ARCHIVED - Report of the Royal College of Physicians and Surgeons of Canada - Expert Panel on Human safety of rbST

Prepared for Health Canada January 1999

Background

The rbST Human Safety Panel was formed in April of 1998 in response to a request from Health Canada for a review of the scientific data used by the Bureau of Veterinary Drugs to determine that meat, milk, and dairy products derived from Canadian dairy cattle treated with Nutrilac (rbST) are safe for human consumption. A detailed chronology of the panel's activities appears below.

In approaching the RCPSC with a request that an expert panel be convened, Health Canada stated that "it is essential that public confidence in the safety of the milk supply be assured at all times. In cases where scientists within the health department lack consensus on matters of drug safety and where there is substantial controversy in the international scientific community about the safety and efficacy of a veterinary drug, then, the creation of an external expert advisory committee to provide independent advice to Health Canada is appropriate". The panel was not asked to comment on the review process within the Bureau of Veterinary Drugs at Health Canada. It was, however, asked to "review international scientific reports and conclusions that have been made on rbST" and "to make observations and recommendations regarding the adequacy of the scientific data submitted by the manufacturer of Nutrilac (rbST) or (to examine scientific information) existing elsewhere to make sound scientific assessments regarding the human health risks associated with the use of Nutrilac (rbST) in Canadian dairy cattle."

The membership of the panel was based on suggestions arising from both Health Canada and the RCPSC. Potential panel members were contacted by RCPSC and agreement to participate was obtained by the College. It was intended from the outset that the panel should include representative expertise in the fields of pediatrics, endocrinology, oncology, pharmacology and toxicology, epidemiology, immunology and nutrition. The panel served on a volunteer basis without remuneration by either Health Canada or the RCPSC.

In view of the extraordinary volume of scientific information existing in conjunction with a file that has been active for more than nine years at Health Canada, it was agreed that a Research Assistant should be hired by the panel to assist with the literature review including the search for scientific data available outside the manufacturer's submission. The Research Assistant, Ms. Cindy Woodland, a doctoral candidate at the University of Toronto (Department of Pharmacology) was appointed in June of 1998.

The panel was initially briefed about the nature of its mandate by Health Canada officials including Drs. Yong, Paterson, and Lachance. Apart from the initial briefing the panel has operated as an agent of the RCPSC and the report which follows is offered as a fully independent assessment.

The panel has received 11 volumes of scientific data from Health Canada representing the files containing salient material on human safety relative to Nutrilac. In August of 1998, the panel also received a report from the Health Canada internal review team, which has been termed the "Gaps Analysis" report. The initial report was submitted to Health Canada on April 21, 1998. A supplementary report submitted to Health Canada by two members of the internal review team on June 10, 1998 was also provided to members of the Human Safety Panel in August of 1998. A summary list of scientific documents and published papers provided to the panel either by officials of Health Canada or by panel members is appended to this report (Appendix 1).

Conflict of Interest Screening

In the course of recruitment of the Human Safety Panel, all prospective members were advised of Health Canada's conflict of interest policy. All members completed a disclosure form describing any professional activities which might be viewed as affecting impartiality. These forms were reviewed by the RCPSC and Health Canada before announcement of the final panel membership. The issues surrounding conflict of interest were also reviewed with the panel during an initial conference call held on May 12, 1998 and at the first meeting of the panel on July 20, 1998.

Panel Membership

Brief biographical summaries of panel members follow. Detailed curricula vitae are available on request.

Dr. Stuart M. MacLeod
Stuart MacLeod (Chair) received his M.D. from the University of Toronto in 1967. Following graduate study in pharmacology and training in internal medicine at McGill University, he received his Ph.D. in 1972 and FRCPC in 1973. He held a number of positions in the University of Toronto between 1973 and 1986. At the time of his departure from the University of Toronto, he was a Professor in the Departments of Medicine, Pediatrics, Clinical Biochemistry and Pharmacology. During the period 1987-1992, Dr. MacLeod was the Dean of the Faculty of Health Sciences at McMaster University. He is currently Professor of Clinical Epidemiology and Biostatistics, Medicine and Pediatrics at McMaster University, where he is the Founding Director of the Father Sean O'Sullivan Research Centre at St. Joseph's Hospital, Hamilton. Dr. MacLeod has served on numerous national and international advisory committees related to his expertise in clinical pharmacology and toxicology, pediatric pharmacology, medical education and international development. He is the author of more than 190 publications and has edited five books. Pediatric Pharmacology and Therapeutics, which he co-edited with Dr. Ingeborg C. Radde (Toronto), is currently in its second edition. Dr. MacLeod is also Vice President, Medical Affairs for

Innovus Research Inc., a Burlington-based contract research organization which was derived from McMaster University in 1984.

Dr. Réjeanne Gougeon

Réjeanne Gougeon received her B.Sc. degree in nutrition from Université Laval in 1963 and then went to Dayton, Ohio for a dietetic internship. From 1964-1966, she worked as an administrative and therapeutic dietitian at Douglas Hospital in Quebec where she was the Director of Food Service. Dr. Gougeon received her M.Sc. in nutrition from Columbia University in 1967. She then instructed in the Department of Home Economics at Marianopolis College in Montreal and in the School of Home Economics at the University of British Columbia. Dr. Gougeon completed her Ph.D. in experimental medicine in 1979 at the Université de Montréal. In 1986-87, she held a post-doctoral fellowship in nutrition at McGill University where she is currently an Assistant Professor in the Faculty of Medicine and the School of Dietetics and Human Nutrition. She has been a Medical Scientist in the Department of Medicine at the Royal Victoria Hospital in Montreal since 1988. Her main research interest addresses protein metabolism in obesity and Type 2 diabetes mellitus. Dr. Gougeon has authored or co-authored 32 publications and 9 reviews, books, and chapters.

Dr. Gerald S. Marks

Gerald Marks received his B.Sc. and M.Sc. degrees in chemistry in 1950 and 1951, respectively, at the University of Cape Town. He completed a D.Phil. degree in organic chemistry at Oxford University in 1954. Dr. Marks took up the position of Assistant Professor in the Department of Pharmacology at the University of Alberta in 1962. In 1969, Dr. Marks was appointed to the Headship of the Department of Pharmacology and Toxicology at Queen's University, Kingston, Ontario. He held this position until 1988, when he stepped down to assume the position of Professor. Dr. Marks has served as the President of the Pharmacological Society of Canada (1984-85), the President of the Federation of Biological Societies (1988-89) and Chairman of the XII International Union of Pharmacology Congress held in Montreal in 1994. From 1994 to 1998, he served as Second Vice-President of the International Union of Pharmacology. He currently serves as a member of the Council of the Medical Research Council of Canada. Dr. Marks served as a member of the Task Force on Chemicals in the Environment and Human Reproductive Problems in New Brunswick (1983-84). He then served as co-editor of the Canadian Journal of Physiology and Pharmacology from 1981 to 1986. Dr. Marks has been a member of the Toxicology Society of Canada for many years and has written numerous papers and reviews in the area of toxicology.

Dr. Michael Pollak

Michael Pollak received his M.D. from McGill University in 1977. He completed his postgraduate training in internal medicine and medical oncology at St. Michael's Hospital, the Toronto Hospital, and Princess Margaret Hospital in Toronto, returning to Montreal in 1985. Dr. Pollak is a Professor of Medicine and Oncology at McGill University. He practices as a clinical oncologist, and also conducts both clinical and laboratory research. He currently holds a Clinician Scientist award from the FRSQ. His research is funded by the National Cancer Institute of Canada and the Medical Research Council of Canada. Dr. Pollak's area of emphasis concerns the role of hormones in carcinogenesis, and he recently has been conducting research concerning insulin-like growth

factors. Based on clues from his laboratory research, he, in collaboration with colleagues at Harvard University, recently published widely cited papers in the *Lancet* and *Science* concerning the relationship between serum IGF-1 levels and the risk of prostate and breast cancers.

Dr. Milton Tenenbein
Milton Tenenbein received his M.D. from the University of Manitoba in 1973. He obtained his specialist certification in Pediatrics in 1978 and in Toxicology in 1982. Dr. Tenenbein is currently a Professor of Pediatrics and of Pharmacology and Therapeutics at the University of Manitoba. He is a member of the Board of Directors of the Canadian Pediatric Society. Dr. Tenenbein is also Past-President of the Canadian Association of Poison Control Centres and President-Elect of the American Academy of Clinical Toxicology. He has authored over 125 publications and over 20 book chapters chiefly within the realm of pediatric toxicology.

Ms. Cindy Woodland
Cindy Woodland is a Ph.D. candidate in the Department of Pharmacology at the University of Toronto. Her research concerns the role of the kidney in human toxicology. She has worked as a Research Assistant in the areas of mammary and renal drug transport and maternal-fetal toxicology in the Division of Clinical Pharmacology and Toxicology at the Hospital for Sick Children in Toronto. Ms. Woodland has won numerous presentation awards from the Canadian Society for Clinical Pharmacology and the University of Toronto. She received a Studentship from the Canadian Cystic Fibrosis Foundation and currently holds a University of Toronto Fellowship Award. Since 1993, Ms. Woodland has been a teaching assistant for courses in pharmacy, pharmacology, and toxicology at the University of Toronto.

Operation of Panel

Chronology of panel meetings and related events

1. Initial discussions between RCPSC and Health Canada
   January 1998
2. Initial contact with Dr. S. M. MacLeod as potential Chair
   February 1998
3. Acceptance of Dr. MacLeod as panel Chair
   March 1998
4. Establishment of panel membership including conflict of interest review
   April 1998
5. Conference call with panel members
   May 12, 1998
6. Conference call with panel members
   June 3, 1998

7. Resignation of original panel member Dr. Richard Gallagher
   une 1998
8. Replacement of Dr. Gallagher by Dr. Michael Pollak
   June 1998
9. Recruitment of Research Assistant Ms. Cindy Woodland
   June 1998
10. Review of literature re: human safety of rbST and IGF-1
    June 1998
11. Meeting of Drs. Marks and MacLeod with Ms. Woodland in Toronto re: pharmacology/toxicology issues
    June 26, 1998
12. Committee meeting, Ottawa
    July 20-21, 1998
13. Call to scientific community for scientific briefs
    August 14, 1998
14. Conference call with CVMA committee re: antibiotic residues
    October 5, 1998
15. Committee meeting, Ottawa
    October 23, 1998
16. Appearance at Senate Committee on Agriculture and Forestry by Dr. S.M. MacLeod
    October 29, 1998
17. Review of progress with RCPSC (Drs. Dinsdale and Brazeau) November 16, 1998
18. Preparation of draft report
    December 1998
19. Circulation of draft report December 21, 1998
20. Finalization of report and re-circulation January 8, 1999
21. Conference call with panel members January 11, 1999
22. Report submission to RCPSC January 12, 1999

Questions posed to the panel

The panel was asked to address four specific questions:

1. Are there potential effects of the residues of rbST and IGF-1 on human immune response?

2. Are there potential effects of the residues of rbST and IGF-1 on the intestinal growth and development of the neonate?
3. Is there a need for chronic toxicity and reproductive studies in laboratory animals in the risk assessment of human food safety of rbST and IGF-1?
4. Is there a potential risk of carcinogenicity in humans as a result of ingestion of residues of rbST and IGF-1 in milk?

The Human Safety Panel was also asked to comment on the quality of the scientific evidence available to the Bureau of Veterinary Drugs, Human Safety Division in its analysis of the Nutrilac file. The committee considered indirectly the information in the Gaps Analysis although that report was concerned primarily with process issues and the question of whether or not the human safety review was conducted in accordance with requirements of the Food and Drugs Act and Regulations. The Gaps Analysis is not, per se, a scientific analysis; however, the questions asked by the authors do relate to scientific data which fall within the mandate of the Human Safety Panel. The report which follows comments on the relevant scientific questions, but does not address issues of process within Health Canada.

The challenge of indirect risk assessment in human toxicology

Under normal circumstances an assessment of human safety for a therapeutic product would call for testing in animal models under acute, subacute and chronic conditions. If no overt toxicities were seen even at doses above those anticipated to be used in humans, then administration to human subjects could be started. This approach does not apply to rbST because it will never be given directly to human recipients. The human counterpart, recombinant human somatotropin is approved for therapeutic use in the treatment of growth hormone deficiency. No major toxicity is recognized at normal therapeutic doses, although colorectal cancer has been associated with pathologically high endogenous growth hormone concentrations in acromegaly. Exogenous human somatotropin is presently being studied for the treatment of intractable heart failure, and delaying the effects of aging, especially in muscle.

The bovine recombinant somatotropin has no effect on human growth hormone receptors. As noted elsewhere in this report, however, rbST does cause increased production of IGF-1 and may, on the basis of rat studies, cause an antibody response in some recipients of oral dosing. The latter response warrants further study in order to determine the likelihood of human hypersensitivity reactions. The implications of human exposure to slightly increased IGF-1 production (1% increment over normal exposure) would be impossible to study in any animal or human model. The enhanced risk of IGF-1 exposure, if any, is entirely indirect and infinitesimally small compared to risk, if any, resulting from exposure to endogenous IGF-1. The human safety panel was unanimous in the view that additional toxicology testing addressing indirect risks of IGF-1 exposure would be unwarranted.

Comments re: review process

The Human Safety Panel undertook its task with the understanding that an objective scientific review of data concerning human safety risks associated with food products from rbST treated dairy cattle was required. It rapidly became apparent that other issues were important to the Canadian public, to some employees of Health Canada, to members of the Senate Committee on Agriculture and Forestry and to the media. The work of the panel has been complicated by suggestions in the press and in verbal communication with members of the panel that one or more of our members, including the Chair, may have conflicts of interest. Such criticisms have persisted although the panel has proceeded on the basis that it is dealing with objective matters of science and a review of evidence on biological matters that are beyond conflict of interest. All members of the panel had previously complied with normal government standards concerning disclosure of professional activities. In the fields important to the current consideration it would be almost impossible to assemble an expert panel in Canada without including individuals who have worked with pharmaceutical companies in a scientific or consultative role. The panel wishes to underscore its role as an objective scientific review body qualified to provide advice as requested to public and private sectors.

The panel's understanding of its mandate relates to the Food and Drugs Act, which requires that the sponsor of a New Drug Submission be responsible for proof of purity, safety, and efficacy of the product under review. The panel did not feel that there was any reason to enter into a review of the file with respect to proof of purity (good manufacturing) and efficacy. Efficacy is within the mandate of the panel struck by the Canadian Veterinary Medical Association. The panel has been focused exclusively on issues of human safety and has operated in the realm of scientific evidence. It was not believed to be within the scope of the committee's work to consider public values, anxieties, or emotional arguments that might be made in favor of or against the introduction of genetically engineered products, such as rbST. The panel accepts that these are legitimate issues of concern to the Canadian public; however, the proper forum for discussion of those concerns lies within the parliamentary process and not within the scientific review of drug products at Health Canada.

Review of international scientific reports and literature

The Human Safety Panel was asked specifically as part of its mandate to review international scientific reports and conclusions that have been made on rbST including those of the Joint Expert Committee on Food Additives (JECFA), the Commission of the European Communities, the U.S. Food and Drug Administration, and the U.S. National Institutes of Health. All available reports have been examined by the panel, but particular attention has been paid to the most recent review published in the WHO Food Additive Series as monograph No. 41. The review prepared by the 50th meeting of the Joint FAO/WHO Expert Committee on Food Additives is entitled "Toxicological Evaluation of Certain Veterinary Drug Residues in Food". The section on recombinant bovine somatotropins and insulin-like growth factor in tissues and milk (section 2.2) is particularly germane to the panel's mandate. The panel found the scientific review in this report to be comprehensive and accurate and the detailed analysis of issues covered adequately in that report has not been repeated here. The report which follows addresses specifically those

questions put to the Human Safety Panel by Health Canada and not covered in adequate depth by the JECFA report.

The literature on biological effects of IGF-1 is extensive. There have been at least 1000 publications in the past twelve months in the peer reviewed scientific literature. The panel has examined, within the limitation of available time, those papers considered to address the questions posed by Health Canada. The relevant references are cited in the following report.

Discussion of Human Safety Issues Related to rbST

Human exposure to bovine food products

Statistics Canada (1992) suggests that Canadians spend more than 10% of their food budget on dairy products. Health authorities have recognized for decades the important place of milk in a balanced diet (1). Since 1944, what is now Canada's Food Guide to Healthy Eating has recommended the consumption of 2 to 4 servings of milk products per day. Despite this recommendation, the average adult Canadian only consumes the equivalent of 1.6 cups of milk per day (1 milk equivalent = 180 g of cheese (except cottage) or 1 litre of yogurt), and dairy product intake varies with age (Table 1) (2,3). An Ontario health survey reports that women consume a larger variety of dairy products than men and that total consumption decreases with age. Furthermore, in persons aged 12 years and older: 40% consume less than 2 servings of dairy products per day, 22% consume from 2 to 2.9 servings per day, and 27% consume more than 3 servings (4). Dairy products are an important dietary source of calcium, vitamins D, A, B_2 and B_{12} for many Canadians.

Table 1. Average daily intake of dairy products per adult in Quebec (1970-1990) (number of servings).

Age (years)	Sex	1970	1990
18-34	male	2.1	2.4
18-34	female	1.3	1.8
35-49	male	1.3	1.8
35-49	female	0.9	1.2

50-64	male	1.6	1.3
50-64	female	0.9	1.1
65-74	male	1.0	1.3
65-74	female	0.9	1.0
all	male and female	1.3	1.6

Cow's milk is an excellent food because it has a high nutrient content, and it is easy to digest and to absorb. It provides 13% of the energy in the Canadian diet. Seventy percent of milk is used in the production of butter, cheese, yogurt, ice cream, powdered milk and other food products (5). The current Canadian trend is to increase consumption of cheese and yogurt and to decrease that of milk (Table 2) (6).

Table 2. Consumption of milk equivalents per person, 1973-1993.

Milk Products	**1973**	**1983**	**1993**
total liquid milk (L)	94.31	96.95	85.62
milk equivalents-cheese (L)	34.44	47.17	63.00
milk equivalents-yogurt (L)	0.61	1.85	3.08
milk equivalents-total by year (L)	129.36	145.97	151.70
milk equivalents-total by day (L)	0.35 (1.4 cups)	0.40 (1.6 cups)	0.42 (1.7 cups)

Type 1 diabetes mellitus has been associated with the early intake of cow's milk by neonates for whom breast feeding is normally indicated (7). In Canada, health professionals universally recommend breast feeding for neonates up to 28 days of life. Virtually all newborns are breast

fed or receive formula. By four months of age, about 25% of Canadian infants are receiving cow's milk (K. Gray-Donald, nutritionist, epidemiologist, McGill University, personal communication), the others are given formula until at least nine months of age as recommended (8), if they are not breast fed.

Although it varies with geographic location, approximately two thirds of mothers breast feed their infant in hospital so that the infants benefit at least from the immune properties of colostrum and possibly from its elevated insulin-like growth factor-1 (IGF-1) content that has been shown to stimulate intestinal development (9). At birth, infants drink approximately 850 ml to 1.2 L (maximum) of milk per day. This same intake of 850 ml (1 L) is maintained throughout the first year with the extra energy requirement met by solid foods. At eight months of age, the recommendations for milk consumption are 850 ml (3.5 cups) per day. Therefore, based on figures taken from Table 3, the amount of IGF-1 consumed daily by a four month-old child drinking breast milk (~16 mg based on the observation that the concentration of IGF-1 in human milk averages ~19 ng/ml by six to eight weeks postpartum) is higher than that consumed by a one year-old child drinking bovine milk from untreated cows (~3 mg) or from cows treated with rbST (~5 m g). Cow's milk is generally avoided in neonates, in the 3 to 5% of older infants who present allergies to its proteins and in individuals with lactose intolerance.

Some human populations have been drinking milk for more than 10 000 years and have benefited from its nutrient contribution, especially protein and calcium, as their diet has become more cereal-based and less dependent on the products of hunting and gathering. In the past, production performance of dairy cows has ensured that adequate supplies of milk have been available to all Canadians as the population has grown. Compared to 1951, 2.9% more milk was produced in 1991 by 849 000 fewer cows (a 49% decrease in herd size) (10).

Pharmacology/toxicology of somatotropin

Somatotropin, commonly known as growth hormone, is a hormone produced in the pituitary gland that is essential for life. Its purpose is to promote growth and to regulate protein, fat and carbohydrate metabolism. The absence of somatotropin in physiologic amounts leads to dwarfism. This outcome is prevented by administering exogenous hormone. Historically, human growth hormone was harvested from cadavers to treat these children. Problems associated with this earlier approach included a limited supply of pituitary glands and, in rare instances, the transmission of disease (Creuzfeldt-Jakob disease). However, with the development of recombinant DNA technology, a synthetic form of human somatotropin can be produced. Now all children with pituitary dwarfism are treated with recombinant human growth hormone.

Recombinant bovine somatatropin (rbST) is the bovine counterpart of human growth hormone. Its pharmacological properties are indistinguishable from its natural bovine counterpart. It is considered to be biologically inactive in humans because it demonstrates negligible binding to human growth hormone receptors. It has negligible acute toxicity with an LD_{50} (oral) in rats of 5000 mg/kg (11) and an LD_{50} (subcutaneous) in mice of 500 mg/kg (12).

Recombinant bovine somatotropin is a protein with a molecular weight of 21 872 Daltons. It has been developed as a commercial product to increase milk production in lactating cows. When cows receive the recommended doses of rbST, the content of bST (measured as natural plus recombinant somatotropin) in milk does not increase (13).

In the product under review, Nutrilac, the N-terminal alanine of one of four natural variants of endogenous bST is replaced by methionine. rbST in cow's milk is largely degraded by the process of pasteurization, with much of the remainder being digested in the human gastrointestinal tract (14,15). Thus, only a small fraction of ingested hormone would be available for absorption. While it is generally believed that this large molecule would not be absorbed intact, some of the data submitted by Monsanto challenge this opinion (see Appendix 2 and discussion under Controversies). In a 90-day study, antibodies to somatotropin were found in rats after the oral administration of high doses (0.1, 5, and 50 mg/kg/day) of this hormone (16). This is evidence that, at least in very high doses, some somatotropin can be absorbed intact and can produce a biologic effect with the risk of immunologic adverse events such as allergy. An identical antibody response would be assumed to occur with either natural or recombinant BST.

Somatotropin has both direct and indirect effects. The direct effects include stimulation of triglyceride hydrolysis and of hepatic glucose output. More importantly, somatotropin stimulates the production of insulin-like growth factor-1 (IGF-1) which is responsible for producing many of the biologic effects associated with somatotropin. While bovine and human somatotropins show differences in amino acid sequence and are species specific in their biological activity, IGF-1 shows no species specificity. In summary, bovine somatotropin has no receptor-mediated effects in humans, however bovine IGF-1 produces identical effects in cows and humans.

IGF-1

IGF-1 is a smaller molecule than rbST with a molecular weight of 7649 Daltons. In considerations of human safety, it is the chief agent of concern because it mediates most of the actions of bST and bovine IGF-1 is identical to its human counterpart. Much is known of this agent with comprehensive reviews found in the medical literature.

Somatotropin binds to receptors in a variety of tissues and stimulates the release of IGF-1 whereupon it circulates to distant tissues where it exerts its biologic effects. Greater amounts of somatotropin in the blood result in an increased production of IGF-1. However, the amount of IGF-1 in the blood of individuals is regulated within narrow limits by a mechanism common to most hormones. This is known as a negative feedback loop. If for any reason the blood concentration of IGF-1 increases beyond the usual level for an individual, the secretion of somatotropin is inhibited so that additional IGF-1 production is slowed. This has been shown in both rats (17) and humans (18). Negative feedback loops maintain blood hormone concentrations within narrow limits.

To a certain extent, the process of protein binding of IGF-1 in blood represents another homeostatic mechanism that controls biologically active IGF-1. Most of the circulating hormone is

bound to specific circulating binding proteins. The IGF binding capacity of serum buffers the IGF bioactivity (19).

It must be emphasized that there is considerable person-to-person heterogeneity in serum IGF-1 levels. That is to say, healthy individuals may have substantially different IGF-1 levels unrelated to dietary intake of IGF-1. The diseases of growth hormone deficiency and acromegaly are associated with very low IGF-1 levels and very high IGF-1 levels, respectively. Between these extremes, individuals have serum levels of IGF-1 that can differ by more than 100 ng/ml (20,21,22). In the context of these large inter-individual variations, the absorption of IGF-1 from the intestinal tract (which has been estimated by some authors to be absent and by other authors to be measurable but small) is considered to be an insignificant determinant of circulating IGF-1 levels. Although some authors have previously stated that IGF-1 cannot be absorbed, there is some evidence from rat studies that IGF-1 can, in fact, be absorbed, especially in the presence of molecules such as aprotinin and casein (23). While we allow for the fact that some absorption may take place, the biological significance of this would be anticipated to be negligible because of the factors listed above. With respect to local effects of the IGF-1 on the intestinal mucosa, because human milk has a higher IGF-1 concentration than milk from rbST-treated or untreated cows (as described below), if the slight increase in IGF-1 concentration of the milk of rbST-treated cattle would have an adverse biological effect, human milk would theoretically have a greater adverse effect.

Somatotropin effects on the composition and characteristics of bovine products

The effects of treating cows with rbST on milk composition and characteristics are as follows:

1. the taste and flavour of milk as determined by panel experts before and after pasteurization do not change (24).
2. .rbST supplementation, administered to multiparous cows 100 days postpartum as recommended does not affect milk protein content (25). Proportions of alpha, beta, and kappa of casein which are important for cheese output remain the same. Studies show that protein content was not affected as long as positive energy and nitrogen balance were maintained. Stage of lactation more than supplementation with rbST determined the relative amounts and types of caseins and whey proteins (26,27). Cow milk protein represents 3.4% (w/w) of total milk and bST and IGF-1 residues account for 0.00001% (w/w) of total milk.
3. the content of lactose and carbohydrate was not affected (4.76 vs 4.77% in 35 controls vs. 32 treated multiparous cows) (28).
4. the total fat content was indistinguishable (3.59% in 35 control vs. 3.52% in 32 treated) in the milk of both supplemented and unsupplemented cows (26,29). The specific fatty acid profile has been observed to be unaffected by rbST supplementation in a number of studies (26,27). A small decrease in saturated fat and an increase in monounsaturates in the milk of rbST supplemented cows has also been reported to occur, especially in the

early stages of treatment (27). Increases in long-chain fatty acids have been shown to occur in the milk of rbST-supplemented cows as a result of negative energy balance and an ensuing mobilization of adipose tissue. However, as voluntary energy intake increased and positive energy balance was achieved, milk fatty acid composition remained no different from that of unsupplemented animals (30). Milk production response was most efficient when dairy herd nutrition and health management were optimal (31).

5. the calcium content (32) and that of vitamins A, D and other minerals were unaffected by rbST supplementation. Milk composition and milk volume are also influenced by the breed, age, health and parity of the cow, the stage of lactation, the feed management, the climate and season (31).
6. milk composition is very much related to the issue of its rbST content. Cow's milk contains bST, whether or not it has been supplemented with rbST. One study measured bST levels in milk pooled from 120 dairy farms. None of the cows had been treated with rbST. BST was, nevertheless, present in the pooled milk in concentrations up to 1 ng/ml (33). Administration of rbST is reflected in increased blood concentrations; however, the cow's mammary gland cells contain no bST receptors (30), only receptors for IGF-1. Therefore, as the BST in blood cannot easily transfer into the milk, the amounts of BST in the milk of rbST-supplemented cows do not increase.

In contrast with BST, IGF receptors are present in the mammary gland of the cow, and therefore, milk levels of IGF-1 reflect serum concentrations. Milk samples taken from 100 raw bulk tanks of milk from untreated cows showed a mean of 4.32 ng/ml of IGF-1 concentrations with a range of 1.27 to 8.10 ng/ml (34). Samples collected from 408 untreated cows varied according to stage of lactation with the highest concentration observed in early stage (6.2 ng/ml) which decreased thereafter reaching a nadir around day 210 (1.85 ng/ml). The mean milk IGF-1 concentration determined seven days post rbST treatments in nine cows treated three times (with the manufacturer's recommended dose of 500 mg every 14 days) and compared to that of nine untreated cows on the same days, gave the following results (35):

3.22, 2.62 and 3.78 ng/ml in untreated cows 3.50, 5.39 and 4.98 ng/ml in treated cows

This increase in IGF-1, which is variable and could amount to 8 m g per litre of milk (36, 23), is considerably less than the normal variation observed to result from factors such as parity and stage of lactation (33). It overlaps with, or is somewhat lower than, levels found in human milk and other physiological fluids (Table 3).

Both BST and IGF-1 are proteins. Therefore, their conformation and activity can be altered by heat. While most BST or rbST activity in the cow's raw milk is destroyed during pasteurization, IGF-1 activity is not altered (14,15). However, IGF-1 is almost completely destroyed in the preparation of infant formulas (15).

Concerning the levels of BST and IGF-1 in tissues, biopsy samples of muscle and liver were taken on days 0, 7, 14, 21, 28 in five lactating cows who were administered 500 mg of rbST on days 0 and 14 (37). Concentrations of BST in these tissues as an effect of treatment at day 21 were not

changed in muscle (4.2 + 2.2 vs. 3.7 + 2.7 ng/g in controls) but were increased in liver (25 + 5 vs. 11 + 4 ng/g in controls) (means + SD). The consumption of 500 g of uncooked meat (muscle) by a person weighing 60 kg would result in a potential exposure to 0.035 mg/kg/day of rbST from treated cows compared with 0.030 m g/kg/day from untreated cows. Likewise, the same consumption of uncooked liver would result in a potential exposure to 0.210 m g/kg/day of rbST from treated cows compared with 0.092 m g/kg/day from untreated cows. Considering that studies show no-hormonal effect at levels of 50 000 mg/kg/day in the 90-day rat study, the exposure for humans suggests an extremely wide safety margin (16).

Seven days post rbST injection (500 mg), treated cows were found to have comparable concentrations of skeletal muscle IGF-1 (312 + 130 ng/g) compared to non-treated cows (272 + 160 ng/g). IGF-1 concentrations in liver were significantly increased in treated (162 + 36 ng/g) versus untreated cows (72 + 9 ng/g) (38). A 60 kg individual eating 500 g of uncooked meat (muscle) or liver from rbST-treated cows is exposed daily to either 2.60 or 1.35 mg/kg body weight of IGF-1, respectively, well below doses expected to have significant biological activity in humans.

Table 3. Concentrations of insulin-like growth factor in body fluids.

Sample	IGF-1 Concentration (ng/ml)	Volume (ml/d)	Reference
bovine milk	3.9 (1-9)	variable	14,39
bovine milk following treatment with rbST	5.9 (1-13)	variable	14,39
human milk[a]	day 1 postpartum: 17.6 day 2 postpartum: 12.8 day 3 postpartum: 6.8 days 4-9 postpartum: ~ 7-8 6-8 wks postpartum: 19 (13-40)	variable	40 41
human plasma	120-380		42
human saliva	3.0-9.8	1500	20
human pancreatic juice	3.8-60.8	1500	20

human bile	4.5-8.2	500	20
human jejunal secretion	24.0-315.8	500	20
human gastric content	12.0-78.8	2000	20

[a]-means (ranges) [b]-ranges

Controversies

Biological impact of IGF-1

It is clear that IGF-1 in milk is not denatured by pasteurisation. The extent of IGF-1 degradation to inactive amino acids and peptides in the gastrointestinal tract after oral administration is unclear. There is also evidence that levels of IGF-1, while low relative to normal physiological concentrations in biological fluids, are significantly elevated in the milk of cows treated with rbST (39). Despite these considerations, the JECFA report of February 1998 has concluded that the additional amount of IGF-1 in milk, assuming no degradation in the gastrointestinal tract, represents 0.8% of gastrointestinal secretion and 0.09% of the daily production of IGF-1 in adults. Since IGF-1 produced by cows has the same chemistry as the IGF-1 produced by humans, it is reasonable to conclude that it is unlikely that the long term consumption of the additional amounts of IGF-1 in milk constitutes a hazard to humans. It is difficult to envisage local effects induced by IGF-1 on the gastrointestinal tract in view of the fact that one is introducing an amount equivalent to a maximum of 0.8% of endogenous IGF-1 into the gastrointestinal tract (39).

It is of interest, as pointed out by the executive summary of the Gaps Analysis (the internal Health Canada review of rbST), that FDA guidelines for toxicology testing of endogenous sex steroids (sex hormones) state that: "if chronic daily ingestion of foods does not result in greater than a 1% increase in exposure to daily synthesis by the segment of the population with the lowest daily production additional animal testing is not required." (These guidelines for sex steroids were accepted, in principle, as appropriate guidelines for other hormones including peptides.) The executive summary of the internal rbST review has requested that the IGF-1 exposures should be verified for neonates and a more detailed analysis of potential human exposure to IGF-1 be carried out, particularly if rbST use becomes widespread. However, even if cow's milk is consumed by human neonates, the increase in exposure to IGF-1 would be expected to fall within the 1% limit proposed by the FDA. More importantly, infants normally consume human breast milk that contains IGF-1 in concentrations considerably higher than those found in bovine milk from cows treated with rbST. Exposure to human milk is clearly not considered hazardous.

The incidence of colorectal cancer is increased in acromegalic patients who have pathologically high endogenous concentrations of free IGF-I in their plasma (43). Given recent epidemiological evidence for a relationship between circulating IGF-1 concentrations and risk of common cancers such as prostate (21), and breast (22) together with multiple prior reports indicating mitogenic actions of insulin-like growth factors, the panel has carefully examined all aspects of this issue in assessing the human safety of consumption of food products from rbST-treated cows.

The relevant physiology is complex, and the documents available for review contain extensive relevant information. Some earlier reviews, including those by Health Canada, may have simplified the physiology. For example, it has been the opinion of some that orally administered IGFs cannot be absorbed. However, in some experimental model systems, absorption of IGF-1 can indeed occur, and this may involve absorption of complexes formed between IGF-1 and the milk protein casein (23,44). For instance, the bioavailability of radiolabelled rhIGF-1 given to rats (1 mg/kg p.o.) increased by 67% when coadministered with casein (23).

The only definitive proof of absolute safety of milk from rbST-treated cattle would be long term follow up data in a population exposed to the resulting food products. Such data are not available, and in the opinion of this panel, requirement of such data would represent a standard of certainty of safety which does not currently exist for any other food product.

The panel has also considered theoretical risks related to rbST and IGF-1. As mentioned, these are deserving of study, as there is evidence that people with higher circulating levels of IGF-1 may be predisposed to certain kinds of cancer (21,22). It is important to highlight, however, that very substantial inter-individual variation in serum IGF-1 concentrations is not at all a function of exposure to foods that contain more or less IGF-1. While some absorption of oral IGF-1 may occur, the amount absorbed is very small relative to the amount made endogenously by individuals. For the reasons detailed above, it is highly unlikely that consumption of food products from rbST-treated cows would raise serum IGF-1 concentrations. While there is evidence that IGF physiology may be related to cancer, and this remains an active area of research, it is the unanimous opinion of the panel that, based on current data, there is no evidence that oral intake of IGF-1 by humans is related to cancer risk or to circulating IGF-1 levels. The IGF-1 content in the diet is more influenced by whether a person consumes milk products at all than by whether a person consumes milk products derived from rbST treated cattle rather than from untreated cattle.

There is theoretical concern about exposure of the mucosal surfaces of the gastrointestinal tract to IGF-1 in milk obtained from rbST-treated cows. However, biologically, this is highly unlikely to be hazardous, as the concentration of IGF-1 in milk from rbST-treated cows is much lower than that normally present in digestive juices secreted by humans (Table 3). Human milk also has much higher IGF-1 concentrations than milk from either rbST-treated or untreated cows (Table 3).

Immunological Effects of rbST

The manufacturer of sometribove (Nutrilac) conducted a three-month (90-day) oral toxicity study of sometribove in the rat which has raised some human safety concerns (Appendix 2). Before this study was released, it was generally believed that sometribove could exert no physiological effects in humans because it is species specific and furthermore, would be degraded in the gastrointestinal tract along with other peptides. However, the finding that some rats treated with sometribove develop antibodies, has challenged this belief.

Given the apparently anomalous result seen at 14 weeks in the 0.1 mg/kg/day group (one out of 30 rats showed an antibody response), the panel feels that the possibility of hypersensitivity reactions deserves further exploration. The sponsor suggested that this finding was either due to a labelling error or to extreme hypersensitivity in the responding rat. The panel is concerned by the continuing uncertainty of explanation and by the sponsor's inability to resolve the question of a labelling error that implies a breach of good laboratory practice. The panel is less concerned about antibody responses to rbST at extremely high doses, but feels that there is a need for clarification of hypersensitivity risk from orally administered rbST at lower dosages. It is the opinion of the panel that the sponsor should repeat the 90-day study of orally administered rbST at doses of 0.1, 0.5 and 5 mg/kg/day. In order to reconcile the antigenicity controversy with Health Canada officials, the sponsor should submit data obtained from studies conducted in accordance with good laboratory practice and analysed to the satisfaction of Health Canada scientists.

Antibiotic Residues

The product monograph for sometribove (Nutrilac) states that treatment of cows with rbST will lead to an increased risk of clinical mastitis. The Animal Safety Panel convened by the CVMA estimates an increase of 25% in the number of cases of clinical mastitis but cautions that this only equates to a real overall increase of about 10% when the following adjustments are taken into consideration:

1. the increase in risk only occurs during the treatment period which should begin at 56-70 days after calving, and
2. the number of cows milked will decrease when cows are treated with Nutrilac due to the implementation of the current supply management milk marketing system which tries to match milk supply with demand.

It can be assumed that this increase in incidence of clinical mastitis will result in a corresponding increase in the use of antibiotics. Concern has been raised that this could result in an increase in the content of antibiotic residues in bovine milk. However, Canada has an enforced testing program for all milk pooled in tank reservoirs, therefore, antibiotic residues will be maintained within nationally accepted standards. Moreover, even though overall use of antibiotics may increase in response to a higher incidence of clinical mastitis, in the unanimous judgment of this panel, this increase would be too small to have any biological significance. Specifically, it is highly unlikely to have any impact on the important public health issue of increasing of antibiotic resistance. Although antimicrobial resistance is linked to the exposure of bacteria to

antimicrobials, the amount of increased exposure as a result of the treatment of this condition would be marginal in comparison to other agriculture and human use. The quantity of antibiotic use for the treatment of infections in animals is insignificant, as compared to their long-term use as growth promoters. (Dr. Donald Low, The Mount Sinai and Toronto Hospital, Toronto, Ontario, personal communication).

Conclusions

The panel is of the unanimous opinion that adequate data have been provided to the Bureau of Veterinary Drugs to permit reasonable decisions to be made about the human safety of meat, milk, and dairy products derived from Canadian dairy cattle treated with Nutrilac (rbST). The panel recognizes that there are extraordinary difficulties in drawing inferences about the potential for indirect human toxicity related to the increased production of IGF-1 in rbST-treated animals. The medical and scientific understanding of IGF-1 will undoubtedly continue to grow; however, the panel does not believe at this time that there is a significant probability of increased human toxicity resulting from the very small increments in IGF-1 concentration observed in the milk and other products from rbST-treated cows.

The panel has reviewed international scientific reports and conclusions concerning rbST and is in agreement with those reports as they concern human safety of meat, milk and dairy products derived from rbST-treated cows.

The panel is concerned about the indication that rbST may cause an immune response in rats exposed to high dosages. This finding is indicative, at least, of a potential for hypersensitivity reactions occurring in humans; however, such reactions would occur in response to natural as well as to recombinant rbST. It is the opinion of the panel that the sponsor should be asked to repeat the 90-day toxicity studies of rbST and to explore whether or not there is a real risk of hypersensitivity reactions occurring at 0.1 mg/kg/day.

The panel was asked to address four specific questions. The questions and conclusions appear below:

a. **Are there potential effects of the residues of rbST and IGF-1 on human immune response?**

   As noted above, the panel considers that the Monsanto 90-day study has demonstrated an antibody response to rbST. It is anticipated that natural bST would also be antigenic. There is no basis on which to expect an immune response to IGF-1. The potential for hypersensitivity reactions to BST or rbST in milk remains open to question. Further study is required to clarify whether or not the observed antibody response is significant in association with very low concentrations of rbST as observed in cow's milk.

b. **Are there potential effects of the residues of rbST and IGF-1 on the intestinal growth and development of the neonate?**

   There is no potential for rbST to influence the intestinal growth and development of the human neonate since the bovine hormone is inactive in humans. IGF-1, however, has equivalent biological effects in both cows and humans. Infants normally consume human breast milk that contains much higher concentrations of IGF-1 than those in bovine milk from untreated or rbST-treated cows. Therefore, the residues of rbST and IGF-1 in bovine milk from rbST-treated cows are not considered hazardous to the neonate. Requirement for further studies would not be justifiable.

c. **Is there a need for chronic toxicity and reproductive studies in laboratory animals in the risk assessment of human food safety of rbST and IGF-1?**

   Given that there is no increment in the milk concentration of somatotropin from rbST-treated cows and that increments in the milk concentrations of IGF-1 would result in ingestion of amounts representing less than 1% of normal endogenous exposure, chronic toxicity and reproductive studies in laboratory animals are not justified. It is possible that reproductive studies are relevant to consideration of animal safety, but that is beyond the mandate of the Human Safety Panel. There is no justification for the performance of chronic toxicity and reproductive studies of rbST since this product has no receptor-mediated effects in humans.

d. **Is there a potential risk of carcinogenicity in humans as a result of ingestion of residues of rbST and IGF-1 in milk?**

The product under review, rbST is devoid of biological effects in humans and therefore poses no carcinogenic risk. As an endogenous substance, IGF-1 may play a role in the pathophysiology of neoplasia; however, there is no evidence that oral intake of IGF-1 is carcinogenic. There is considerable scientific interest in IGF-1 effects and the worldwide enquiry will undoubtedly continue with further clarification of any role in cancer played by IGF-1. In the opinion of the panel, Health Canada would not be justified in requiring further studies of IGF-1 to be conducted by the manufacturer of Nutrilac with respect to pathogenesis of human malignancy.

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Glossary

BST
bovine somatotropin

casein
the principal protein in milk; present in milk curds; (the concentrations in human and bovine milk are 4.4 g/l and 20 g/l, respectively)

CVMA
Canadian Veterinary Medical Association

Dalton
a unit of mass equal to a single hydrogen atom

FAO
Food and Agricultural Organization of the United Nations

FDA
Food and Drug Administration (U.S.A.)

formula
commercial product substituted for human breast milk in infant feeding

FRSQ
Fonds de Recherches Scientifiques de Québec

Gaps Analysis
report prepared by the rbST internal review team

IGF-1
insulin-like growth factor-1

internal rbST review
review of rbST performed by the rbST internal review team

JECFA
Joint Expert Committee on Food Additives

LD_{50}
dose required to cause death in 50% of treated animals

Nutrilac
registered trade name for rbST (sometribove) manufactured by Monsanto Company

pasteurization
the process of heating milk and other substances at a moderate temperature for a definite period of time in order to destroy undesirable bacteria

plasma
the liquid part of the lymph and of the blood

plasma vs. serum concentrations
for the substances discussed in this report, the distinction is unimportant and we have followed the measurements determined by the original authors

rbST
recombinant bovine somatotropin

rbST internal review team
a coordinator plus two members of the Human Safety Division, Bureau of Veterinary Drugs, Food Directorate, one member of the Chemical Health Hazards Assessment Division, Bureau of Chemical Safety, Food Directorate, and one member of the Office of Science, Therapeutic Products Directorate appointed by Health Canada to "determine if any gaps exist in the scientific data regarding the human health risks associated with the Nutrilac (rbST) in Canadian dairy cattle."

RCPSC
Royal College of Physicians and Surgeons of Canada

rhIGF-1
recombinant human insulin-like growth factor-1

SD
standard deviation

serum
the watery portion of the blood after coagulation

somatotropin
growth hormone

sometribove
rbST manufactured by Monsanto (Nutrilac)

ST
somatotropin

WHO
World Health Organization

Appendix 1

Information Distributed to Human Safety Panel Members

- Human Safety Report by D.R. Casorso, 1990 (Nutrilac File)
- Human Safety Report by M.S. Yong, 1995 (Nutrilac File)
- Human Safety Reports by M.S. Yong, 1995, General rbST and IGF-1 Reports (General Report File)
- Human Safety Report by M.S. Yong, 1998, Immunogenicity, Need for Chronic Toxicity, (General Report File)
- Copies of major references from the above Human Safety Reports
- U.S. FDA Freedom of Information Summary for Posilac, 1993
- JECFA Evaluation Reports
- U.S. National Institute of Health Report on Human Safety
- European Union Evaluation Report on rbST
- Third Party Submissions (e.g. Toronto Food Policy Group Position Paper)
- FDA responses to concerns expressed by Dr. Epstein, Ms. Mullarkey, Johanna Dairies
- Monsanto Submissions (including: MSL 8531, 8633, 8671, 8673, 9134)
- Three month (90 day) Oral Toxicity Study of Sometribove in the Rat, SA-88-353
- Effect of Recombinant Bovine IGF-1 on Growth Performance of Rats HLA Study No. 88-338
- A Comparison of the Growth Promoting Effects of Bovine Somatotropin Administered Orally or Injected Subcutaneously, MSL 5900
- Four week Gavage Study (rats) with zinc salt of Methionyl Bovine Somatotropin, HL 84-222
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Appendix 2

Monsanto 90-day Study: Determination of Sometribove Immunoglobulin in Rat Serum

Information not available