e. rrST and rmST). Daily subcutaneous administration of rrST and rmST to groups of rats and mice, respectively, for 2 years did not show any carcinogenic effects. Although the Committee considered these data not directly relevant to the risk assessment of rbSTs, these observations do illustrate that other somatotrophins are not potential carcinogens.

Concentrations of rbSTs and IGF-I in milk and tissues

Previous meetings of the Committee have concluded that owing to the structural dissimilarity between bovine and human somatotrophins and species-specific receptor binding, rbSTs are not biologically active in humans. Also, similar concentrations of total bST are detected in milk and tissues of rbST-treated and untreated cows. Very few new publications investigating the concentrations of bST in milk and tissues following treatment with rbSTs were available in the literature since the 50th Meeting of the Committee. Available information supports the conclusions of the previous Committee that there is no significant change in the concentrations of total bST detected in milk and tissues of rbST-treated cows when compared with untreated controls.

Available new information supports previous conclusions that the IGF-I concentration in milk varies widely in lactating cows and is influenced by parity, stage of lactation, nutritional status, season and somatic cell counts (an indication of udder health) of the milk. IGF-I concentrations measured in colostrum are substantially higher than concentrations in milk produced subsequently. Treatment of cows with rbSTs transiently increased the mean IGF-I concentration in milk by up to 50%, but such increases were within the physiological variations observed in untreated cows.

A new cross-sectional study of retail milk in the United States of America suggests that the IGF-I concentrations in retail milk labelled as conventional, which includes milk from both rbST-treated and untreated cows (3.1 ±0.1 ng/ml), were not different from concentrations in milk labelled to be from rbST-free cows (3.0 ±0.1 ng/ml). However, the percentage of conventional milk that comes from cows treated with rbSTs is not known.

The 50th Meeting of the Committee considered that some milk-borne IGF-I may escape degradation by gastrointestinal tract enzymes and get absorbed from the gastrointestinal tract. *In vitro* digestion studies indicated that IGF-I is rapidly degraded by gastrointestinal tract enzymes. However, subsequent studies in experimental animals showed that the rate of degradation could be reduced by the components in milk and colostrum. *In vivo* studies in laboratory animals suggested that up to 25% of IGF-I fed with milk could be absorbed from the gastrointestinal tract, although only a fraction of it would reach the systemic circulation. Studies in infants showed that feeding a formula supplemented with a 20-fold higher

concentration of IGF-I did not increase the IGF-I concentrations in serum compared with feeding a standard formula. Randomized trials in active adult athletes did not detect any difference in IGF-I concentrations in plasma from an intervention group fed up to 120 000 ng IGF-I per person per day from bovine colostrum for up to 8 weeks when compared with controls fed whey protein during pre-treatment, treatment or post-treatment periods.

The literature suggests that the concentration of IGF-I in serum in humans is influenced by a number of factors, including age, physiological stage and nutritional status. Consumption of milk *per se* was associated with increased blood IGF-I concentrations in humans. There is evidence that orally administered IGF-I has some local bio-activity in the gastrointestinal tract. However, given the large quantity of IGF-I secreted in the digestive tract of humans, the small additional quantity of IGF-I in milk from cows treated with rbSTs is unlikely to make a biologically relevant contribution to the effects of endogenous IGF-I. The endogenous IGF-I production in humans will be more influenced by the consumption of milk *per se*, irrespective of whether it is from rbST-treated or untreated cows.

The present Committee concluded that some milk-borne IGF-I may not be degraded by gastrointestinal enzymes. However, even if some of the IGF-I in milk were absorbed, the incremental human exposure would be negligible when compared with total daily human production of IGF-I of 10 mg/day, as reported by the Committee at the 50th Meeting. This is consistent with the previous conclusion of the Committee.

Expression of retroviruses and prion proteins

The 50th Meeting of the Committee concluded that the available studies provided no evidence that rbSTs affect the expression of retroviruses in cattle. The Committee also concluded that the possibility of a link between rbST treatment and BSE was highly speculative, as there was no evidence for a direct link. No new information on the role of rbSTs in the expression of retroviruses or prion proteins in ruminants was available from the literature.

Risk of type 1 diabetes in genetically susceptible infants

There is evidence that in infants genetically susceptible to type 1 diabetes, exposure to cow milk early in infancy, when an infant's gastrointestinal tract is not fully developed, may stimulate the production of antibodies that can cross-react with pancreatic islet β-cell surface antigens. This may be a risk factor for the development of type 1 diabetes. Stimulation of aberrant immune response in infancy, however, is not limited to milk components alone, as infants genetically predisposed to type 1 diabetes also have a generalized aberrant immune response to several other proteins (e.g. cereals, fruits, bacteria, viruses).

Animal and human studies suggest that IGF-I is unlikely to have an adverse impact on the pathogenesis of diabetes in humans. The composition of milk from cows treated with rbSTs did not differ materially from that of untreated cows, and therefore consumption of milk from rbST-treated cows would not pose an additional risk for the development of diabetes.

Risk of cancer

The Committee also considered the potential cancer risk in humans associated with the consumption of milk from rbST-treated cows. The Committee concluded that any carcinogenic risk from rbSTs themselves was negligible, because they are not absorbed from the gastrointestinal tract, they are not bio-active in humans and the respective orthologues did not cause cancer in rats or mice when administered subcutaneously.

As stated above, the IGF-I exposure from consumption of milk from cows treated with rbSTs represented a small fraction of the physiological amounts produced in humans, and endogenous IGF-I production in humans will be influenced more by the consumption of milk *per se* than by whether the milk is from rbST-treated or untreated cows. Circulating IGF-

I concentrations at the higher end of the normal physiological range were observed in some cancer patients, although these were inconsistent between studies and between different types of cancers. Moreover, these observations came from epidemiological studies in which the impact of reverse causation cannot be excluded.

Risk to human health from use of antimicrobial agents

The 50th Committee Meeting concluded that the use of rbSTs would not result in a higher risk to human health due to the use of antimicrobial agents to treat mastitis and that increased potential for drug residues in milk could be managed by practices currently in use within the dairy industry and by following the directions for use.

The potential risk to human health due to the potential for increased use of antimicrobial agents to treat mastitis or increased incidence of non-compliant residues in milk of cows treated with rbSTs was also considered by the present Committee. A meta-analysis published in 1998 observed that cows treated with rbSTs had a higher incidence (up to 25%) of mastitis compared with untreated cows. A systematic review of the literature published since the 50th Meeting of the Committee did not find any significant difference in the incidence of mastitis between rbST-treated and untreated cows. However, the Committee did not have data to determine the use of antimicrobial agents to treat mastitis on farms using rbSTs.

The 50th Meeting of the Committee had assessed the data from a post-approval monitoring programme established in the United States of America to monitor the effects on animal health, including mastitis and non-compliant drug residues in milk. Additional monitoring data for 1996–2012 from the same programme were assessed for the long-term trend in antimicrobial residues in bulk milk. Since 1996, there has been a consistent decrease in the number of bulk milk samples positive for non-compliant antimicrobial residues, with only 0.017% of samples testing positive in 2012, compared with 0.1% in 1996. Several factors could influence the observed results, including adherence to good veterinary practice and improved animal husbandry practices. Moreover, the available data did not provide individual animal-level data to correlate with the use of rbSTs. Nonetheless, the Committee considered that the available evidence suggested that in the United States of America, the approval of rbSTs did not lead to an increased incidence of non-compliant antimicrobial residues in bulk milk. The Committee found no relevant monitoring data from other jurisdictions where rbSTs are authorized for use.

Although the Committee was aware of the concern regarding potential antimicrobial resistance, its systematic review of the literature did not find specific studies correlating the use of rbSTs with the development of antimicrobial resistance in mastitis pathogens.

Based on the data reviewed, the Committee concluded that there was no evidence to suggest that the use of rbSTs would result in a higher risk to human health due to the possible increased use of antimicrobial agents to treat mastitis or the increased potential for non-compliant antimicrobial residues in milk.

Evaluation

Based on the above assessment, the Committee's responses to the issues raised by the Codex Alimentarius Commission are as follows:

(i) Update the toxicological evaluation

No new toxicological studies were available. Owing to structural differences between bovine and human somatotrophins, species-specific receptor binding of somatotrophins and lack of bio-activity of rbSTs following oral intake, the Committee concluded that if any rbST residues are present in milk or tissues, they would pose a negligible risk to human health.

(ii) Update the exposure assessment based on any new occurrence data in food

The Committee concluded that similar concentrations of total bST were present in milk and tissues of rbST-treated and untreated cows.

(iii) Consider new data and information related to the possibility of increased levels of IGF-I in the milk of cows treated with rbSTs

There is a transient increase in IGF-I concentrations in milk of rbST-treated cows, which fall within the normal physiological range. IGF-I is substantially, if not completely, degraded in the gut and is unlikely to be absorbed from the gut and be bio-available at biologically relevant exposures. Therefore, the contribution of exogenous IGF-I resulting from the ingestion of milk from rbST-treated cows is extremely low in comparison with endogenous production.

(iv) Evaluate potential adverse health effects, including the possibility that exposure of human neonates and young children to milk from rbST-treated cows increases health risks (e.g. the development of insulin-dependent diabetes mellitus)

Exogenous IGF-I from milk makes no significant contribution to circulating levels of IGF-I in humans, and there are no significant differences in the composition of milk from rbST-treated cows when compared with the milk from untreated cows. The Committee concluded that there was no additional risk for the development of type 1 diabetes due to the consumption of milk from rbST-treated cows. The Committee also concluded that the literature did not support a link between exposure to IGF-I in milk from rbST-treated cows and an increased risk of cancer.

(v) Consider new data and information related to the potential effects of rbSTs on the expression of certain viruses in cattle

There was no new information on the link between rbST use and either potential stimulation of retrovirus expression or prion protein expression in cattle. The present Committee considers that the position expressed by the previous Committee remains valid.

(vi) Consider new data and information related to 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

The Committee concluded that there was no evidence to suggest that the use of rbSTs would result in a higher risk to human health due to the possible increased use of antimicrobial agents to treat mastitis or the increased potential for non-compliant antimicrobial residues in milk. The Committee found no specific studies linking the use of rbSTs with the development of antimicrobial resistance. The present Committee considers that the position expressed by the previous Committee remains valid.

(vii) Consider the need to revise or maintain the ADI and MRLs for rbSTs

The Committee reaffirmed its previous decision on ADIs and MRLs "not specified" for somagrebove, sometribove, somavubove and somidobove.

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