Report on Public Health Aspects of the Use of Bovine Somatotrophin - 15-16 March 1999

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INTRODUCTION

Mandate

The Scientific Committee on Veterinary Measures relating to Public Health is asked examine the use of bovine somatotrophin (BST) to dairy cows as a productivity aid to milk production. In particular the Committee is invited to assess the possible direct and indirect adverse effects on public health caused by the use of BST under normal conditions.

In a parallel exercise, the Scientific Committee on Animal Health and Animal welfare is asked to report on the incidence of mastitis and other disorders in dairy cows and on other aspects of the welfare of dairy cows.

Background

Council Decision 94/936/EC of 20 December 1994 amending Decision 90/218/EEC concerning the placing on the market and administration of bovine somatotrophin (BST) prohibited the marketing and the use of BST in the EU until 31 December 1999.

The Council asked the Commission to entrust a Working Party of independent scientists with the task of assessing the effects of using BST, in particular as regards the impact of the use of this product on the incidence of mastitis. In this request it is stated that "BST is an issue which gives rise to considerable interest among consumer, agricultural and industry interests. In this context, concerns have been expressed about the safety to humans, animals and the environment, the quality of milk, the economic and social consequences in agriculture, the climate for research and development, industrial competitiveness and trade implications".

Comment

The present report is limited to the public health aspects. The abbreviation BST is generally used to indicate recombinant bovine somatotrophin (rBST).

EFFECTS OF rBST ON PUBLIC HEALTH

Products containing or consisting of rBST are veterinary medicinal products within the meaning of Directive 81/851/EEC on the approximation of the laws of the Member States relating to veterinary medicinal products. In the case of veterinary products derived from biotechnology, Community concertation procedures established by Directive 87/22/EEC have to be taken into account as well, implying that the advice of the Committee for Veterinary Medicinal Products (CVMP) must be obtained before any decision on the authorisation of individual products can be accepted. Recombinantly derived BST products (rBSTs) may have slightly different chemical structures from natural BST produced by the pituitary gland, by adding a number of amino acids. Thus, each product must be considered on its own merits by the CVMP and it should be emphasised that it is not the aim of this report to provide an expert opinion on certain veterinary medicinal products.

In drawing up this report, the working group has made use of previously compiled reports by regulatory and advisory authorities in which aspects of the safety, quality and efficacy of rBST products has been examined. In particular, reference is made to:

1. 1st report concerning Bovine Somatotrophin (BST) COM89, 379 final

2. 2nd report from the Commission tot the Council and to the Parliament concerning Bovine Somatotrophin (BST) SEC(91) 2521 final (16.01.1992)

3. CVMP-European Commission, DOCs. No. III /3006-7/93, 23 January, 1993

4. FAO FNP 41/5: Food and Nutrition paper: Residues of some veterinary drugs in animals and foods. Bovine Somatotropins (1993)

5. Communication from the Commission to the Council concerning Bovine Somatotrophin (BST) update SEC(94), 1713 (25.10.1994)

as well as the recent

6. Report of the JOINT FAO/WHO Expert Committee on Food Additives, presented at the 50th meeting in Rome, 17/26.02.1998, (WHO: Food Additive Series 41, pp. 125-146, 1998)

7. Health Canada Report on BST (1999)

8. Ongoing discussions in Codex Alimentarius

In addition, recent scientific literature, in particularly those which became available after 1994 have been considered, as indicated in this report (references section A). Finally a number of reports and opinion statements have been considered as summarised in section B of the references.

1. The rationale of risk assessment and risk management in the context of public health

Risk assessment and risk management are not only scientific and technical activities, but also represent a task attributed to science from the society. In principle, risk assessment should represent a formally defined and socially accepted evaluation process, which is separate and independent from the decisions concerning risk reduction or risk elimination. This separation and independence was considered appropriate for preventing possible biases in the risk assessment process, which could be caused by influencing the desired neutrality of the evaluation. Based on this principle, risk assessment should be a matter of scientific evaluation, whilst risk management should be a matter of political and social decision making. Thus, additionally to the scientific procedure of risk assessment the following issues may be considered in risk management:

The perception of involuntary risk factors (consumer's expectations and concerns).

The uneven distribution of risk and benefits (e.g. health and/or economic advantage).

Risk assessment should cover the following items:

1. Hazard identification

2. Hazard characterisation: dose (concentration) - response (effect) assessment

3. Exposure assessment

4. Risk characterisation

As far as risk assessment is concerned, the following definitions are applied:

Hazard identification

Identification of the adverse health effects related to the intrinsic properties of a substance.

Hazard characterisation

Qualitative and/or quantitative evaluation of the nature of the adverse health effects. This implies a dose (concentration) - response (effects) assessment and an estimation of the relationship between dose (or level of exposure) to a substance and the incidence of a biological effect (response).

Exposure assessment

Qualitative and/or quantitative estimation of the concentrations/doses to which human populations (here: consumers) are exposed. Exposure assessment requires information about the effects of production, processing, handling, and consumption of respective food commodities.

Risk characterisation

Estimation of the incidence and severity of the adverse effects likely to occur to a human population. Thus, the risk characterisation should " *include a qualitative and/or quantitative estimation, including attendant uncertainties of the probability of occurrence and severity of known or potential adverse health effects*" as specified in a document on "Risk Assessment; Towards internationally acceptable standards for food additives and contaminants bases on the use of risk analysis" (Hugett et al., (1998).

In conclusion, risk assessment can be regarded as scientific essentiality directed to provide suitable answers to two questions:

1. What is the probability or likelihood of an undesired event to occur, and

2. What are the consequences of this undesired event in qualitative and quantitative terms.

Thus, by definition, basic risk assessment excludes in its initial phase concerns of the decision making bodies and neglects the comparison and balance of risk and benefits and societal requests related to ethical, economic, technical and political aspects (EU, 1996, Technical Guidance Document in Support of the Council Directive 93/67/EEC on Risk Assessment of New Notified Substances and Council Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances).

2. Public health aspect in terms of safety of milk and milk products derived from rBST treated lactating cows

2.1. Hazard identification

2.1.1. BST and its metabolites

Growth hormone (GH, somatotrophin ST) belongs to the protein family of somatolactogenic hormones. In the 1980s advances in recombinant DNA techniques made sufficient quantities of recombinant bovine growth hormone (rBST) available for the use as milk production enhancing agent. No therapeutic applications of rBST have emerged in veterinary medicine (Burton et al., 1994).

The application of rBST to dairy cows involves a parenteral application due to the instability of BST in the gastrointestinal tract. Following application and based on the peptide nature of rBST, rapid degradation by cytosolic proteases and lysosomal enzymes which are virtually present in all cells takes place. Residual amounts of rBST may be expected at the site of injection and in muscle and connective tissues especially following improper administration of rBST formulations. The major identified metabolite of rBST in plasma was the same as the physiological thrombin cleavage product of BST. This was demonstrated by sequence analyses in which two fragments were found in a close to equimolar ratio. One sequence was homologous to the N-terminus of the BST protein, whilst the other sequence represented a fragment produced by cleavage at the same site as the thrombin cleavage site of the BST molecule (Bang et al., 1994b, Bang, 1995, Bang and Fielder, 1997). No formal risk assessment has been applied to these cleavage products.

Hence there is no evidence that intact BST or one of the above mentioned cleavage products exert any direct biological effect after oral ingestion in humans and in consideration of the heat-lability of rBST during pasteurisation, non-specified ADI- and MRL values have been considered for rBST (FAO/WHO Expert Committee on Food Additives, Rome, 1998).

2.1.2. IGFs

Elevated levels of pituitary growth hormone are associated with increased liver secretion of IGF-I and its binding proteins and chronic inhibitory control of GH secretion is mediated by IGF-I feeding back to all upper levels of the GH regulatory pathway.

Thus, in particular long-term metabolic effects of GH or its analogues (rBST) are considered to reflect the regulation of expression of certain genes. GH regulated genes in the liver include the gene encoding for IGF-I and recent work indicated that other tissues including adipocytes and chondrocytes increase IGF-I mRNA expression in response to GH. IGF-I has a high affinity for a family of IGF-binding proteins, which modulate its biological actions. Regulation by GH of these genes encoding for binding proteins is considered as another relationship between GH and IGF-I. In addition, several other genes have found to be regulated by GH including the *spi*2.1. gene, encoding a liver specific serine protease inhibitor and the genes encoding cytochrome P450 enzymes (particularly the CYP2C family, see also section 3) responsible for the biotransformation of numerous pharmaceuticals and other xenobiotics (for review see Carter-Su et al., 1996).

As the increase of circulating IGF-I under the control of GH is considered as one of the physiological mechanisms of GH, the application of rBST is expected to induce the same mechanism. Indeed following the zootechnical application of BST an increase in circulating IGF-I concentrations has been found in lactating dairy cows (for details see section 2.3.). Hence IGF's are single chain polypeptides, they are excreted into milk. This has been confirmed in different animal species including humans. The amino acid sequence of IGFs is highly conserved in mammals, and bovine and porcine IGF-I are identical to human IGF-I (Honegger and Humbel, 1986, Francis et al., 1989a,b), while IGF-II sequences exhibit a greater variation among different animal species.

IGFs possess endocrine, paracrine and autocrine activities. IGF-I acts as a progression factor in the cell cycle and has mitogenic and anti-apoptotic properties. IGFs are involved in numerous physiological cell differentiation processes embodying for example cellular differentiation in perinatal development as well as processes such as maturation of ovary cells and regular apoptosis, and cell proliferation. The numerous medical reports (more than 1000 per year in the last two years) focus on both aspects, the possibility of the use of IGF-I in the treatment of distinct diseases, among others insulin independent diabetes and renal failure, whilst others describe the detrimental role of IGF-I as cellular growth regulator and tumour promoter. The plethora of biological effects exerted by IGF-I in vitro needs to be translated to the complexity of mechanisms in the intact organism before a final evaluation of dose-dependent effects can be made.

2.1.3. Additional hazards

In identifying the potential hazards, secondary risks related to the use of rBST in dairy cows need to be considered as well. These arise from possible changes in milk composition of treated animals and impairment of animal health, in particular the increased incidence of mastitis resulting in a more frequent use of antimicrobial substances (as discussed in more detail in the report on the animal welfare aspects).

2.2. Hazard characterisation: Qualitative and quantitative evaluation of the nature of intrinsic biological properties of IGFs

As it has been mentioned above, during the last five years, an explosion of new information has confirmed and extended the understanding of the pleiotropic effects of the IGF system on growth, development, and intermediary metabolism (Stewart and Rotwein, 1996). The insulin-like growth factors (IGFs) comprise a conserved pair of secreted proteins, **IGF-I** (previously termed somatomedin C) and **IGF-II** (termed somatomedin A). IGF-I is a single-chain basic protein of 70 amino acids, and IGF-II is a slightly acidic single-chain peptide of 67 residues (Rinderknecht and Humbel, 1978a,b). By molecular cloning it could be demonstrated that both IGFs are highly conserved proteins found in an array

of vertebrate species (for recent reviews, see Rotwein, 1991, Dugay et al., 1995).

Circulating IGFs are bound to carrier proteins, denoted **IGF bindings proteins** (IGFBPs). It soon became evident that IGFBPs comprises a family of at last six members, and a diversity of functions has been attributed to these proteins, which prolong the half-life of circulating IGFs, facilitate the transport of IGFs from the circulation to the peripheral tissues, and thus potentiate or inhibit IGF action (Bach et al., 1994; Jones and Clemmons, 1995; Chan and Spencer, 1997; Hossner et al., 1997; Lee and Giudice, 1997)

The cellular effects of IGFs are mediated by two distinct receptors. The **IGF-I receptor (IGF-IR)** is a hetero-tetrameric glycoprotein which may be produced by mRNAs derived from a single 21-exon IGF-IR gene, located on chromosome 15q25-q26 although several receptor variants have been described (Abbott et al., 1992). The IGF-IR is similar in topography and sequence to the insulin receptor and shares >50% amino acid identity (Ullrich et al., 1986). The receptor is composed of two ligand binding a -subunits and two transmembrane b -subunits. Ligand binding to the a -subunit triggers activation of the intracellular tyrosine kinase, leading to receptor autophosphorylation by an intra-molecular trans-mechanism similar to that used by other receptor-tyrosine kinases (Leroith et al., 1995).

Functional analysis of IGF-IR revealed a complex signal transduction pathway as activation of the IGF-IR by ligand binding causes not only rapid tyrosine phosphorylation but also the intracytoplasmatic assembly of a complex consisting of a variety of proteins (SH2-containing proteins including Grb2, GAP, SH-PTP2, p85, Nck and Sc), which link this receptor to the stimulation of the protooncogene p21 *ras* and the mitogen-activated protein (MAP) kinase pathway and thus overall regulation of gene expression (Davis, 1994). Activation of phosphatidylinositol-3-kinase via IGF-I signalling pathways leads to the induction of several biological effects, including stimulation of hormone-sensitive glucose transport (Cheatham and Kahn, 1995) and activation of the enzyme p70S6 kinase, which may be involved in mitogenesis (Cheatham et al., 1995; Baserga, 1995).

Over-expression of human IGF-IRs in mouse and rat fibroblasts has been found to induce neoplastic transformation and development of tumours when transfected cells were introduced into immunodeficient nude mice (Kaleko et al., 1990). These findings indicate the potential role for IGF-IR in tumour genesis (see below).

Finally IGF-IR is involved in the signalling pathway of other growth factors including epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) (Coppola et al., 1994; Deangelis et al., 1995) and at least two dominant oncogenes (large T antigen of *simian virus 40* and the *ras* and SRC oncogenes and tumour suppress genes (Sell et a., 1993, Valentis et al., 1994, Sell et al., 1994; Werner and Leroith 1995, Neuberg et al., 1997).

The IGF-II receptor (IGF-IIR) is a single-chain membrane-spanning glycoprotein that also is known as cation-independent mannose-6-phosphate receptor. The IGF-IIR is highly conserved among different species, with ~80% identity being found among bovine, rat, mouse, and human receptors (Kornfeld, 1992). The IGF-IIR is uniquely involved in the clearance of lysosomal enzymes from the extra-cellular environment. For example, the receptor plays a role in the uptake of thyroglobulin after its secretion by thyroid follicular cells and its subsequent degradation in lysosomes (Herzog et al., 1987). It has been shown that IGF-IIR binds the latent form of transforming growth factor- b 1 (TGF- b 1) and that this binding seems to be essential for growth factor activation pointing to the role of IGF-II in fetal development (Korner et al.., 1995, Lau et al., 1994).

However, genetic studies have not depicted a signalling function for the IGF-IIR and thus the role for the receptor in mediating IGF-II actions remains to be substantiated (Flaumenhaft et al, 1993, Korner et al., 1995).

The physiological actions of IGF-I and IGF-II relate to growth and development of the embryo and fetus and to cellular differentiation, proliferation and cancer.

Over-expression of bovine, murine or rat GH causes increased growth in transgenic mice accompanied by two- to threefold elevations in serum IGF-I concentrations (Mathews et al., 1988a). Transgenic mice expressing human IGF-I in the liver and other tissues also showed enhanced growth (Mathews et al., 1988b), while mice over-expressing IGF-II did not (Wolf et al., 1994). Over-expression and subsequent increase of serum IGF-I levels manifest as selective organomegaly rather than increase in skeletal size. This indicates that the effects of GH or IGF-I on rate of growth on individual organs and in the entire animal are not identical. IGF-I stimulates a greater increase in kidney, spleen and

thymus weight than GH (Skottner et al., 1989). These qualitatively different responses to GH and IGF-I might be related to the fact that GH induces IGF-I synthesis in multiple tissues and also enhances the expression of the major serum carrier protein IGFBP-3 and its cofactor ALS (acid labile subunit) in liver. The consequence of the induction of the expression of this ternary complex is a more sustained exposure of all tissues to IGF-I. IGF-I can stimulate the expression of IGFBP-3 but has no effect on ALS synthesis.

Depending on the study, mice with a disrupted IGF-I gene were significantly smaller in weight and length than wild-type litter mates (Powell-Braxton et al., 1993). Although this confirms that IGF-I and IGF-IR are necessary for normal embryonic and fetal growth, IGF-II seems to be essential. Despite numerous reports on IGF-II gene expression and its regulation by parenteral imprinting in rodents, comparable information from humans is scarce. However, a concordant loss of imprinting of the human IGF-IIR gene promoters has been found in certain cancers (Zhan et al., 1995).

In conclusion, the results described above, in conjunction with other known growth factor signalling pathways and oncogene-mediated cell transformation, provide the evidence for the role of IGFs in tumorigenesis (Yang et al., 1993; Sell et al., 1995, Minniti et al., 1995). However, when critically examining this information it has to be concluded that IGF action is involved in multiple biological processes thus rejecting the possibility to define a dose-effect relationship which describes all individual events.

2.3. Exposure assessment: Occurrence and detection of BST, rBST and IGF-I

Exposure assessment of food contaminants comprises direct measurements indicating the presence and quantity of the compound under investigation in certain food commodities and molecular epidemiology providing evidence of past exposure based on the analysis of typical biomarkers (for example DNA- or protein adducts), or selected somatic cell mutations, if appropriate.

Exposure assessment as applied to chemically defined feed supplements or veterinary medicinal products, e.g. compounds which are used on purpose (intentionally) in food production processes comprises the evaluation of the fate of the compound in the target animal species (distribution and disposition of the parent compound and its biological active metabolites) with the aim to describe the time dependent (target animal) body clearance and thus the quantity and likelihood of the occurrence of residual amounts of the parent compound or its biologically active metabolites in edible tissues, milk and eggs.

rBST closely resembles the physiologically expressed, endogenous bovine growth hormone and is designed to exert the same effects as this natural hormone in dairy cows. Thus, provided that rBST is used in animal husbandry, two general questions need to be addressed:

1. What is the state of art in analytical methodology for the discrimination between endogenous growth hormone profiles and zootechnically applied rBST?

2. What is current knowledge on the occurrence of residual amounts of rBST remaining at the injections site and to what extend secondary, biologically active metabolites such as IGFs are detectable in edible tissues and milk as a consequence of rBST treatment.

2.3.1. Analytical methodology: State of the art in the discrimination between non-treated and rBST-treated cows

2.3.1.1. GH and BST

Formerly, the analytical methods used to determine GH (bovine growth hormone; bovine somatotrophin (bST)) concentrations in plasma, milk and tissue of cows were exclusively radio-immunoassay procedures. None of them were able to distinguish between the endogenous bST and the recombinant growth hormone (rBST) products.

However, this assay was applied to compare bST and IGF-I levels in tissues of control animals and rBST treated animals (Choi et al., 1997). Although a tendency towards a dose-related increase of tissue (muscle) was observed, the differences between control animals and rBST treated animals were statistically not significant.

Since 1990 a number of interesting developments have been launched. Electro-spray mass spectrometry has been used to determine the differences in molecular mass between the natural bST and one of the recombinant products (Somagrebove®). Purified preparations of bovine pituitary bST and rBST were used (Scippo et al., 1997) and the accuracy of the technique was proven to be about 0.05 % of the mass of the protein. This corresponds to 11 Dalton for a protein of about 22000 Dalton, which is more than enough to detect a difference of one amino acid, as the average molecular mass of an amino acid is 115 Dalton. For rBST (Somagrebove®) a molecular weight of 22103 Dalton was measured, whereas the theoretical molecular mass is 22094 Dalton. For the natural bST the mass spectrum is much more complicated because theoretically four variants exist. These have either 190 or 191 amino acids (phenylalanine or alanine-phenylalanine at the N-terminal) with a heterogenecity at position 127 (valine or leucine). The two most dominant variants (190 and 191 amino acids with leucine at position 127) give peaks corresponding to molecular masses of 21725 Dalton and 21796 Dalton respectively, whereas the theoretical values are 21720 Dalton and 21791 Dalton. For the detection of rBST treated cows, the authors suggest to apply this technique on milk and plasma samples after purification and concentration by immuno-affinity chromatography. The minimum amount needed to be obtained by this concentration steps is approximately 5 to 10 pmoles, which corresponds to 0.1 to 0.2 µg. Considering that the minimal concentration of bST in plasma is in the range of 1 ng/mL in non-treated cows, this means that approximately a 100 mL plasma sample will be required for analytical procedures as described.

Several attempts have been made to measure bST concentrations in milk or in plasma by non-radioisotopic immunoassays. A biotin-avidin sandwich enzyme-linked immunosorbent assay for the determination of bovine growth hormone in plasma has been developed by Secci et al. (1988). Affinity-purified antibodies are immobilised on microtiter plates. Bovine GH bound to the specific antibody is then detected with a second anti-bovine GH antibody labelled with biotin and peroxidase-conjugated avidin. This method has a sensitivity as low as 0.25 ng/mL plasma. No applications for the detection of rBST administration are reported as of yet.

An avidin/biotin ELISA assay for bovine somatotrophin is described by Zwickl et al. (1990). The method uses affinity-purified polyclonal antisera raised in rabbits to immobilize bST from blood or milk samples on the wells of microtiter plates. Bound bST is quantitated by adding biotinylated anti-bST antibody during the sample incubation step, followed by incubations with horseradish peroxidase labelled avidin D and ABTS substrate. Because high-affinity anti-bST antibody is used, and the biotinylated antibody is added directly to the sample, the assay can be performed in less than 4 h while sensitivities of 0.2 and 20 ng/mL in milk and blood, respectively, are obtained.

Another competitive enzyme immunoassay for bST was described by Hennies and Holtz (1993). Antiserum, raised in rabbits, is preincubated with samples and free antibodies from the reaction mixture are immobilised using a microtiter plate coated with bST. Bound antibodies remaining from the pre-incubation are visualised using a biotinylated second antibody as a bridge for subsequent amplification by an avidin-biotin-peroxidase complex. The measuring range covers concentrations between 0.5 and 100 ng/mL. A similar competitive enzyme immunoassay (EIA) for growth hormone in bovine pituitary cell culture medium has been developed by Roth et al. (1997).

Ehrard et al. (1994) developed a sandwich ELISA which is able to detect various rBSTs with different N-terminal amino acids and thus allowed the discrimination between rBST and pituitary bovine GH by an affinity factor of 2.0. The authors believe that it might be possible to identify rBST treated cows, but a field study is needed for confirmation. No results of such a field study have been reported as of yet. The other methods for the control of treated and untreated animals are all indirect methods. Various possibilities are under development.

The injection of rBST into animals gives rise to the production of antibodies against these compounds. Their presence in plasma is an indirect proof of the treatment, even after discontinuation of the treatment. A first assay measuring these specific antibodies has been elaborated (Scippo et al., 1997). ELISA plates are coated with rBST and incubated with the cows serum. In the case of antibodies present in the serum they are detected by the addition of a second antibody against bovine IgG, coupled to a peroxidase label.

2.3.1.2. IGF-I

The second type of indirect methods are related to the fact that GH and rBST application increase IGF-I levels in milk. A radio-immunoassay for IGF-I in bovine milk was developed by Zhao et al. (1991). The technique was used for the

analysis of milk samples obtained from three control cows and three rBST-treated cows (41-44 weeks post partum). Mean concentrations of IGF-I were 2.77 ± 1.36 ng/mL in control cows and 3.30±1.40 ng/mL in treated cows, respectively.

The results of IGF-I quantitative assays are controversial: the physiological reference values vary from 1 to 30 ng/mL. This variation is not only based on the reference populations of cows (inter-individual variation) but also reflects the sensitivity of the antibodies applied for the radioimmuno assays (Malven et al., 1987, Bang et al., 1994b).

In conclusion, the analytical procedures described, were designed to discriminate between treated and non-treated animals. No formal intercomparisons of the different analytical procedures are available allowing a conclusive comparison of the reported IGF-I levels in milk and dairy products.

An enzyme immuno-receptor assay for the quantitation of IGF-I and insulin receptors in bovine muscle tissue was developed by Boge et al. (1994). After solubilization with Triton X-100 receptors were immobilised in microtiter plates using receptor specific monoclonal antibodies that recognise the intracellular beta-domain of the respective receptors. The immobilised receptors were labelled with either biotinylated IGF-I or insulin. The bound ligands were detected with a streptavidin-horseradish peroxidase technique. The assay, which was fully validated, had a detection limit of 1 fmol receptor/well. The assay system was used to study the effect of growth hormone treatment upon IGF-I and insulin receptors in bovine skeletal muscle. Three groups of 12 heifers (13 months old) were treated with 320 or 640 mg rBST (slow release preparation) every fortnight for 3 months. When samples of the M.splenius were assayed for IGF-I and insulin receptors, there was no difference between groups neither with respect to receptor concentration nor affinity.

Finally, a third analytical procedure was introduced, again designed to identify rBST treated cows. This procedure is based on the fact that treatment with rBST results in a decrease of the blood levels of specific IGF binding proteins (IGFBP). The use of an immunological method (Scippo et al., 1996) allows to estimate this decrease. The concentration of IGFBP seems to be 7 times lower in treated cows compared to untreated animals. Thus, these methods would allow a reliable identification of rBST treated animals.

2.3.2. Excretion of IGF-I in milk of non-treated and treated (rBST) cows with particular reference to physiological variation during lactation

Evaluating more than 60 scientific articles covering the period 1987-1998 it can be concluded that mammalian milk contains various biological active growth factors including IGF-I peptides (for review see Xu, 1998). In bovine milk, concentrations of IGF-I have been observed in the range of:

1-34 ng/mL, normal milk (Malven et al., 1987; Campbell & Baumrucker, 1989; Juskevich and Guyer, 1990; Collier et al., 1991; Schams, 1991; Zumkeller, 1992).

100-300 ng/mL, colostrum (Francis and Read, 1986, Malven et al., 1987; Campbell & Baumrucker, 1989; Zumkeller, 1992).

4.3 ng/mL (range 1.3 - 8.1 ng/mL) average bulk tank milk prior to BST use (Collier et al., 1991)

A comparison of retail milk originating from 'labelled' milk (from non-treated cows) and 'non-labelled' milk (non-specified samples originating from treated and non-treated cows) demonstrated a small, insignificant increase of IGF-I concentrations in the non-labelled milk samples (Eppard et al., 1994). However, in this study the actual number of animals treated with commercial rBST is not known.

During a lactation period, a typical IGF-I profile in cow's milk varies from 150 ng/mL after parturition to 25 ng/mL at the end of the first week of lactation, to 1 to 5 ng/mL at day 200 of lactation (Prosser, 1988; Xu, 1998).

In 1989, the first full report on milk concentrations of IGF-I in cows treated with rBST appeared. Prosser et al. (1989) showed a 3.6-fold increase in the IGF-I concentration over a 7-day period of treatment. In 1994, Burton et al. highlighted several studies demonstrating a two to fivefold increase of IGF-I as a consequence of rBST treatment (Van den Berg, 1989; Gluckman, 1990; Groenewegen et al., 1990; Juskevich and Guyer, 1990).

A broad experiment comprising daily injection and administration of a sustained release formulation of rBST, respectively, was performed with 74 lactating cows (Zhao et al., 1994). Treatments began in the fourth week of lactation and lasted 40 weeks. IGF-I was monitored through early, mid- and late lactation. rBST treatment resulted in a significant increase of plasma IGF-I in all lactation periods for both treatment groups. A higher milk IGF-I concentration, however, only occurred in mid- and late lactation periods for the daily injection group. It is worthwhile to mention that application of rBST is restricted in most cases to the mid- and late lactation.

The JECFA Report (1998) cites average control values for IGF-I in milk of 3.7 ng/mL for untreated cows, and a significant increase to an average of 5.9 ng/mL as a consequence of rBST-treatment (see FAO FNP 41/5, 1993). Similarly, studies of different pharmaceutical companies report an increase of IGF-I levels in milk between 25 and 70 percent in individual animals (Burton et al., 1994). Thus, the quantities present in the daily human consumption of milk and dairy products are much lower than the total amount of IGF-I secreted daily in the gut (saliva, gastric juice, jejunal chyme, bile, and pancreatic juice (Chaurasia et al., 1994, Bauman, 1995).

The IGF-I concentration in human breast milk at weeks 6 to 8 is 22 ng/ml (Prosser, 1988). Likewise to animals, IGF-I levels are high in the colostrum (17-30 ng/mL) and decline during lactation period (1-10 ng/mL) (Xu, 1998 and references therein).

Milk secretions of mammals, however, also contain amino acid N-terminally truncated forms of IGF-I, which have a potency that is up to ten times greater than normal IGF-I (Francis et al., 1988; Lemmey et al., 1991). Regarding milk from cows, 3% of the IGF-I is reported to be of the N-terminally truncated form (Shimamoto et al. 1992).

Consequently, even at a 3% level, the des(3N)IGF-I contributes substantially to an increase in bioactivity.

Bovine IGF-I is not denatured by pasteurisation (79 ° C for 45 seconds; Miller et al., 1989). However, following processing of milk for infant formula (121 ° C for 5 minutes) IGF-I is no longer detectable (Collier et al., 1991). In contrast, an increase of measurable IGF-I levels up to 70% following pasteurisation have been reported as well (Juskevich and Guyer, 1990). However, the different analytical methods applied allow no direct comparison of these different reports. It is worthwhile to mention here again that bovine IGF-I has been shown to be identical in structure to human IGF-I (Honegger and Humbel, 1986; Burton et al., 1994) as mentioned before.

In conclusion, even though factors such as stage of lactation, parity, level of nutrition and age influence IGF-I levels in milk, the daily administration of rBST will increase the concentration of IGF-I in milk throughout the lactation period.

IGF-I in milk is resistant to pasteurisation and even elevated levels of IGF-I have been reported after pasteurisation. The latter might be related to the standard analytical procedures which fail to detect protein-bound (IGFBP-bound) IGF-I (see section 2.3.1.2.). Consequently, consumption of milk from rBST treated dairy cows will increase the daily intake of IGF-I.

2.4. Risk Characterisation: Bioactivity of GH and IGF-I

2.4.1. Effects of rBST and IGF-I in the Gastrointestinal Tract

Although - at least theoretically - measurable residual amounts of rBST may occur in edible tissues (including the site of application) these residues are not considered to be of public health concern as the bovine growth hormone fails to interact with human growth hormone receptors (*In contrast:* human recombinant GH is under investigation for therapeutic use in the treatment of inflammatory bowel diseases).

Thus, even persistent rBST residues in meat and milk are unlikely to be absorbed from the gastrointestinal tract and would be biologically inactive in humans. In addition, rBST in cow's milk is inactivated by pasteurisation.

In contrast, IGFs are highly conserved throughout mammalian species and bovine and human IGF-I are identical. This implies that possible biological effects of persistent and even slightly increased IGF-I levels in milk (as discussed in section 2.2) have to be evaluated. The following questions deserve attention:

- Does the IGF-I molecule remain undestroyed in the gastrointestinal tract of humans (when products from rBST-treated animals have been consumed)?

- Based on the biological activity of IGF-I activity as cellular growth factor and assuming that IGF-I is not immediately destroyed in the gastrointestinal tract, what is the consequence of the direct exposure of the gut mucosa?

- What evidence can be provided that orally ingested IGF-I enters systemic circulation and what are the possible consequences of this systemic bioavailability?

2.4.1.1. Physiological properties and functions of IGF-I in the gastrointestinal tract

Until 1991 little attention has been focused on IGF actions in the gut (Read et al. 1991), although it had been described earlier that particularly in the fetal period the stomach contains one of the highest concentrations of IGF-I mRNA and thus the IGF-I content of the intestine exceeds that in liver. In human foetal stomach and intestine IGF immuno-reactivity is localised in epithelial cells with higher concentrations in the villus than in the crypt cells. Adult rat intestines contain slight to moderate IGF-I immuno-activity in scattered epithelial cells covering the Peyer's patches. It was concluded that gut expression of IGF-I and IGF-II is under developmental regulation. IGF-II expression was found to be maximal in foetal life declining rapidly in the early postnatal period. This pattern parallels the postnatal decline in liver IGF-II, but contrasts with the marked increase in liver IGF-I in neonatal rats.

In addition, gut tissues express several types of IGF bindings proteins including IGFBP-2 and IGFBP-3. The expression pattern differs between stomach and intestines.

Finally, IGF-I and IGF-II receptors have been identified throughout the gut of several species, including human, pig, rat and rabbit, again exhibiting tissue-specific distribution patterns. Epithelial receptor-binding activity is higher in the colon than in other parts of the gastrointestinal tract, while receptor density in the intestinal epithelium is greater in the crypts than the villi. These findings suggest that receptor expression declines with cellular differentiation (Read et al., 1991 and references therein).

Evidence that exogenous supplementation (via the intake of milk containing IGF-I) with IGF-I is essential in the postnatal phase was provided by Dvorak et al (1996). Applying a sensitive RT-PCR assay, IGF-I gene expression was measured in different age groups (rats) indicating 3 fold higher levels of IGF-I mRNA transcripts in the rat small intestine of adults than in sucklings. The authors concluded that the obvious limitation for IGF-I synthesis in suckling rats may relate to significant enteral IGF-I intake via milk. However, exogenous IGF-I peptide as present in milk may be also responsible for the down-regulation of IGF-I mRNA expression in the developing rat gastrointestinal tract.

Of interest are also previous findings in rats and pigs indicating high postnatal concentrations of IGF-receptor specific mRNA in gastrointestinal tissues relative to the mRNA concentrations of IGF-I (and IGF-II). The temporal changes in IGF-receptor density have been found to correlate with other indicators of intestinal growth and functions (Schober et al. 1990, Burrin, 1997)

2.4.1.2. Trophic effects of exogenous IGF-I:

In animals and humans there are specific IGF-I receptors on the luminal surface of the gastrointestinal epithelium (Donovan and Odle, 1994; Zumkeller, 1992, Oguchi et al., 1997). IGF-I stimulates growth and developments of the tissue and it has been demonstrated that it increases cell proliferation in a dose-dependent manner. Investigating the rate of cell replacement in primary cultures of small intestinal epithelium, Booth et al (1995) found a dose dependent increase in epithelial growth at concentrations ranging between 0 and 20 ng IGF-I per mL.

Initial experiments by Young et al. (1990) had indicated that IGF-I administered either by oral or parenteral routes, stimulated brush border enzymes including maltase, lactase, alkaline phosphatase and aminopeptidase, but had no effect on sucrase activity. In contrast, IGF-II stimulated lactase and aminopeptidase, but only by the oral route.

Comparative experiments in which the effect of GH, IGF-I and GH plus IGF-I was measured revealed, that all intestinal growth parameters were increased following the administration of IGF-I and GH plus IGF-I, whilst GH alone had no

effect (Peterson et al., 1997). These findings are in contrast to in vitro data in which GH was found to significantly increase crypt epithelial cell proliferation in explants of the human small intestine (Challacombe and Wheeler, 1995; Wheeler and Challacombe, 1997). However, this might be attributed to indirect effects of GH as well, mediated by IGF-I.

Additional in vitro studies clearly indicate the mitogenic nature of IGF-I on adult human duodenal mucosa (Wheeler and Challacombe, 1997). The trophic effects of IGF-I to increase crypt epithelial cell proliferation in test explants, exceed those of GH and insulin (Michell et al., 1997a). However, no comparative studies have been conducted in vivo as of yet. As it could be demonstrated that subcutaneous administration of IGF-I improved mucosal structure and absorptive function after small bowel transplantation in rats, the possibility to use IGF-I therapeutically with the aim to improve adaptive changes after surgical resections, has been discussed (Sanderson 1997, Zhang et al., 1995; Chen and Nezu, 1997).

Taken together it can be concluded that there is convincing evidence that IGF-I and other growth factors excreted via milk play an important role in growth and differentiation of gastrointestinal tract tissues and support the concept of a physiological role of colostrum-borne IGFs on the neonate (Baumrucker and Blum, 1993; Fholenhag et al., 1996; Fholenhag et al., 1997)). In addition, clear evidence is provided that orally ingested IGF-I reaches the receptor sites in the gut in its biologically active form.

While the prominent role of IGF-I in the modulation of somatic and gastrointestinal growth in the neonatal was confirmed in several other experiments with rats (Philipps et al., 1997, Steeb et al., 1997, Steeb et al., 1995) and pigs (Burrin et al., 1996), it also became evident, that oral administration of IGF-I results in systemic effects (increase in body weight, liver and brain weight) in suckling rats, and thus indicated the resistance of IGF-I to degradation by gastrointestinal juices of the suckling rat. Radio-labelled IGF-I, when administrated orally remained receptor-active in gastrointestinal tract tissue for at least 30 min post-ingestion (Philipps et al., 1995).

The appearance of IGF-I in mammary secretions has been shown to vary with physiological state. Colostrum of all species contains high concentrations of IGFs when compared with concentrations in mature milk (Baumrucker et al., 1994). This implies that under physiological conditions exposure to high levels of IGF-I occurs only during the short perinatal period. The possible trophic biological effects of a consistent IGF-I exposure via milk throughout the entire life-span needs to be established. Assuming a dose-dependent mitogenic effect of IGF-I, the question remains to be answered, to what extend exogenous IGF-I, being additive to the amount of IGF physiologically present in the gastrointestinal tract (via pancreatic and biliary excretions; Chaurasia et al., 1994), is able to induce any adverse effect as a consequence of long term exposure. This question needs to be addressed as several in vitro studies indicated that IGF-I is mitogenic to several colon carcinoma cell lines (Lahm, 1992; Michell et al., 1997a; Guo et al., 1998)

2.4.1.3. Bio-availability of orally administered IGF-I.

As IGF-I might be important in the treatment of Laron dwarfism and insulin-resistant diabetes, the oral application of recombinant (human) IGF-I has been studied experimentally (the structures of human and bovine IGF are identical). It could be demonstrated that the initial low oral bioavailability of 9.3% could be increased by the co-administration of aprotinin, and, more importantly, by simultaneous application of casein. Casein enhances the oral bio-availability of IGF-I in adult rats to 46% and 67%, respectively (Kimura et al., 1997). The orally administered IGF-I was present in the plasma as the 50-kDa and 150-kDa complexes, indicating that transmucosal transport is facilitated by a specialised transport mechanism.

These data confirm previous experiments, in which an increase of the oral bioavailability of IGF-I in the presence of milk casein had been reported in neonatal calves and neonatal pigs (Xu and Wang, 1996; Vacher et al., 1995), whereas other studies report a poor absorption rate only (Donovan and Chao, 1997). These experimental data allow the hypothesis that IGF-I possess a considerable oral bioavailability also in humans after consumption of IGF-I enriched milk as the casein acts as inhibitor of several proteases (Playford et al., 1993). This hypothesis needs to be reflected in the light of epidemiological studies indicating a positive correlation between dairy product consumption and breast cancer risk (see section 2.4.2.2.; Del Guidice et al., 1998).

2.4.2. Systemic effects of rBST and IGF-I

2.4.2.1. rBST

Although recombinant BST has been considered "essentially chemically the same as natural bovine growth hormone", certain specific differences are worthwhile to mention:

Recombinant rBSTs differ from the natural growth hormone by 1-9 amino acids. In most cases, the N-terminal alanine is replaced by methionine. Dairy industry experiments indicated that the additional, terminal methionyl residue makes rBSTs more immunogenic (FDA Veterinary Note, 1988).

Short-term studies provided no evidence of carcinogenic properties of rBST in Rhesus monkeys. Although the study design is questionably these data fit into the general concept of species-specificity of peptide hormones. However, the possibility that growth hormone cleavage products might retain certain biological properties including the stimulation of the production of growth factors like IGF-I has never been properly addressed.

Furthermore, the role of other milk constituents, which might be altered in their relative concentration in milk, requires further evaluation as not only milk fat quantity and composition is modified by rBST administration but also an increase in the excreted amount of IGF-I, truncated IGF-I ((des3N-IGF-I) and IGFBPs in bovine milk has been reported (Shimamoto et al, 1992; Groenewegen et al., 1990) (see also section 2.3.2.).

2.4.2.2. IGF-I

Previous epidemiological studies have indicated a positive correlation between dairy product consumption and breast cancer (for review see Outwater et al., 1997).

Detailed analyses on the relative risk (RR) including adjustment of RR coefficients for age at first birth and economic variables provided further evidence that milk and cheese were the only dietary variables to remain significantly positive. It was concluded that the relative risk of breast cancer increases with the amount of dairy products consumed; this trend was not evident with respect to meat consumption.

Hence *in vitro* studies indicated that IGF-I is a potent mitogen for breast cancer cells, the link between milk IGF-I concentrations and the relative risk for human breast cancer was established. This hypothesis is supported by the fact that human and bovine IGF-I are identical (as mentioned before) and also IGF-I in milk is present in its unbound form.

These mitogenic effects on cell proliferation rate of breast cancer cells could be observed at concentration as low as 1 ng/mL (Zapf et al., 1981). The average concentration in milk varies between 1-34 ng/mL (see section 2.3.2.).

Nearly all breast cancer cell lines and breast cancer cells from fresh tumour biopsies have receptors for IGF-I, and IGF-I binding to both, benign and metastatic human breast tumours is increased compared to normal mammary tissue binding (Macaulay, 1992; Peyrat et al., 1992; Jammes et al., 1992). In addition, highly malignant human breast cancers produce and secrete IGF-I. This observation has been used as diagnostic tool in clinical oncology but also indicates that IGF-I might be directly involved in tumorigenesis. IGF-I causes changes in the cell cycle and activates oncogenes such as *c-fos* (Li et al., 1997). Evidence suggests also that oncogenes may encode IGF-IRs whose over-expression seems to be involved in the transformation from natural mammary tissue growth to breast cancer (Kaleko et al., 1990).

As IGF-I receptors are over-expressed in virtually all breast cancer cell lines they are considered to be related to enhanced proliferation whilst inhibiting programmed cell death (apoptosis). Recently, Resnik et al. (1998) could demonstrate that IGF-IR expression was 14-fold higher in malignant breast tissue than in normal breast tissue and receptor function, as demonstrated by kinase activity, was 2-4 fold higher in purified receptor preparations from malignant breast tissue.

Epidemiological data stressing the role of IGF-I in breast cancer became available with the nested case-control study within the prospective Nurses' Health Study (Hankinson et al., 1998). This well-known study started in 1976 and includes women of different ages (including pre-menopausal and post-menopausal cohorts). Plasma concentrations of IGF-I and IGFBP-3 were measured in blood samples collected in 1989-1990. These IGF-I concentrations were

compared by logistic regression with adjustment for other breast cancer risk factors.

A positive relation between circulating IGF-I concentration and risk of breast cancer was found among pre-menopausal women (top *vs* bottom tertile: relative risk 2.33 (1.06 - 5.16) with *p* for trend 0.08; selecting pre-menopausal women less than 50 years old, the relative risk amounted to 4.58 (1.75 - 12.0) with *p* for trend 0.02). After adjustment for plasma IGFBP-3 concentrations, the relative risks increased to 2.88 and 7.28, respectively. Neither in post-menopausal women nor among the whole study group, a comparable association between circulating IGF-I concentration and breast cancer could be established (Bohlke et al., 1998).

Del Giudice et al., (1998) found in another case control study a positive association between IGFBP-3, circulating insulin levels and the incidence of pre-menopausal breast cancer. These recent studies confirm previous case control studies, also reporting a positive relation between plasma IGF-I concentration and breast cancer risk (Bruning and Clemmons., 1995; Peyrat et al., 1993). However, it should be taken into account that the recent studies are also in favour of the suggestion that plasma IGF-I concentrations are an early marker in the identification of women at high risk, rather than indicating a causal relationship between cancer incidence and circulating IGF-I levels. In addition, these epidemiological data suggest a correlation between IGF-I and IGFBP-3, however, the individual contribution to the overall bio-activity in the tissues remains unclear.

Further evidence for the relation between IGF-I and breast cancer originates from experiments with rodent species. Energy restriction can decrease tumour development in multiple models. As energy restriction also lowers IGF-I levels, thereby favouring apoptosis over cell proliferation, energy restriction slows tumour progression. Recent studies (Dunn et al., 1997) confirmed this hypothesis as the protective effect of energy restriction could be abolished by supplementation of IGF-I.

The responsiveness of breast epithelial cells to IGFs is modulated by estrogens and estrogens appear to act at several points of the IGF signal transduction and to regulate both, IGF-I and IGF-II expression as well as IGF binding proteins and type I IGF receptors (Westley et al., 1998; Koval et al., 1998). These data confirm previous studies describing that estrogens increase the level of IGF-I in human breast tissue (Osborne and Arteaga, 1990). Furthermore, IGF-I stimulates estrone sulphatase activity (Purohit et al., 1992) and the number of IGF-I receptors has been found to be positively correlated with the number of estradiol receptors, suggesting synergistic mechanisms (Peyrat et al., 1992).

It is worthwhile to mention that in breast cancer as well as in prostate cancer, bladder tumours, gastric cancer and paraganglioma tumours an increased expression of IGF-II was demonstrated providing further evidence for the role of IGFs in autocrine cancer cell growth *in vivo* (Li et al., 1998).

Finally, IGF-I has been found to be a mitogen for prostate epithelial cells. A prospective case control study of men, participating in the Physician's Health Study revealed a strong positive association between IGF-I levels and prostate cancer risk (Chan et al., 1998; Brower, 1998). Relative risk (RR) varied in an univariate analysis between 0.62 and 4.74 with *p* for trend of 0.006 (test for linear trend calculated by assigning the medians of the quartiles as scores). Multivariate analysis (with simultaneous adjustment for IGF-I or IGFBP-3) revealed quartiles associated RR values between 0.83 and 10.6 with a *p* for trend of 0.001.

Again the question remains to be answered whether or not an increased level of circulating IGF-I has to be considered an early marker, predicting prostate cancer risks, rather than indicating a causal association.

3. Secondary risks related to the use of rBST in animal production

Based on the nature and intrinsic activity of GH in the target animal, a number of secondary effects can be anticipated:

3.1. Effect of rBST on drug metabolism in the target animal species

Growth hormone has been shown to exert its biological effect by regulating the expression of different genes, including the expression of enzymes of the cytochrome P450 family.

Particular reference is made to the CYP2C family, which comprises a considerable percentage of total P450 activities in bovines. CYP2C is involved in the bio-transformation of a wide range of pharmaceuticals facilitating their bio-inactivation and elimination. Down-regulation of CYP2C would result in delayed body clearance and increase the biological half-life of these drugs (Witkamp et al., 1993, Chilliard et al., 1998). This comprises a virtual risk towards an increase of undesirable residues in edible tissues and milk and might lead to an intensified drug residue monitoring.

3.2. rBST and clinical mastitis

The use of rBST might comprise the risk of an increased incidence of mastitis in dairy cows (for a detailed discussion of this item we refer to the corresponding report devoted to Animal Welfare aspects). The public health and food safety aspects of mastitis in dairy cows are exclusively associated with the potential problems of side effects from using antimicrobials in the treatment or prevention of such cases. Treatment of clinical mastitis cases with antimicrobials is not limited to those cases which may be classified as *severe*, although such cases are probably more likely to receive systemic treatment. Also *mild* clinical cases are often treated with local application of antimicrobials, such as the application of formulations for intra-mammary use. Even cases of *sub-clinical mastitis* are sometimes treated with antimicrobials, depending on other factors in the herd, as are cows being dried off before calving (Radostits et al., 1994). The result is that mastitis is the one condition in dairy cows which is associated with use of the largest amount of antimicrobials. It is therefore not surprising, that by far the most frequent reason for residue violations in milk are related to mastitis treatment (Leslie and Keefe 1998). This applies in particular in cases where the principles of Good Clinical Practice are not respected.

The public health reasons for limiting as far as possible the use of antimicrobials in dairy cows are the risk of:

- an increased incidence of allergic reactions from drugs and their metabolites in consumers of milk and dairy products;

- an increased selection of bacteria resistant to antimicrobials.

Allergic reactions:

It is estimated that 3 - 10% of the human population is allergic to penicillin and other beta-lactam antibiotics, which constitute the most common therapeutic treatment for clinical mastitis. There are a few reported cases in the literature on allergic reactions following consumption of contaminated milk.

There is no available data on how the risk of such residues vary with occurrence of mastitis in the source cows, but as a general assumption one may consider that increasing risk of mastitis which is treated by antimicrobials will increase the risk of such residues (Kaneene and Ahl, 1987).

The extent to which this risk is modified or prevented by testing for residues by routine monitoring is also not known, but of course any violation which is detected before the milk is processed will lower the risk of residues in milk for consumption. The test characteristics (sensitivity and specificity) of the test procedures used for detection of residues will, therefore, influence the outcome of the monitoring, and critical evaluations of some of the tests used have been published (Gardner et al. 1996).

Antimicrobial resistance:

The risk of antimicrobial resistance following veterinary, including mastitis related, use of antimicrobials is the subject of another scientific report currently being prepared by the Scientific Steering Committee. Recent publications referring to the specific issue of bacterial resistance following mastitis related use of antimicrobials vary in their evaluation of the phenomenon (Hillerton 1998, Sandgren 1998, Wegener 1998, Aarestrup and Jensen 1999). The issue of antimicrobial resistance in general is subject of several ongoing evaluations in the EU and Codex Alimentarius.

It can be anticipated that with an increase of the incidence of bovine mastitis more veterinary medicinal products will be used. This practice comprises a virtual risk toward an increase of undesirable residues in milk and other edible tissues and might lead to an intensified drug residue monitoring program within the European Community. Furthermore, the increased use of antimicrobial substances in the treatment of rBST related mastitis might lead to the selection of

resistant bacteria.

3.3. Adverse effects related to alteration of milk composition

Several reports express concerns about undesirable allergic reactions which might occur after the consumption of milk obtained from rBST-treated cows. Previously, the antibody response to rBST has been investigated as indirect measure of the possible absorption of rBST from the (rat) gastrointestinal tract. However, the question whether or not a change in milk protein composition as a consequence of rBST application to dairy cows might pose an additional risk factor in the development of food allergies has so far not been addressed adequately.

4. Summary and Conclusions

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

Based on its peptide nature, rBST has to be applied parenterally and the concept of species - specificity implies that residual amounts of unchanged rBST fail to induce a biological response in species (including humans) other than bovines. However, the nature of rBST cleavage products and their biological activity has not been investigated in detail.

Comparably to the endogenous growth hormone, rBST is known to increase the level of circulating IGF-I in the target animal followed by an increased excretion of IGF-I in milk. Consequently increased levels of IGF-I in milk have to be included in the estimation of potential health hazards originating from the zootechnical use of rBST.

IGF-I is a physiological constituent of bovine milk. Data on the actual amount of IGF-I in milk are inconsistent as physiological levels show a considerable variation depending on the age of the animals, state of lactation and nutritional status. The highest IGF-concentrations in milk are found at the initial phase of lactation (colostrum) and decline as lactation progresses.

The various analytical techniques for the determination of IGF-I and its truncated forms need to be evaluated in validated procedures. Present data do not provide a conclusive answer to whether or not previously applied analytical techniques have underestimated the actual IGF-I level in milk by neglecting the protein-bound fraction, and to what extent the ratio between free and bound IGF-I in milk has changed as a consequence of rBST treatment resulting in a relative increase of the free IGF-I fraction.

Application of rBST increases the amount of excreted IGF-I in milk by 25-70 % in individual animals. The Committee noted that bovine milk may contain truncated IGF-I (des(1-3)IGF-I) which was found to be even more potent than IGF-I in the anabolic response when given subcutaneously to rats. No quantitative data are available indicating the additional level of this truncated form of IGF-I in milk from rBST-injected dairy cows.

The biological activity of IGF-I comprises endocrine, paracrine and autocrine effects and IGF-I has been identified as cellular growth factor with mitogenic, anti-apoptotic properties and may thus directly interfere with physiological mechanisms involved in the removal of transformed cells. Evidence on the physiological essentiality of IGF-I in foetal and perinatal development is accumulating. Biomedical research focuses on the possible use of IGF-I in the therapy of distinct diseases, whereas the detrimental role of IGF-I in tumour progression is disputed.

Experimental evidence for an association between IGF-I and breast and prostate cancer is supported by epidemiological studies. The bimodal activity of IGF-I being essential in the process of cellular differentiation regulating the expression of several genes, and acting as cellular growth factor with anti-apoptotic properties hinders the definition and establishment of a no-adverse-effect level, a paradigm in conventional risk assessment.

Advocates of the medical (therapeutic) use of IGF-I refer to the short half-life and the auto-regulatory mechanisms sequestering free biologically active IGF-I via endogenous binding proteins (IGFBPs).

Opponents refer to the epidemiological evidence arising from the recently published cohort studies indicating an association between circulating IGF-I levels and the relative risk of breast and prostate cancer, respectively.

Elevated plasma IGF-levels may be considered as a predictive marker for breast and prostate cancer. However, it should be emphasised that all these epidemiological studies refer to a time interval in which exposure to dairy products originated exclusively from non-rBST treated animals. Whether or not the use of rBST will modify the level of risk, remains to be substantiated.

Following the globally accepted concept of risk assessment it is concluded that:

- Direct risks associated with the use of rBST in dairy cows appear to be related to the possible increase of IGF-I levels in milk. The diverse biological effects attributable to the intrinsic activity of IGF-I, exerting a broad variety of metabolic responses through endocrine, paracrine and autocrine mechanisms, make the definition of an in vivo quantitative dose-effect relationship virtually impossible.

- Risk characterisation has pointed to an association between circulating IGF-I levels and an increased relative risk of breast and prostate cancer. In addition, the possible contribution of life span exposure towards dietary IGF-I and related proteins, present in milk from rBST treated cows, to gut pathophysiology particularly of infants, and to gut associated cancers need to be evaluated.

- The available data basis for exposure assessment, i.e. the amount of IGF-I and/or its truncated forms excreted in milk following the administration of rBST to dairy cows, is incomplete.

In addition secondary risks associated with the use of rBST in dairy cows are:

- Potential changes in milk protein composition which might favour allergic reactions.

- An increased use of antimicrobial substances in the treatment of rBST related mastitis which might lead to an increased risk of residue formation in milk and to the selection of resistant bacteria.

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5.2. Section B: Reports and opinion statements

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