BOVINE SOMATOTROPINS

First draft prepared by
Prof. Fritz R. Ungemach
Institute of Pharmacology, Pharmacy and Toxicology
Veterinary Faculty, University of Leipzig, Leipzig, Germany
and
Dr. Nicholas E. Weber
Food and Drug Administration, Center of Veterinary Medicine
Rockville, MD, USA

ADDENDUM
to the Bovine somatotropins monograph prepared by
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INTRODUCTION

The four analogues of bovine somatotropins somagrebove, sometribove, somavubove and somidobove that are produced by recombinant DNA-technology (rbST) were previously evaluated by the Committee at its fortieth meeting (WHO TRS 832, 1993, WHO FAS 31, 1993, FAO FNP 41/5, 1993). At that time the Committee established an ADI "not specified" for rbSTs. The term ADI "not specified" was used because of the lack of oral activity of rbSTs and of insulin-like growth factor I (IGF-I), as well as the low levels and non-toxic nature of the residues of these compounds, even at exaggerated doses, resulting in an extremely large margin of safety for humans consuming dairy products from rbST-treated cows. The Committee concluded that the use of these drugs according to good practice in veterinary medicine does not represent a dietary hazard to human health and that there was no need to specify a numerical ADI. Accordingly, no MRLs need to be set. In the meantime (1993), the Committee of Veterinary Medicinal Products of the EEC (CVMP) also concluded that the use of the rbSTs sometribove and somidobove in dairy cattle did not present any risk to the health of the consumers of meat and milk obtained from treated animals, and that due to their safety, the somatotropin products may be used without any withdrawal period for meat and milk. The CVMP considered that it is not necessary for the protection of public health to establish maximum residue limits (MRL) for rbSTs.

The recommendations of the 40th JECFA were deliberated by the Codex Alimentarius Commission, at its 22nd session in June, 1997. The Commission voted that the proposal to adopt the ADI and MRL proposal of "not specified" for rbSTs be postponed pending a reevaluation of new scientific data by JECFA at its 50th meeting in February 1998.

Information was submitted by organizations and individuals relating to the following concerns about the safety of the consumers of dairy products from rbST-treated cows:

- the increased use of antibiotics with a higher rate of violative drug residues in milk due to a possible increased incidence of mastitis in rbST-treated cows,
- the possibility that increased levels of IGF-I in milk of rbST-treated cows lead to increased cell division and growth of tumors in humans,
- the potential effect of rbST on the expression of certain viruses in cattle, particularly the retroviruses,
- the possibility that the incubation period of bovine spongiform encephalopathy (BSE) is shortened due to an IGF-I induced increase of the production of pathogenic prion proteins, and
- the possibility that early exposure of human neonates to milk from rbST-treated cows increases the risk for developing insulin-dependent diabetes mellitus.

BIOLOGICAL DATA

Use of antibiotics

The effect of rbST treatment to induce an increase of mastitis and somatic cell count in milk of treated cows was not reviewed by the Committee at its 40th meeting. These effects on animal health were considered outside the terms of reference of the Committee.

At its 50th meeting the Committee considered the literature data and the results of a post-approval monitoring program for sometribove (Posilac®) in the United States on the influence of rbST on mastitis and animal health. It was concluded that the effects of rbST on the incidence of mastitis and general animal 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 results of the post-approval monitoring program (PAMP) on the percentage of milk discard due to violative drug residue as a consequence of antibiotic use after the launch of Posilac® was considered by the Committee. The PAMP was initiated by the US FDA at the time of approval of sometribove (Posilac®) in November 1993 and started with its commercialization in February 1994. The objectives of this program were to determine whether mastitis incidence and antibiotic use was manageable under actual conditions of use, and whether label directions were adequate (FDA, 1996). The program was designed to address the following areas (Collier, 1996):

- the incidence of mastitis and responses related to herd health (not within the terms of reference of the Committee),
- the treatment with any medications in a 28-herd study with rbST-treated cows (not within the terms of reference of the Committee),
- to examine the incidence of milk discard due to violative drug residues in key dairy states representing at least 50 % of the U.S. milk production.

The PAMP was closely monitored by the FDA and performed according to the sponsor's Quality Assurance Standard Operating Procedures. The FDA confirmed that data integrity was acceptable and that data records and analysis showed excellent fidelity (FDA, 1996).

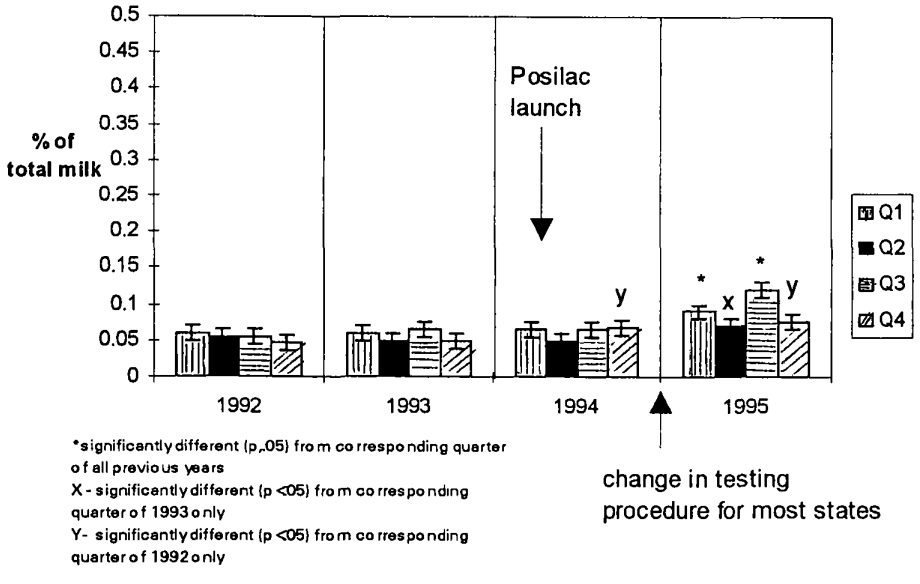
A program was designed for tracking milk residues by key dairy states before and after the approval of sometribove to reveal whether a possible increase of violative drug residues in milk is associated with increased frequency of use of antibiotics for mastitis and other health problems in rbST-treated herds of dairy cows (Veenhuizen *et al.*, 1996). The data from the milk monitoring program for the two years prior to the commercial use of sometribove (1992-1993) was compared to the discard data for two years after the launch to the market (1994-1995). The tracking of residues in milk was recorded by the National Drug Residue Milk Monitoring Program in which all bulk milk tanker trucks are routinely sampled and tested. The data set represented greater than 50% of the total U.S. milk supply. The data were analyzed quarterly by comparing the milk discarded prior sometribove launch with data after launch.

As seen in Figure 1, no change was observed in 1994 after Posilac® was approved. The average percentage of milk discarded was 0.06 % for 1992 as well as for 1993 and after launch 0.07 % in 1994. In 1995 the number of violations slightly, but significantly, increased to 0.09%. This increase, however, coincided with a change of the testing procedures in most states to include more sensitive screening tests in 1995 and that is believed to be responsible for the slight increase seen in 1995. Data reported by Veenhuizen et al. (1996), and submitted to FDA in the drug experience report of May 17, 1996 demonstrated that for New York State there was no significant change in milk discard rate for both years after approval of Posilac compared to the two years prior to approval. In New York, the same testing protocol had been in use throughout the entire four-year period (1992-1995). The values were 0.062% pre-approval and 0.064% post-approval. As reported by the Company launching sometribove, rbST was purchased by nearly 37% of the farms in the state, and these farms represented approximately 50% of the state's cows. These data indicate that:

- no product related increases in violative residues occurred in the years following commercialization of sometribove,
- the rate of positive tests is even slightly lower as compared with the monitoring results for antibiotics in Grade A milk in the U.S.,
- the use of sometribove will not have an impact on the safety of milk and dairy products due to violative drug residues resulting from a slightly higher medication rate in rbST-treated animals due to the procedures used by the milk monitoring program.

It was concluded that the use of rbST will not result in a higher risk to human health due to the use of antibotics 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 (FDA, 1996).

Figure 1. Percent of Milk Discarded Due to Violative Residues from 1992 Through 1995 (52% of 1995 U.S. Milk Supply represented)



*significantly different (p<.05) from corresponding quarter of all previous years
X - significantly different (p <05) from corresponding quarter of 1993 only
Y- significantly different (p <05) from corresponding quarter of 1992 only

adapted from Veenhuizen et al., 1996

bST and IGF-I levels in tissues and milk

Tissue levels of bST and IGF-I

A recent study by Choi *et. al.* (1997) reported the findings on the tissue levels of bST and IGF-I in cattle that had been treated with a 14-day sustained release product containing the natural variant of rbST, somavubove. The authors ran two experiments, A and B. Experiment A was comprised of three groups (12 animals per group, except the low dose group where only 6 animals were employed) of beef cattle whose average weight was 450 kg. The animals were treated by subcutaneous injection for 20 weeks. The controls received no treatment or vehicle, whereas the low dose (LOW), and the high dose (HIGH) groups received 250 mg of rbST at one-week, and 500 mg of rbST at two-week intervals, respectively. It was noted that the control and high dose groups were further divided into a low- and high-energy feed. The stated total dose was 5 and 10 grams of rbST, respectively; however, the dose calculated from the stated regimens would be 5 grams for both groups. Two weeks after final treatment, the animals were slaughtered and muscle samples were obtained and stored at -20°C for analysis.

In the second experiment (B), four groups of beef cattle were employed. They were a control group (CONT) that did not receive any drug or vehicle, a sustained-release low-dose (SR-L), a sustained-release medium-dose group (SR-M), and a sustained-release high-dose group(SR-H). The drug treated groups were administered the drug by s.c.-injection every two weeks for 24 weeks as follows: SR-L, 0.42 mg rbST/kg b.w. (0.03 mg/kg b.w./day); SR-M, 0.84 mg rbST/kg b.w. (0.06 kg b.w./day); and SR-H, 1.26 mg/kg b.w. (0.09 mg/kg b.w./day). The total dose was 2.3 g, 4.5 g, and 6.8 g for the respective dosing regimens. Animals were slaughtered and samples of muscle, kidney, liver and fat were taken two weeks following the final treatment and again stored at -20°C.

Frozen samples were assayed for bST and IGF-I residues by radioimmunoassay (RIA) procedures. The assays employed five grams of tissue and used acid ethanol for the extraction of muscle, and acetic acid for extraction of kidney, liver, and fat samples. The RIA procedures used standard double antibody techniques and iodinated tracers. The detection limit for the assays (the amount that could be distinguished from zero concentration with 95% confidence) was 0.17 ng/g and 0.61 ng/g for bST and IGF-I, respectively. Coefficients of variation for the two assays were approximately 6% or less, and

recoveries in liver, kidney and fat were 64.4% and 84.3% for bST and IGF-I, respectively. Similar recoveries were obtained in muscle samples. A summary of the results are seen in the following Tables 1 and 2.

Table 1. bST Levels* in Tissues after rbST Treatment

Experiment A	Tissue	CONT	LOW[1]	HIGH	
	Muscle (n=12)	1.87 ± 1.82 (a)	1.55 ± 1.62 (a)	3.25 ± 2.17 (a)	
Experiment B		**CONT**	**SR-L**	**SR-M**	**SR-H**
	Muscle (n=5)	3.38 ± 1.51 (a,b)	4.94 ± 1.47 (b)	3.78 ± 1.96 (b)	1.47 ± 0.86 (a)
	Fat (n=4)	5.05 ± 1.67 (a)	9.33 ± 5.23 (a)	4.82 ± 1.95 (a)	11.24 ± 11.95 (a)
	Liver (n=4)	5.18 ± 0.59 (a)	3.56 ± 1.73 (a)	5.36 ± 1.21 (a)	4.63 ± 1.96 (a)
	Kidney (n=4)	3.58 ± 1.14 (a)	4.45 ± 1.62 (a)	4.49 ± 1.83 (a)	3.92 ± 0.94 (a)

1 -number of animals in LOW group = 6; * levels in ng/g are expressed as the mean ± SD;
a and b -the same letter means that there is no statistically significant difference between values

Table 2. IGF-I Levels* in Tissues after rbST Treatment

Experiment A	Tissue	CONT	LOW[1]	HIGH	
	Muscle (n=12)	88.1 ± 21.0 (a)	131.8 ± 24.6 (a)	115.4 ± 32.1 (a)	
		(a)	a	a	
Experiment B		**CONT**	**SR-L**	**SR-M**	**SR-H**
	Muscle (n=5)	44.5 ± 6.5(ab)	34.9 ± 15.2 (b)	39.7 ± 5.1 (b)	54.5 ± 18.5 (a)
	Fat (n=4)	210.2 ± 84.8 (a)	204.3 ± 64.6 (a)	203.6 ± 52.6 (a)	339.1 ± 229.2 (a)
	Liver (n=4)	349.7 ± 23.5 (a)	389.6 ± 132.3 (a)	383.9 ± 168.1 (a)	294.4 ± 88.4 (a)
	Kidney (n=4)	913.5 ± 133.5 (a)	997.0 ± 140.2 (a)	821.1 ± 124.0 (a)	979.4 ± 219.4 (a)

1 -number of animals in LOW group = 6 * levels in ng/g are expressed as the mean ± SD
a and b -the same letter means that there is no statistically significant difference between values

The authors conclude that two weeks after administration of rbST for extended times in two dosage forms and at two or three levels, the tissue concentrations of bST and IGF-I are not significantly different from untreated control animals.

IGF-I residues in milk

Information on the residues of bST and IGF-I residues in milk of rbST-treated cows was evaluated by the Committee at its fortieth meeting (FAO FNP 41/5, 1993).

In bovine milk IGF-I is a normal, but highly variable constituent with the concentration depending on the state of lactation, nutritional status, and age. Over an entire lactation the IGF-I levels range from 1-30 ng/ml with highest concentrations in colostrum followed by constant decline thereafter. Multiparous animals have higher IGF-I concentrations in milk than primiparous (first calf) cows (Burton *et al.*, 1994). Bulk tank milk from cows not receiving rbST had IGF-I concentrations ranged from 1 to 9 ng/ml (Juskevich and Guyer, 1990). The JECFA monograph on BST (FAO FNP 41/5, 1993) cited average control values of 3.7 ng/ml for untreated cows. In milk from rbST-treated cows the levels of IGF-I ranged from 1-13 ng/ml in most studies and were about 25-70% greater as compared with untreated animals (Burton *et al.*, 1994). The JECFA monograph reported average IGF-I concentrations of 5.9 ng/ml (FAO FNP 41/5, 1993). The increase was significant even though most of the measured IGF-I levels were below 10 ng/ml.

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Since the original reported work was reviewed, very little additional residue data has appeared in the literature or in reports made available by sponsors. Monsanto, manufacturer of POSILAC® - previously identified as sometribove which is one of the forms of rbST approved in a number of countries - submitted additional information on levels of insulin-like growth factor-I (IGF-I) in milk. The study (Eppard *et al.*, 1994) was designed to determine the levels of IGF-I in retail milk samples and to compare IGF-I levels in milk which was specifically labeled that it did not come from bST treated cows with IGF-I levels in milk which was not labeled. While the sponsor assumes that the unlabeled milk contained milk from cows treated with bST, the extent to which herds contributing to the unlabeled milk were treated with bST was not ascertained. The study was conducted under FDA's GLP regulations (21CFR 58).

The labeled and unlabeled status of 127 of 129 retail milk samples (4 goats and 125 cows) was determined and subjected to analysis by radioimmunoassay (RIA) for IGF-I. Seventy eight samples were labeled as farmer certified that rbST had not been used. As indicated above, the remainder of the samples did not identify any absence of treatment and were inferred by the sponsor to include herds from rbST treated cows. The samples were collected from retail outlets as 2%-fat cow's milk in 34 cities located in Wisconsin, Minnesota, and Iowa. Fifty-one brands and 51 stores were represented. Note that rbST is not approved for use in goats in the United States and is therefore assumed to reflect untreated IGF-I values for this species. The results are shown in Table 3.

These values for IGF-I are not unlike those reported in the first JECFA monograph on BST (FAO FNP 41/5, 1993). Even though it is not known to which extent milk from rbST-treated cows contributed to the unlabeled milk, the data indicate that in the first year after launch of rbST the IGF-I concentrations in retail milk did not increase.

Table 3. Insulin-like growth factor-I (IGF-I) concentrations in milk identified as farmer certified that rbST was not used (labeled) and non-labeled milk.

	SAMPLE		
	Labeled (ng/ml)	**Non-labeled (ng/ml)**	**P-value**
Raw mean	4.3 ± 0.09	4.5 ± 0.12	
Log_e[1]	1.47 ± 0.044	1.55 ± 0.031	0.1769
Antilog (95% confidence interval)	4.4 (4.0, 4.7)	4.7 (4.4, 5.0)	

[1]n = 78 labeled, 45 non-labeled samples. Least square means adjusted for state where purchased and dairy brand.

Assay values

Because of variations of IGF-I values in different studies, questions have been raised in submissions to the JECFA regarding the accuracy of the IGF-I milk values. In some studies lower values were measured because they were obtained by an assay that used acid ethanol extraction. The availability of another assay which employs acidic gel filtration has been quoted as achieving superior recovery when compared to the acid ethanol procedure. Vega *et al.* (1991) have reported on

the difference in these two assays when determining the IGF-I in pre- and postpartum mammary secretions. These authors calculated that the acid ethanol assay underestimated IGF-I levels by 24 ± 6.6 % when comparing values obtained with acid gel filtration. The problem is that the IGF-I binding protein which has a respectable binding affinity for IGF-I competes with the antibody used in the RIA procedure; however, the gel filtration removes the hormone when it extensively dissociated by the acid that is common to both assays. Studies on the acid ethanol assay by Hadsell (1991) examined the recovery of ^{125}I-IGF-I in bovine serum and colostrum which also contain binding proteins. The author obtained values of 86 ± 6% and 88 ± 7%, respectively when testing the amount of label recovered by the antibody. These studies suggest that the acid ethanol assay probably underestimates milk and plasma values by 15-25 %. It is also apparent that if we are examining the relative difference in milk, the difference will probably not affect the outcome of any decision based on these low levels, although higher control values may affect the inferences being made. Though incomplete removal of IGF-binding proteins or variation of standard source might influence reported results, the Committee considered these factors not to materially alter any conclusions.

Bioavailability and bioactivity of IGF-I residues in milk

At its 40th meeting, the Committee concluded that many of the physiological effects of rbSTs are mediated by bovine insulin-like growth factor I (IGF-I) which is structurally identical to human IGF-I (WHO TRS 832, 1993). It was noted that there is a substantial synthesis of IGF-I mainly in the liver but it is also present in human milk, saliva and pancreatic secretions. It was further concluded that IGF-I had no bioactivity when administered orally to normal and hypophsectomized rats at doses up to 2 mg/kg bw/day. The role of dietary IGF-I was evaluated with the result that it is degraded by digestive enzymes and not active in the upper gastrointestinal tract.

Concerns have been expressed that a broad use of rbSTs in dairy production would lead to a sustained increase of the levels of IGF-I in bulk cow's milk and that the higher exposure of consumers would cause adverse health effects, if IGF-I survives digestion (Hansen *et al.*, 1997).

For a quantitative risk assessment the slight increases of IGF-I in milk of rbST-treated cows have to be compared with the physiological variations of this growth factor during lactation as well as with the levels in human breast milk, in the secretions of the gastrointestinal tract, and in serum.

In human milk the concentrations of IGF-I range from 8-28 ng/ml in the colostrum and from 5-10 ng/ml thereafter (Zumkeller, 1992, Burton *et al.*, 1994) indicating that breast-fed human neonates are normally exposed to IGF-I levels equal or higher as compared with milk of rbST-treated cows.

Assuming a daily intake of 1.5 l of milk from rbST-treated cows with an average IGF-I concentration of 6 ng/ml the ingested amount of IGF-I is 9000 ng/day. The additional daily ingestion of IGF-I as compared with milk from untreated animals with an average IGF-I level of 4 ng/ml or 6000 ng/1.5 l would be 3000 ng.

By ingestion of milk from rbST-treated cows the slightly increased IGF-I levels contribute to the endogenous levels of IGF-I in the gastrointestinal tract of the consumers. The major site of IGF-I production is the liver in animals and humans. This peptide is further produced in the human gastrointestinal mucosa and is also found in saliva, bile, and pancreatic juice (Olanrewaju *et al.*, 1992). Chaurasia, et al. (1994) have determined the secretion of IGF-I in human gastrointestinal secretions. The five secretions and their average concentrations of IGF-I are as follows: saliva, 0.9 nM; gastric juice, 3.5 nM; jejunal chyme, 24.6 nM; pancreatic juice, 3.6 nM; and bile, 0.9 nM. Using a molecular mass of 7.5 kD for IGF-I (Zumkeller, 1992) and the volume for production of each of the fluids (Vander et al.,1990) permits calculation of the total production for IGF-I emptying into the gastrointestinal tract at 383,000 ng/day. The following Table 4 uses the results of the concentration data, along with daily production values for the various secretions and shows the calculated values for total IGF-I secreted.

The data in Table 4 indicate that the amount of endogenous IGF-I emptying into the gastrointestinal tract on a daily basis is more than (383000/9000) 42 times greater than the amount present in 1.5 liters of milk of rbST-treated cows. The 9000 ng value is 2.3% of the estimated daily gastrointestinal secretion of IGF-I in the adult. The additional daily ingestion of IGF-I of 3000 ng as compared with milk from untreated animals represents 0.78% of the gastrointestinal secretion.

Table 4. Gastrointestinal secretion of IGF-I from various sources in the gastrointestinal tract.

	Volume[1]	Concentration	Total mass of IGF-I
Secretion	ml/day	average ng/ml	secreted in ng
Jejunal chyme	1500	184.5	276750
Pancreatic juice	1500	27.0	40500
Gastric juice	2000	26.2	52400
Bile	500	6.8	3400
Saliva	1500	6.8	10200

[1] Vander *et al.* (1990) after Bauman, 1995

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Based on recent studies discussed below, it is postulated that, in contrast to the previous conclusion of the Committee (WHO TRS 832, 1993) of a complete and rapid degradation of IGF-I in the gastrointestinal tract, milk-borne IGF-I may partially escape digestion by proteases. It may, therefore, be bioactive in the intestine (Hansen *et al.*, 1997), or even be absorbed as intact peptide into systemic circulation (Epstein, 1996). In a study designed to investigate the potential of IGF-I peptides as therapeutics in the gut and to check the possibility of orally active formulations the degradation of IGF-I in various segments of the gastrointestinal tract of the rat *in vivo* and *in vitro* was determined (Xian *et al.*, 1995). Compounds that reduce the rate of degradation were also studied. The authors employed ^{125}I labeled IGF-I and monitored the extent of degradation by three methods. These included receptor binding, immunoprecipitation, and trichloracetic acid (TCA) precipitation. The model used two gut segments from each anaesthetized male Sprague-Dawley rat that had been fasted for 24 hours. Ligated segments of duodenum, and ileum, or whole stomach, and part of the colon were used. A bolus of labeled IGF-I (8.6 ng/ml in 0.2% BSA w/v saline) was injected into each segment and incubated for various times up to 60 minutes. The reactions were stopped and the flushed luminal contents were examined for the intactness of the labeled IGF-I by the three methods. The parallel set of *in vitro* experiments utilized flushed luminal contents from each of the four gut segments as a source of degredation enzymes. The results are found in the following Table 5.

Table 5. Half-life of intact ^{125}I-labeled IGF-I in ligated Sprague-Dawley Rat gut segments (A) and in in-vitro flushings (B)

Test for Intactness	Duodenum/Ileum		Stomach		Colon	
	A	B*	A	B*	A	B*
TCA	2 min	2 min	8 min	50 min	38 min	>60 min
Ab binding	2 min		5 min		33 min	
Receptor binding	2 min	2 min	2.5 min	3 min	16 min	ND

* for *in vitro* values (B), Ab and membrane receptor values reported as receptor binding; ND = not done

The data show the most rapid degradation is encountered in the duodenum and ileum segments and in their flushings (in vitro, B) followed by the stomach and then by the colon. In all cases the *in vitro* values were equal to or greater than the *in vivo* values.

The authors also examined the effectiveness of slowing the degradation rate of IGF-I in the gut by protecting the molecule in several ways. Among those tested, casein was the most protective exhibiting >90% protection in both the TCA and receptor assays in stomach flushings at concentrations of 10 mg/ml. In duodenal flushings, casein exhibited 80% (TCA

assay), but only 36% (receptor assay) protection against IGF-I degradation when the maximal casein concentration of 40 mg/ml was employed. The half-life of IGF-I (measured with the receptor assay) in the upper gastrointestinal tract increased from 2-3 min (Table 5) in the absence of casein and to up to 35 min in its presence.

This experiment appears to demonstrate a significant amount of protection by casein, at a concentration to the levels of this protein in milk. However, the authors acknowledge that the observed effects can be explained by the simple argument that there is competition by the additional proteins for degradation by the proteases in the respective segments. The experiment demonstrates that even with a high amount of protection from the protease activity, biological receptor binding activity, which is the best indicator of biological activity, is dramatically reduced.

These results were interpreted with regard to milk residues of IGF-I that "the protective effect of casein makes irrelevant the argument that human saliva contains IGF-I at levels greater than the quantities that would be consumed in milk. As the IGF-I produced by salivary glands is free IGF-I, without protective effect of casein, it is unlikely to survive digestion" (Hansen *et al.*, 1997). This argumentation neglects the following facts:

- Saliva is not the only source of IGF-I in the gastrointestinal tract. The majority is secreted in the gastrointestinal tract and the high concentration in intestinal chyme indicates that IGF-I is secreted in substantial amounts by the mucosa throughout the whole gastrointestinal tract (Olanrewaju *et al.*, 1992, Chaurasia *et al.*, 1994).
- Casein is flexible in its structure and is known to be readily degraded in the stomach and in the small bowel (Xian *et al.*, 1995). Thus, the protective effect will only be present in the upper gastrointestinal tract.
- The half-life of IGF-I in the presence of casein is only 35 min in the intestine (Xian *et al.*, 1995). Therefore, less than 5% of the initial IGF-I dose will survive more than 2 hours during the passage through the upper gastrointestinal tract.
- In the presence of casein ingested with milk the endogenous IGF-I in the gastrointestinal tract will also be protected.

It is therefore concluded that even considering a limited protective effect of casein the amount of bioactive IGF-I ingested with milk from rbST-treated cows will still be negligible.

Due to the protective effect of casein, some IGF-I might escape digestive degradation, being absorbed in intact form. A recent article by Kimura *et al.* (1997) studied the absorption of large oral doses of ^{125}I-rhIGF-I in a fasted adult rat model. Oral administration of 1 mg/kg doses of the labeled growth factor was followed by trichloroacetic acid precipitation of plasma proteins to evaluate the absorption of IGF-I. The baseline bioavailability of the administered IGF-I was determined to be 9.3% of the dose but was increased by co-administration of 4 mg/kg aprotinin (46.9%), and 10 mg/kg casein (67.0%). RIA analysis of the plasma further confirmed the bioavailability of IGF-I in this fasted rat model and the administered radioactivity was found in the form of high-molecular weight complexes. It should be noted, however, that the receptor assay which has the highest accuracy in measuring biological activity was not used.

The relatively large bioavailability of intact IGF-I in this adult rat model is not supported by the results of a lack of any oral bioactivity of IGF-I in adult animals (WHO TRS 832, 1993, WHO FAS 31,1993) as well as by the results of various studies with neonatal animals which have an incomplete mucosal barrier and a reduced intestinal proteolytic activity (Burrin, 1997). Studies in neonatal rats and piglets indicate that, although 30% of an orally administered dose of ^{125}I-IGF-I can be recovered in the intestinal mucosa, there is very limited absorption into peripheral circulation (Phillips *et al.*, 1995, Donovan *et al.*, 1997). In suckling transgenic rats, despite the ingestion of 1000-fold higher concentrations of des(1,3) human IGF-I, no des(1,3) IGF-I was detected in the plasma of the pups (Burrin, 1997). Furthermore, in studies with newborn calves and piglets given large doses of IGF-I in milk replacers no substantial increase of the plasma levels of this growth factor could be measured (Donovan *et al.*, 1997, Hammon and Blum, 1997, Houle *et al.*, 1997). In one study with newborn calves fed milk replacer, a small amount of orally administered ^{125}I-IGF-I could be detected in the blood plasma (Baumrucker *et al.*, 1992). However, the increase of plasma levels was not observed before three days of administration and was only detected in three of six animals. These results indicate that IGF-I even in neonates is absorbed to only a very small extent, and absorption is rather unlikely in adults.

Furthermore, the absorbed amount has to be compared with the normal levels of IGF-I in human serum which show considerable variation depending on age. The lowest values are observed in infants < 2 years, and constantly increase to reach a maximum in late pubertals and afterwards decrease to adult values as indicated in Table 6.

From the values in Table 6 and assuming a blood volume of 5% of the body weight (Ganong, 1971) a serum load of IGF-I of 49500 ng in a 15 kg child, 714000 ng in a 60 kg adult person and 1220000 ng in a 50 kg teenager can be calculated. The total IGF-I production in adults has been estimated as 10000000 ng per day (Guler *et al.*, 1989). These high amounts have to be compared with the IGF-I amount of 9000 ng in 1.5 l of milk, which constitutes only 0.09% of the daily IGF-I

Table 6. IGF-I concentrations reported in human blood plasma according to Schaff-Blass *et al.*, 1984

Age	Males [ng/ml]		Females [ng/ml]	
	Mean	Range	Mean	Range
0-2 years	42	14-98	56	14-238
3-5 years	56	59-210	84	21-322
6-10 years	98	28-308	182	56-364
Prepubertal > 10 years	126	84-182	182	70-280
Early pubertal	210	140-240	224	84-392
Late pubertal	364	224-462	434	224-686
Adult > 23 years	112	42-266	140	56-308

Total daily production of IGF-I in an adult = 10^7 ng/day (Guler *et al.*,1989)

production. Since only one third of the milk levels can be attributed to IGF-I caused by rbST treatment and only a very small amount if at all will be absorbed, the milk-borne IGF-I reaching systemic circulation is negligible and this small amount will be immediately sequestered by unsaturated binding proteins.

It can therefore be concluded that the slight increase of IGF-I in the milk of rbST-treated animals is many orders of magnitude lower than the physiological amounts of IGF-I produced in the gastrointestinal tract as well as in the body and will cause no relevant exposure of the consumer neither locally in the gut nor systematically.

Concerns have been expressed about possible adverse health effects in consumers exposed to increased IGF-I concentrations in milk from rbST-treated cows (Hansen *et al.*, 1997, Epstein, 1996). The most important potential adverse effects of IGF-I arise from the fact that it is a mitogen for a number of cell types and has been associated with the growth of various tumours including colon and breast cancer, osteosarcoma and lung cancer (National Institute of Health, 1995, McCaulay, 1992, Pines *et al.*, 1985). The mitogenic effect is further supposed to cause proliferative reactions locally in the gut. Thus, orally administered IGF-I to rats increased the in vivo cellularity of the intestinal mucosa (Olanrewaju, 1992) or increased the proliferation rate in cultures of human epithelial crypt cells from the duodenum (Challacombe and Wheeler, 1994). Since IGF-I receptors could be detected throughout the epithelium of the intestine and with a high density in the colon (Laburthe *et al.*, 1988) and the incidence of colorectal cancer is increased in acromegalic patients having excessively high levels of free IGF-I in the plasma (Ezzat and Melmed, 1991) concerns have been expressed that increased levels of milk-borne IGF-I may increase the risk of colonic cancer.

Although IGF-I has as a consequence of its normal biological effects the potential hazard to promote the growth of tumors, this hazard can only become a risk if there would be an adequate exposure of the consumers to increased amounts of IGF-I. Since the exposure to IGF-I by ingesting milk from rbST-treated cows is negligible when compared with the endogenous IGF-I production it is extremely unlikely that the IGF-I residues can cause any systemically or local adverse mitogenic reaction.

Expression of lentiviruses and prion proteins

Somatotropins and the immune system

Somatotropin (ST) has immunomodulatory effects. Immunoenhancing activity has been documented in many different species including cattle (Comens-Keller *et al.*, 1995). The primary effect appears to be altered responsiveness of the immune system even though data of a substantial nature of this effect are incomplete (Burton *et al.*, 1994). Information on changes in cytokine concentrations or secretion as well as their binding site populations are needed to define the nature of ST-immunoenhancing effects. The literature is inconsistent regarding the source of ST used, the ST treatment schedule and the age of the animals. Differences between *in vitro* and *in vivo* findings may be explained by ST-stimulated release of mediators in vivo such as IGFs and cytokines which are not present during in vitro studies (Kelley, 1989). A better understanding of ST-mediated immunoenhancement as homeorhetic regulation of the overall health and disease resistance of animals is needed. It has been reported that lymphocytes from rbST-treated cows have greater average maximum

lymphoblastogenic response to rbST as compared to other mitogens in the periparturient period (Comens-Keller et al., 1995). It is postulated that this effect might prove to be beneficial for prevention of mastitis or other infectious diseases that occur during the immunosuppressed periparturient period.

Effect of bST on the expression of retroviruses

Concerns have been expressed that the immunomodulatory effect of bST might effect retrovirus expression in treated animals and cause resurgence of latent retrovirus and lentivirus infections in the ruminant population and cause the occurrence of such viruses in somatic cells in milk. The concerns are largely based on a review by Lerondelle *et al.* (1994) discussing evidence of induction of these viruses in small ruminants by steroid hormones along with discussion of induction of lentiviruses in other species by other hormones including growth hormone and/or IGF-I and on an unpublished study of Lerondelle *et al.* (1996) who investigated the effects of rbST on the expression on Caprine Arthritis Encephalitis Virus (CAEV) in goats. This virus belongs to the group of lentiviruses which like Maedi/Visna can infect small ruminants.

There are at least three reasons why there might be interest in ruminant lentiviruses. First, they might be of concern to persons consuming the milk in that these viruses may cause illness in humans. Second, there may be additional concern that if the use of rbST causes increases in the ruminant viruses presumably through the presence of rbST itself, or the action of IGF-I, the presence of the small additional amounts of growth hormone present in milk of treated cows may also somehow affect the retroviruses which affect the human immune system known as HIV-1 and HIV-2. Finally, the potential to increase the severity or kinetics of expression of the disease in the ruminant itself.

The study by Lerondelle and coworkers (1996) attempts to address the last question as whether rbST increases the expression of Caprine Arthritis Encephalitis Virus (CAEV), a member of a family of retroviruses that infect small ruminants. Measurements of viral expression included assay of reverse transcriptase activity in cells in milk, a clinical examination of the udders and joints of animals at the beginning and end of the study, and evidence of infection by use of an immunodiffusion assay. Twelve pregnant Saanen goats, seronegative for CAEV were experimentally infected by treating them intramammarily with *in vitro* infected monocytes with the Cork strain of CAEV at the time of drying off. Groups of four goats were treated as follows beginning seven weeks after giving birth: one group treated with rbST (sometribove); a second group treated with thyroxine; and the control group was untreated. Doses of 5 mg/day/goat for rbST, and 10/mg/goat/day for thyroxine were administered in suspension in sterile water. The drugs were given for 30 days followed by a 45 day observation period. Milk samples were taken for reverse transcriptase activity on treatment days 7,14, 21 and 28, and three times during the observation period. As indicated previously, udder and joint health as well as immunodiffusion tests were also done at the beginning, and end of the study. In addition, milk production, and milk cell counts were evaluated every two days. The results shown in Table 7 were presented to describe the occurrence and onset of the appearance of CAEV viral effects.

The results show the time in days for the cultured milk cells to exhibit evidence of viral expression in the cells harvested at the designated milk sampling times. The data show that there is greater evidence of positive cultured cells from the control treatment than either of the hormone treatments. Perhaps the most striking effect is the lack of a positive increase in the rate of infectivity, and even the suggestion of a decrease in infected cells as seen in goats 9431 and 9436, as a result of the treatment with rbST in particular after the first milk sampling period.

Among the studies the authors carried out on the milk of the CAEV infused goats, the one shown in Table 8 examines the infectivity challenge and its attempted augmentation by treatment with the hormones, thyroxine and rbST. The test employed the reverse transcriptase assay and measures activity in cells considered positive for virus in culture and expressed as a ratio of transcriptase activity over number of positive cell cultures

The results of the study showed no positive correlation of the effect of rbST or thyroxine on the activity of reverse transcriptase in the milk samples as seen in the summary of results shown as Table 8. In fact, there appears to be no evidence of increased transcriptase activity in any of the groups. This is particularly interesting in the rbST group which appears to have a lower initial rate of infection than the two other groups including the controls, yet neither is there an increased rate of infection as measured by number of positive cultures nor is there an increase in transcriptase activity. The authors interpreted their results as a tendency of an increased virus expression with increased milk production. The results with rbST were, however, biased by the fact that only two animals were included in the final evaluation. The authors concluded that due to the heterogeneity of the effects on milk production and the small number of animals tested the data base for establishing the promoting effect of rbST on virus expression was insufficient to demonstrate a clear effect.

Table 7. Onset of appearance of the cytopathic effect (in days) for each of ten milk samplings from the control, thyroxine, and rbST groups as a function of the period of hormonal treatment.

Goats	Before Treatment			During Treatment				After Treatment		
Milk Samplings	1	2	3	4	5	6	7	8	9	10
Control										
9433	4	8	6	10	6	6	6	8	10	8
9434	4	6	6	6	8	6	6	6	-	10
9435	4	6	4	6	4	4	6	4	4	6
9439	6	8	10	6	10	6	-	-	-	-
M ± SD (n)	6 ± 1.91 (12)			6.4 ± 1.71 (15)				7 ± 2.39 (8)		
Thyroxine										
9430	8	6	10	4	6	6	-	-	6	4
9441	6	4	4	4	4	4	4	4	ND	4
9442	4	4	4	4	4	4	6	4	ND	4
9443	4	8	6	4	4	4	6	4	ND	4
M ± SD (n)	5.67 ± 2.06 (12)			4.53 ± 0.92 (15)				4.25 ± 0.71 (8)		
rbST										
9431	8	-	-	-	-	-	-	-	-	ND
9436	4	8	8	-	8	-	-	-	4	ND
9438	ND	-	-	-	-	-	-	-	-	ND
9440	4	4	4	4	4	4	4	4	4	4
M ± SD (n)	5.71 ± 2.41 (7)			4.8 ± 1.79 (5)				4 (4)		

Table 8. **Number of positive samples by the Reverse Transcriptase-Positive Culture Ratio for the virus in the goats of control, thyroxine, and rbST treated goats.**

Goats		Before treatment	During treatment	After treatment	Total
Control	9433	3/6	4/7	3/4	10/17
	9434	3/6	3/5	1/3	7/14
	9435	6/6	4/5	5/6	15/17
	9439	4/4	2/8	3/6	9/18
	Total	16/22	13/25	12/19	41/66
Thyroxine	9430	0/3	2/4	0/3	2/10
	9441	4/4	5/7	6/6	15/17
	9442	6/6	8/8	6/6	20/20
	9443	6/6	8/8	4/6	18/20
	Total	16/19	23/27	16/21	55/67
rbST	9431	2/4	0/6	0/1	2/11
	9436	6/6	7/8	2/5	15/19
	9438	0/2	0/4	0/3	0/8
	9449	4/4	4/5	5/6	12/14
	Total	12/16	11/23	7/15	29/52

These data provide no evidence that rbST treatment of cows infected with lentiviruses will cause resurgence of virus infections in ruminants or give rise to any risk of human health. Lentiviruses are a type of retrovirus which only replicate in activated immune system cells. They may stay dormant for months and years before they gradually wear down an immune system to the point of collapse. The phylogenetic tree of lentiviruses includes the subfamily of Bovine Immune Deficiency Virus (BIV) also called Bovine Leukemia Virus (BLV) as well as the subfamily of HIV-1 and HIV-2 virus which are causative agents of AIDS in humans. BIV and HIV are not the same viruses and are highly separated phylogenetically (Robertson, 1997). Furthermore, BIV is not known to cause disease in humans although the virus has shown the ability to infect human cells in vitro where host defense mechanisms would not be present (Georgiades *et al.*, 1978, Van Der Maaten and Miller, 1990). Infection of human cells in vivo could not be demonstrated. All attempts to obtain direct evidence of infection in exposed human populations have yielded negative results (Straub, 1981, Van Der Maaten and Miller, 1990). The failure of human infections has also been shown for other ruminant lentiviruses such as CAEV and Maedi/Visna (Straub, 1981). Excretion of virus with milk somatic cells can cause infection of the offspring of infected cows. This transmission can effectively be blocked by procedures similar to pasteurization of the milk which will destroy the virus at 60°C within 30 sec (Abramova *et al.*, 1974, Baumgartner, 1976, Van Der Maaten and Miller, 1990). Therefore pasteurization may also prevent the transmission of BIV to consumers of milk.

It is concluded that BLV cannot induce diseases in humans and is completely inactivated by routine pasteurization. Furthermore, according to a not further qualified statement of the company commercializing sometribove, there is no indication that the incidence of BLV has increased in cattle after 4 years of continuous use of rbST in the U.S. and 8 years in Mexico and Brazil (Collier *et al.*, 1998).

An increase of the expression of HIV-viruses in humans by ingestion of milk from rbST-treated cows is extremely unlikely due to the negligibly small residues of rbST and IGF-I. It has further been shown that treatment of AIDS patients for 6 weeks with recombinant human growth hormone and IGF-I had no influence on HIV levels associated with peripheral blood mononuclear cells, CD3, CD4, or CD8 counts in peripheral blood as well as serum HIV p24 antigen levels (Waters *et al.*, 1996).

Effect of rbST on Prion Proteins

Concerns have been expressed that rbST treatment could increase the risk of bovine spongiform encephalopathy (BSE) in dairy cows (Hansen *et al.*, 1997). Little evidence to support this concern has been provided, and that provided is indirect.

The present theory is that the infectious agent of BSE is a prion protein (PrPc) (Prusiner, 1982). PrPc's are normally found in all animals and are encoded by a prion-protein gene. BSE is associated with a post-translationally modified protease-resistant protein (PrPsc) which differs in its three-dimensional structure to the normal protease-sensitive PrPc. Normal PrPc's are found membrane-bound on the surface of all nerve cells, some lymphocytes and other tissues (Prusiner, 1991). To date no function has been ascribed to normal PrPc. The most widely accepted theory of BSE is the conversion of normal PrPc's to the abnormal PrPsc's form which in turn causes more normal PrPc's to convert to PrPsc's The mechanisms of the conversion to the disease-causing PrPsc are not clearly understood. In contrast to the normal form, PrPsc cannot be turned over in cells, and builds up in the cell forming large oligomers observed as plaques (amyloids) in the brain of affected individuals (Gajdusek, 1993).

It has been demonstrated that IGF-I increases the production of PrP-mRNA in vitro in a rat phaeochromocytoma cell line (PC12 cells) with a rather flat dose response curve with a 40% increase at 10 ng/ml, and doubling at 100 ng/ml (Lasmézas *et al.*, 1993). In transgenic mice harbouring multiple copies of the PrP gene the speed of progession of Scrapie was increased (Prusiner, 1991). It has not been shown that IGF-I increases the formation of the PrPsc form of the protein, and thereby shortening the incubation period for BSE.

It is speculated that the increased IGF-I levels in rbST-treated cows would lead to an increased PrPc production and possibly speed up the progression of BSE. However, there are no data that directly address whether rbST or IGF-I increases the formation of normal PrPc or its pathogenic protease-resistant mutant in the brain of the cattle. Therefore, the possibility of a link between rbST treatment and BSE is highly speculative.

Cows milk and insulin-dependent Type I diabetes mellitus in childhood

In epidemiological studies performed in various geographical regions it could be demonstrated that among other environmental factors such as chemicals or virus infections the short duration of breast-feeding and the early dietary exposure of newborns to cow's milk containing formulas will increase the risk of insulin-dependent Type I diabetes mellitus (IDDM) by about 1.5 times (Scott, 1990, Dahlquist *et al.*, 1991, Jorgensen *et al.*,1991, Virtanen *et al.*, 1993 and 1994, Gerstein, 1994, Verge *et al.* 1994). IDDM develops as a consequence of autoimmune destruction of the insulin-producing ß-cells of the pancreatic islets. The precise trigger of the autoimmune reaction is unknown (Gerstein, 1994). It is hypothesized to be a genetically acquired immune defect in susceptible individuals (Gerstein, 1994). Epidemiological evidence exists that IDDM is geographically and temporally related to neonatal feeding practice with cow's milk and that avoidance of cow's milk in the first few months of life can protect genetically predisposed individuals (Gerstein, 1994, Verge *et al.*, 1994).

Serological evidence supports the view that this immune defect may be triggered by exposure to proteins of cow's milk (Gerstein, 1994). It is postulated that in neonates, milk proteins may cross the immature gut wall initiating an immune response that crossreacts with a ß-cell surface antigen (Verge *et al.*, 1994). It could be shown that older children (5-9 years) with intact intestinal barrier are not at risk to acquire IDDM by exposure to cow's milk (Dahlquist *et al.*, 1991). The possible triggering factors in cow's milk have not been precisely identified. Casein seems to be unlikely since in diabetes-susceptible rats replacement of milk proteins by casein completely prevented diabetes (Jorgensen *et al.*, 1991). It is supposed that increased levels of IgA antibodies to cow's milk and beta-lactoglobulin are associated with increased risk if IDDM (Dahlquist *et al.*, 1991, Virtanen *et al.*, 1994). It is unlikely that exposure of human neonates to milk of rbST-treated cows increases the risk of IDDM for the following reasons:

- the composition of milk from rbST-treated cows is well within the normal variations observed during the course of lactation,
- the slightly increased IGF-I levels in cow's milk can be excluded as a triggering factor because of the identical nature of bovine and human IGF-I and that levels of IGF-I in breast milk are equal and initially higher than those found in cow's milk.

APPRAISAL

General

Information was submitted by organizations and individuals relating to the following concerns:

- the increased use of antibiotics with a higher rate of violative drug residues in milk due to a possible increased incidence of mastitis in rbST-treated cows,
- the possibility that increased levels of IGF-I in milk of rbST-treated cows might lead to increased cell division and growth of tumors in humans,
- the potential effect of rbST on the expression of certain viruses in cattle, particularly the retroviruses,
- the possibility that the incubation period of bovine spongiform encephalopathy (BSE) is shortened due to an IGF-I-induced increase of the production of pathogenic prion proteins, and
- the possibility that early exposure of human neonates to milk from rbST-treated cows increases the risk for developing insulin-dependent diabetes mellitus.

Use of antibiotics

After reviewing the data the Committee considered the risk of mastitis induced by rbST as an issue of animal health which is not within the terms of reference of the Committee. However, the possible increased use of antibiotics was considered.

A post approval monitoring program (PAMP) was established in the United States to address the following areas:

- the incidence of mastitis and responses related to herd health (not within the terms of reference of the Committee),
- the treatment with any medications in a 28-herd study with rbST-treated cows (not within the terms of reference of the Committee),
- the incidence of milk discard due to results from antibiotic residue testing in key dairy states representing at least 50 % of the U.S. milk production.

In New York State the percentage of milk discard resulting from antibiotic residue testing was not significantly changed after introduction of rbST. In other states a small, but statistically significant, increase was observed in 1995 which

coincided with a change to a more sensitive testing method. The Committee concluded that the use of rbST will not result in a higher risk to human health due to the use of antibiotics to treat mastitis and that the increased potential for drug residues in milk could be managed by practices currently in use by the dairy industry and by following label directions for use.

IGF-I levels in milk and tissues

IGF-I is a normal component of milk and is found in abundance in variety of body fluids (see Table 9).

Table 9. IGF-I in milk and body fluids

Fluid		[ng/ml]	Fluid		[ng/ml]
Milk	human	5 - 10	Gastrointestinal secretions (human)	Saliva	6.8
	human colostrum	8-28		Gastric juice	26
	bovine – untreated*	1 - 9		Pancreatic juice	27
	bovine - rbST-treated*	1 - 13		Bile	6.8
Plasma	child	17 - 250		Jejunal chyme	180
	adolescent	182 - 780	**Daily production by adult humans = 10^7 ng/day**		
	adult	123 - 460			

*bulk milk

The presence and concentrations of IGF-I were at the center of much of the scientific discussion in the original scientific review of bST undertaken by the 40th meeting of the Committee and in submissions to the present JECFA meeting. Information that was previously reviewed is summarized in FAO Food and Nutrition Paper No. 41/5 (1993). IGF-I concentrations in milk are variable and have been shown to depend on state of lactation, nutritional state, and age.

Methods for assaying IGF-I were considered by the 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 any conclusions. Relatively high values previously reported in milk were considered to reflect inadequate extraction procedures.

Since the previous evaluation, very little additional data on residues have appeared in the literature and in reports provided by interested parties. However, the manufacturer of sometribove submitted additional information on levels of IGF-I in retail milk after the approval of rbST in the United States. The results showed no difference in the IGF-I concentrations between labeled (certified to be derived from cows not treated with rbST) and unlabeled milk. However, the percentage of milk derived from cows receiving rbST was not specified for the unlabeled milk.

Concerns have been expressed that any rbST-induced increase of IGF-I in milk contribute to the endogenous levels of IGF-I in the gastrointestinal tract and in serum if not biodegraded, and if absorbed. A recent study in rats confirms that IGF-I is rapidly degraded in the gastrointestinal tract. However, in these studies a protective effect of casein on IGF-I could be demonstrated. It is postulated that the retarded degradation leads to increased serum levels of IGF-I (as has been shown in one study in rats) as well as to prolonged exposure of the gut as well as to increased serum levels of IGF-I. The Committee also noted that 7 days of oral administration of high doses of IGF-I in milk replacer did not increase circulating concentrations of IGF-I in newborn calves and piglets indicating that significant absorption of IGF-I is unlikely to occur under physiological circumstances in these food animals.

Considering the decreased rate of degradation observed in the small intestine in rats in the presence of casein, levels of the growth factor would likely deplete to less than 5% of their initial values within two hours indicating that milk-borne IGF-I would not be expected to contribute to levels of IGF-I in the large intestine.

Assuming the ingestion of 1.5 liters of milk per day, the average ingested amount of IGF-I will be 6000 ng for milk from untreated animals containing an assumed IGF-I concentration of 4 ng/ml and 9000 ng for milk of rbST-treated animals with an approximate average concentration of 6 ng/ml. It has been calculated that IGF-I in gastrointestinal secretions amounts to

about 380000 ng/day. Therefore, the additional amount of IGF-I in 1.5 liters of milk from rbST-treated cows as compared with milk from untreated cows is only about 0.8 % of the gastrointestinal secretion.

The total amount of IGF-I in serum has been calculated to range from approximately 50000 to 1220000 ng depending on age. The total daily IGF-I production in adult humans has been estimated as 10^7 ng. Therefore, the daily value of IGF-I ingested with milk from rbST-treated cows compared with the daily production will be less than 0.09% for adults. Even if the total amount of milk-borne IGF-I were absorbed the additional amount would be negligible.

The Committee concluded that any increase of IGF-I in milk from rbST-treated cows is orders of magnitude lower than the physiological amounts produced in the gastrointestinal tract as well as in other parts of the body. Thus, the Committee concluded that there will be no increased exposure of the consumers either locally in the gut or systemically. Consequently, the potential for IGF-I to promote tumor growth will not increase when milk from rbST treated cows is consumed, resulting in no appreciable risk for consumers.

Recent studies have been performed in which sustained release rbST was administered for 20 weeks. Tissue levels of of rbST and IGF-I were measured two weeks after the final administration of rbST. No significant increases in the rbST and IGF-I levels were observed.

Expression of retrovirus

Concerns that rbST treatment of cattle would increase the expression of retroviruses including Bovine Leukemia Virus (BLV), were addressed by experiments in a goat model that used caprine arthritis encephalitis virus. Infectivity was not increased when measured by numbers of infected cells, and there was no evidence of increased reverse transcriptase activity. These studies provided no evidence that rbST affects the expression of BLV, a lentivirus in cattle. Furthermore, it has been shown that BLV will be destroyed by simulated pasteurization conditions by heating milk to 60°C for 30 sec. In addition, there is no evidence of human susceptibility or responses to ruminant retroviruses.

Expression of prion proteins

Concerns have been expressed that rbST treatment could shorten the incubation period for bovine spongiform encephalopathy (BSE). This hypothesis is based on *in vitro* results in a neuronal cell line indicating an increased formation of mRNA of prion proteins (PrP) in response to IGF-I. Furthermore, in transgenic mice harbouring multiple copies of PrP gene, an increased formation of PrP shortened the incubation period of Scrapie. However, no data were available that directly address whether rbST or IGF-I increases the formation of normal PrP or its pathogenic protease-resistant mutant in the brain of cattle. The Committee considered that the possibility of a link between rbST-treatment and BSE to be highly speculative.

Risk of insulin-dependent diabetes mellitus (IDDM)

It has been shown, that exposure of neonates to cow's milk increases the risk of IDDM by about 1.5-fold. The Committee considered whether exposure of human neonates to milk from rbST-treated cows further increases this risk, and concluded that, because of its unchanged composition, the milk of rbST-treated cows is not expected to represent an additional risk to the development of IDDM.

On the basis of the following:

- insignificant changes in quantities of milk discarded due to results from antibiotic residue testing after introduction of rbST into commercial use;
- low levels residues of rbST and IGF-I in milk;
- the degradation of IGF-I in the gut and its abundance in gut secretions;
- the extremely low levels of ingested IGF-I when compared to endogenous production;
- the lack of evidence that rbST stimulates expression of retroviruses;
- lack of information directly linking rbST-treatment and BSE; and
- the absence of significant changes in composition of milk from rbST-treated cows which may contribute to the additional risk of development of IDDM

the Committee concluded that rbST can be used without any appreciable health risk to consumers. The Committee reaffirmed its previous ADI and MRLs "not specified" for somagrebove, sometribove, somavubove, and somidobove.

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