# FINDING OF NO SIGNIFICANT IMPACT

for

Sterile Sometribove Zinc Suspension
(Methionyl Bovine Somatotropin, POSILAC®)

For Use in Lactating Dairy Cows

NADA 140-872

Monsanto Agricultural Company
St. Louis, MO

FOR PUBLIC DISPLAY

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The Center for Veterinary Medicine has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

The Monsanto Agricultural Company submitted a new animal drug application (NADA) for the use of Sterile Sometribove Zinc Suspension (Methionyl Bovine Somatotropin, POSILAC®) in lactating dairy cows. The submission includes the attached environmental assessment (EA), dated September 1992, that addresses environmental and occupational exposure concerns for the manufacture and use of the product.

Since the preparation of this EA, the firm has changed the chemical designation of the product from *Sometribove-Zinc Complex* to *Sterile Sometribove Zinc Suspension* to more accurately describe the chemistry of POSILAC®. On March 30, 1993, the firm submitted a letter to NADA 140-872 (copy attached) referring to Monsanto's decision to support the nomenclature change and affirming that this name change has no significant effect on the information in the September 1992 EA. CVM agrees with this name change and did not require further revisions to the EA to reflect the name change.

**Sometribove Production**

The production of Sterile Sometribove Zinc Suspension, including fermentation, purification, formulation, filling and packaging, will take place at Biochemie GmbH in Kundl, Austria. The product can also be filled into the final container and packaged at Solvay Duphar B.V., Olst, The Netherlands. Both of these facilities were inspected by FDA in October 1991 to confirm details of biocontainment described in the attached EA. No FDA 483 was issued for either facility. The finished product will be shipped to and stored in distribution warehouses until shipment to distributors/customers.

**Recombinant DNA *E. coli* Production Strain**

Bulk sometribove will be produced by large-scale aerobic fermentation utilizing a recombinant DNA-derived (rDNA) *Escherichia coli* K-12 strain at the Biochemie facility. This parent strain of *E. coli* is classified as a Class 1 agent, as outlined in Appendix B-I-A in the National Institutes of Health's (NIH) "Guidelines for Research Involving Recombinant DNA Molecules" (7). It is not considered pathogenic to man or other animals. As per Appendix C-11 of the NIH Guidelines, this strain does not contain conjugation-proficient plasmids or generalized transducing phages.

The sometribove-encoding genetic information, transformed into the recombinant production *E. coli* strain, has been cloned into a nonconjugative, poorly mobilizable and scientifically well-known plasmid, pBR322. pBR322 is one of the most widely used cloning vehicles and extensive literature is available on its structure and function (1). This combination of host bacteria and plasmid vector was listed as a certified Host - Vector System (EK2 Plasmid Systems) in Appendix E - III of the NIH Guidelines.

Monsanto conducted tests on the recombinant production *E. coli* strain to confirm that it exhibited similar biological characteristics to non-recombinant *E. coli* K-12 strains. Monsanto also conducted studies to determine the viability of the strain when introduced into environmentally relevant microcosms and whether the novel genetic information could be transferred under these conditions to indigenous microorganisms residing in the test microcosms. It is widely accepted that aquatic and terrestrial laboratory microcosms are useful for examining the fate and effects of introduced microorganisms, as well as their survival and persistence in specific environments (9).

**Viability of the Recombinant *E. coli* K-12 Strain in an Aquatic Microcosm**

In order to determine whether the recombinant production *E. coli* strain has environmental survival characteristics consistent with those observed for non-recombinant *E. coli* K-12 strains, Monsanto conducted a study to determine the viability of its production *E. coli* strain (W3110G[pBGH1]), a plasmid-free *E. coli* strain (LBB269) and a plasmid-containing *E. coli* strain (LBB269[pBGH1]) in an environmental water source (the Missouri River).

To conduct a well-controlled study, it was necessary to use, for comparison purposes, a plasmid-free *E. coli* strain, that was closely related to the production strain . Monsanto created strain LBB269 (resistant to nalidixic acid) in order to facilitate tracking of a plasmid-free *E. coli* strain in an environment populated with other species of microorganisms.

Strain LBB269 was subsequently transformed with pBGH1 to create a new strain LBB269[pBGH1] yielding a nearly isogenic pair of strains (i.e., LBB269 & LBB269[pBGH1]). The two strains differ only in the presence of the recombinant production plasmid and allows the examination of the effect the plasmid had on survival of the LBB269 strain. This allows Monsanto to directly determine the effect of the addition of the plasmid coding for sometribove on an *E. coli* K-12 strain.

The results of this study demonstrate that there were no significant differences in die-off rates between strain W3110G[pBGH1] and strain LBB269[pBGH1] and between strain LBB269 and strain LBB269[pBGH1]. The study demonstrated that these strains did not survive in an environmental source of water in detectable numbers ($< 1.5 \times 10^2$ cfu/ml) for longer than eight days. This result is consistent with what is known concerning the survival of *E. coli* K-12 strains in other environmental settings (6). A review of the scientific literature, summarized in section 7 of the EA, demonstrates that strains of *E. coli* K-12 do not persist in non-sterile water, soil, sewage or the mammalian intestinal tract. This study was inspected by FDA on April 27-29, 1992, to confirm details of the experiment, and no adverse findings were issued for this study.

## Gene Transfer From the Recombinant *E. coli* K-12 Strain to Indigenous Microorganisms

In order to determine whether the recombinant production *E. coli* strain would demonstrate the expected low rate of *E. coli* K-12 gene transfer, Monsanto studied the potential for gene transfer from the production strain *E. coli* W3110G[pBGH1] to indigenous bacteria in a Missouri River water microcosm. The test for such an occurrence involved the detection of DNA containing the sometribove structural gene in indigenous microbes isolated from Missouri River water that had been inoculated with the production strain. DNA from the indigenous microbes was examined for the sometribove gene using the polymerase chain reaction (PCR) assay.

The results of the gene transfer study show that neither the intact plasmid, pBGH1, nor the portion of pBGH1 that includes the sometribove structural gene, was transferred from *E. coli* K-12 strain W3110G[pBGH1] to indigenous microorganisms in Missouri River water. The absence of observed gene or plasmid transfer in this study demonstrates that if either event occurred, it would be at a frequency of less than 1 transfer event per $2.7 \times 10^7$ bacterial cells. This result is consistent with what is known concerning transfer of pBR322 plasmids in microcosm settings (4). The literature review, in attachment 7 of the EA, also examined the possibility of conjugative transfer of genetic material from *E. coli* strains containing recombinant plasmids derived from pBR322. The gene transfer study was inspected by FDA on April 27-29, 1992, to confirm details of the experiment, and no adverse findings were issued for this study.

## Production Facility Biocontainment

Biochemie GmbH is in compliance with NIH Biosafety Level 1 - Large Scale (BL1-LS) biocontainment conditions for all procedures involving the handling of viable recombinant production *E. coli*. These procedures are designed to minimize accidental and ephemeral releases and to minimize the potential for human colonization by the recombinant production organism. The EA provides descriptions of the firm's implementation of BL1-LS biocontainment parameters. The firm states that the facility and operations comply with the relevant NIH Guideline recommendations in Appendix K for a BL1-LS facility and operation.

The entire biocontainment area (fermentation/isolation/solubilization) of the production facility is a closed system providing minimal opportunity for operators to come into contact with the recombinant production *E. coli* strain. The fermentors used in the production of sometribove are pressure tested annually by the Association for Technical Control. Biochemie personnel check the fermentor tanks routinely to ensure that valves and cooling coils are not leaking and that stirring gear bearings are properly adjusted.

Gaseous emissions from the fermentor tanks are passed through a 0.2-micron air sterilization filter to minimize release via off-gas. Post-filtration exhaust gases are monitored monthly for the presence of the recombinant production *E. coli* strain. If viable recombinant production *E. coli* are detected, the plant supervisor and biosafety officer are notified and the observation is documented and investigated. The need for corrective action is evaluated by the Institutional Biosafety Committee (IBC) as called

for under the NIH Guidelines.

Biochemie operates two validated biowaste inactivation systems. The systems service the fermentation plant and the isolation/purification facility. Liquid wastes include residual fermentor broth, wash water, dilute caustic solutions used for cleaning, and steam-sterilization condensate. The wastes are passed through the biowaste inactivation system before discharge into Biochemie's waste treatment facility.

The fermentation equipment and plant are designed to minimize the release of the recombinant production *E. coli* strain. However, procedures and equipment are in place to ensure proper management of releases should they occur. Operators and supervisors are trained annually on each operation of the fermentation and spill inactivation. The main fermentor is fitted with a weight control system that will trigger an alarm in the event of a sudden reduction of fermentor weight. In the event of a leak or minor spill, the affected area is treated with 0.5% peracetic acid solution, rinsed with water, and collected for further decontamination in the fermentation plant biowaste inactivation system. In the event of a catastrophic accident, the biowaste inactivation system holding tanks, the heat-inactivation tanks and fermentor operating sump are adequate to contain the entire fermentor contents.

**Compliance with Requirements of Austria and The Netherlands**

Monsanto Agricultural Company has demonstrated that the overseas production facilities (Biochemie GmbH in Kundl, Austria and Solvay Duphar B.V., Olst, The Netherlands) are currently in compliance with all the applicable emissions requirements of Austria and The Netherlands. Monsanto has provided current English-translated copies of its permits verifying the facilities compliance.

Biochemie holds a permit issued by the Minister for Public Health and Public Services, Republic of Austria, for the production of sometribove. An additional permit from the same organization to cover expanded production and filling and packaging operations was issued in 1990. Biochemie also holds permits issued by the Tirolean State Government for the existing waste water disposal plant and discharge from the plant into the River Inn and for expansion of the facility. A permit issued by the local administrative district of Kufstein also allows operation of the new formulation area and syringe filling and labeling operations.

Solvay Duphar holds permits to allow the discharge of effluents to the River Ijssel and to incinerate pharmaceutical and chemical wastes. A general operating permit has been issued by the Netherlands Secretary of State for Welfare, Human Health and Culture.

**Production Worker Exposures**

Sometribove is a protein that does not possess any unusual toxicological properties. However, worker exposure to airborne concentrations of sometribove-containing dust has produced respiratory symptoms such as coughing, sneezing, inflammation of the mucous membranes of the nose, and, in one case, an asthmatic reaction. Frequent skin contact with foreign proteins may also cause dermatitis in susceptible individuals. Therefore, in those areas where product exposure can occur, the firm requires workers

to wear appropriate protective clothing including gloves, suits and masks covering the nose and mouth. Material Safety Data Sheets (MSDSs) are available and included in the EA package for the lyophilized bulk product, sometribove, and for the final formulated product, POSILAC®.

An environmental monitoring program is in effect at the manufacturing site in Austria. This program provides for an evaluation of the air-borne dust removal capabilities of the facility and a microbial analysis of the organisms present in the facility. The microbial analysis includes assays for the recombinant organism. No environmental monitoring is in effect at Duphar, the Netherlands, as there are no gaseous emissions at this packaging facility

**Dairy Farmer Exposures**

The warnings section of the product package insert advises people administering the product to dairy cows how to minimize the possibility of an allergic reaction to POSILAC®:

> "Avoid prolonged or repeated contact of POSILAC® with eyes and skin. POSILAC® is a protein. Frequent skin contact with proteins in general may produce an allergic skin reaction in some people. Always wash hands and skin exposed to POSILAC® with soap and water after handling. Clothing soiled with the product should be laundered before reuse."

The EA also discusses the disposal of unused product and of expended syringes still containing small quantities of POSILAC®. To prevent exposure to the product the firm has included the following instructions on the outside of the product container and in the product package insert:

> "Used syringes and needles should be placed in a leak-resistant, puncture-resistant container for disposal in accordance with applicable Federal, state, and local regulations."

These handling instructions provide adequate information for dairy farmers who will be using the product to provide for the safe and legal disposal of used syringes and needles on the farm.

In addition, Monsanto Agricultural Company has entered into an agreement with Browning-Ferris Industries (BFI) to provide dairy farmers with a complete sharps waste management program. Monsanto will provide customers with sharps mail-back kits that comply with U.S. Postal Service regulations. Dairymen can mail spent POSILAC® syringes and needles to a medical waste treatment facility where the contents will be destroyed by incineration or by autoclaving and shredding.

**Sometribove in the Environment**

Sterile Sometribove Zinc Suspension (POSILAC®) is an amino-terminal methionylated, recombinant DNA-derived analogue of bovine pituitary somatotropin (BPS), generically referred to as bovine somatotropin (BST) or bovine growth hormone (BGH). Zinc is added during the production process to form the finished POSILAC®. The EA describes

introductions of sometribove into the environment. Monsanto considered potential environmental introductions of sometribove from BST-supplemented cows via exhaled gases, milk, feces, and urine. Sometribove has a large molecular weight (22,000 daltons) and a negligible vapor pressure. The large molecular weight of sometribove prevents the exhalation of the intact compound. Sometribove is present in significant amounts only in the urine. However, Monsanto demonstrated in studies that the majority of immunoreactive sometribove in cow urine is partially degraded (i.e., the majority of residues were pieces of sometribove and not the intact protein).

In addition, it can be reasonably expected that any sometribove entering the aquatic and terrestrial ecosystems will be degraded into small peptides and free amino acids by proteases naturally present in soils and bodies of water. There are many bacterial proteases which would be expected to degrade sometribove into peptides and free amino acids. Some of these proteases perform both endo- and exopeptidase activities and as examples include: 1) subtilisn from *Bacillus subtilis*, 2) thermolysin from *Bacillus thermoproteolyticus*, 3) V-8 protease from *Staphylococcus aureus* and 4) pronase (a mixture of proteases) from *Streptomyces griseus* . Identical peptide bonds are cleaved in sometribove as compared to BPS (10). Based on these considerations, it can be predicted that sometribove will be unstable in both aquatic and terrestrial environmental compartments and that the degree of instability will generally reflect the abundance of microorganisms and microbial proteases.

Any sometribove entering the environment via excretion, or as a result of improper disposal, will be unstable and thus will be unlikely to have any detectable impact on the environment. Monsanto has also provided information that demonstrates that sometribove has growth-stimulating effects in only a few species and that, even in those species in which it is active, it is active only at doses far greater than any likely to be achieved in the environment.

**Impacts on Land Use and the Dairy Industry**

Changes in land use are recognized environmental effects under the National Environmental Policy Act (NEPA), and the potential for the use of sometribove to cause such changes and to contribute to restructuring of the dairy industry has been reviewed (5). The Monsanto Agricultural Company provided an analysis in the EA on the impact of sometribove commercialization on land use. The starting point for this analysis is a report published by the Economic Research Service of the U.S. Department of Agriculture (USDA) on the potential effects of the introduction of BST on the U.S. dairy industry (Fallert Study) (3).

The 1987 Fallert study predicted that the effects of BST will depend on the flexibility of government price support systems, with higher price support levels favoring greater numbers of cows, higher BST adoption rates, and greater potential for effects on the dairy industry due to BST use. Four government price support scenarios, bracketing the most probable expected support level, were discussed. The 1990 Farm Bill, which will be in effect until 1995, calls for a price support level of $10.10 per hundred weight (CWT). This level corresponds to Fallert's Scenario I. The Fallert study concluded that, even at the highest price support level (Scenario IV), the effects of the introduction of BST are likely to be relatively minor and will not fundamentally change structural trends already underway in the dairy industry.

In 1990, Blayney and Fallert updated the 1987 Fallert study in response to a request by Senator Leahy (2). The updated report states that Scenarios I and III used in the 1987 Fallert study are the most applicable as potential bases for analysis under current conditions. The authors concluded that:

> "The reevaluation of the 1987 study and the review and analysis undertaken in response to Senator Leahy's request indicate that most of the previously listed general trends, results, and implications of the 1987 study remain valid today."

Preckel and co-workers used the 1987 Fallert study to investigate the potential environmental effects of the use of BST, including its effects on crop production, use of agricultural chemicals, and manure production (8). At the national level, the Preckel study found that the effect of BST adoption on crop production (due to changes in feed requirements for dairy cows) was likely to be negligible. Preckel further predicted that any associated changes in agricultural chemical use would also be negligible.

According to the 1987 and 1990 Fallert studies and the 1988 Preckel study, dairy industry acceptance of BST is not likely to lead to any significant shift or impact on agricultural land use, agricultural chemical usage, surface or ground water quality, non-target species, soil tillage, or manure production.

**Impacts on Greenhouse Gas Emissions**

The Monsanto Agricultural Company has provided in the EA two reports analyzing sometribove's potential effects on greenhouse gas emissions. The two reports (the G.F. Hartnell and G.H. Irwin reports) cover three areas: 1) a calculation of the potential effects of POSILAC® use on emissions by the dairy industry in the U.S. (Hartnell report), 2) a calculation of greenhouse gas emissions due to the manufacture and transport of POSILAC® (Irwin report), and 3) a calculation of the net effects of POSILAC® use, manufacturing, and transport on greenhouse gas emissions (Irwin report).

The results of the Hartnell analysis demonstrate that the use of POSILAC® will either slightly increase or slightly decrease emissions depending on whether milk yield increases resulting from POSILAC® use results in a reduction in the number of dairy cows in the national herd. In either case, the magnitude of the changes will be extremely small and insignificant compared to total worldwide emissions of carbon dioxide and methane.

The results of the Irwin analysis demonstrate that, according to Hartnell's Scenarios 1 and 2, the manufacture and transport of POSILAC® will result in incremental increases in carbon dioxide and methane emissions. These increases will be insignificant when the net effects of usage, manufacturing and transport are calculated and compared to total worldwide emissions of these gases. The Irwin analysis states that "the magnitudes of the changes are so small compared to worldwide emissions that they are unlikely to be of environmental significance."

**EA Conclusions**

We have reviewed the EA and supporting documentation and find that together they provide adequate information to conclude that the approval of NADA 140-872 is not expected to have a significant effect on the quality of the human environment.

5/7/93 — Roger A. Jones
Date — Preparer, Environmental Sciences Staff, HFV-152

5/7/93 — John C. Matheson III
Date — Senior Regulatory Environmental Scientist, HFV-150

5/11/93 — Suzanne G. Sinclair
Date — Primary Action Officer, HFV-126

5/7/93 — Neil E. Sigler
Date — Chief, Environmental Sciences Staff, HFV-152

**Attachments:**

1. Environmental Assessment, dated September 1992, and attachments 1-15. in volumes 1-9.

2. Amendments to the EA in volume 10.

**References:**

1. Balbas, P., X. Soberon, E. Merino, M. Zurita, H. Lomeli, F. Valle, N. Flores, and F. Bolivar. 1986. Plasmid pBR322 and its special-purpose derivatives- a review. Gene 50: 3-40.

2. Blaney, D.P., and R.F. Fallert. 1990. Biotechnology and agriculture: emergence of bovine somatotropin (bST). USDA, ERS, CED, Staff Report AGES 9037. Washington, D.C.

3. Fallert, R., T. McGuckin, C. Betts, and G. Bruner. 1987. bST and the dairy industry: a national, regional, and farm-level analysis. USDA, ERS, Agricultural Economic Report Number 579. Washington, D.C.

4. Gealt, M., M.D. Chai, K.B. Alpert, and J.C. Boyer. 1985. Transfer of plasmids pBR322 and pBR325 in wastewater from laboratory strains of *Escherichia coli* to bacteria indigenous to the waste disposal system. Appl. Environ. Microbiol. 49: 836-841.

5. Geisler, C., and T. Lyson. 1991. The cumulative impact of dairy industry restructuring. Bioscience 41(8): 560-567.

6. Jain, R.K., R.S. Burlage, and G.S. Sayler. 1988. Methods for detecting recombinant DNA in the environment. CRC Critical Reviews in Biotechnology 8(1): 33-84.

7. National Institutes of Health. 1986. Guidelines for research involving recombinant DNA molecules. 51 FR 16958.

8. Preckel, P.V., R.F. Turco, M.A. Martin, and C.H. Noller. 1988. Environmental effects of the introduction of bovine somatotropin under alternative dairy price support programs. Purdue University. Attachment 11, Environmental Assessment, dated September 1992.

9. Tiedje, J.M., R.K. Colwell, Y.L. Grossman, R.E. Hodson, R.E. Lenski, R.N. Mack, and P.J. Regal. 1989. The planned introduction of genetically engineered organisms: ecological considerations and recommendations. Ecology 70: 298-315.

10. Violand, B.N. 1989. Proteolytic degradation of bovine somatotropin. Attachment 10, Environmental Assessment, dated September 1992.

ENVIRONMENTAL ASSESSMENT - SOMETRIBOVE-ZINC COMPLEX
THE AGRICULTURAL GROUP OF MONSANTO COMPANY

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Section 10. Environmental Assessment - Sometribove-Zinc Complex

1. Date: September, 1992

2. Name of Applicant/Petitioner: Animal Sciences Division
The Agricultural Group of
Monsanto Company

3. Address: 800 North Lindbergh
Boulevard
St. Louis, Missouri
63167, U.S.A.

4. Description of the proposed action:

Sometribove-zinc complex is a recombinant-derived methionyl bovine somatotropin to be marketed by Monsanto Company in the United States under the trademark name "POSILAC" as an over-the-counter new animal drug for use in healthy lactating dairy cows to increase milk production.

The production of sometribove including fermentation, purification, formulation, filling, and packaging will take place at Biochemie GmbH in Kundl, Austria. Kundl is in western Austria, approximately 35 miles NE of Innsbruck and approximately 75 miles SE of Munich. Biochemie is a subsidiary of Sandoz. The controlled-access production facility is in the village of Kundl proper with residential areas on two sides and open fields and/or highway on the remaining sides.

Address: Biochemie GmbH
Dr. Hans Bachmann Strasse 7
A6250 Kundl-Tirol
AUSTRIA

Biochemie manufactures and exports human drug products to the United States including several penicillin products. It also exports an animal drug intermediate. The facility has been inspected routinely by the Food and Drug Administration (FDA) since 1959, annually for 22 years and more recently on a bi-annual basis. In May, 1988, the FDA inspected the sometribove production facilities (fermentation, purification, and formulation) and they were found to be satisfactory. The Biochemie filling operations for sometribove-zinc complex were inspected by the FDA in October, 1990, and also were found to be satisfactory. In addition, an October, 1991, FDA environmental inspection found operations to be acceptable. The sometribove production facility was the subject of an inspection (March 3-6, 1987) by the Austrian Ministry of Health and Environment for the purpose of licensing production of sometribove at the new facility. A permit was issued in May, 1987. A second inspection was conducted by the Austrian Federal Chancery in November, 1989, to approve expanded operations at Biochemie including the addition of filling and packaging operations (product finalization) and a permit for the same was issued in April, 1990. Relevant Biochemie permits are included in Attachment 1.

Following bulk formulation, the product can also be filled into the final container (plastic, disposable syringes) and packaged at Solvay Duphar B.V., Olst, The Netherlands. Olst is located in northeastern Holland, approximately 75 miles from Amsterdam. The filling and packaging facility is surrounded by residential areas on three sides and a canal on the fourth.

Address: Solvay Duphar B.V.
Veerweg 12
8121 AA Olst
HOLLAND

Solvay Duphar is currently licensed to sell a smooth muscle relaxer (Yutopar*) in the United States. Solvay Duphar's facility at Olst has been inspected by the FDA. Sometribove filling and packaging operations were inspected by the FDA in June, 1988, and again in October, 1991, and were found to be satisfactory.

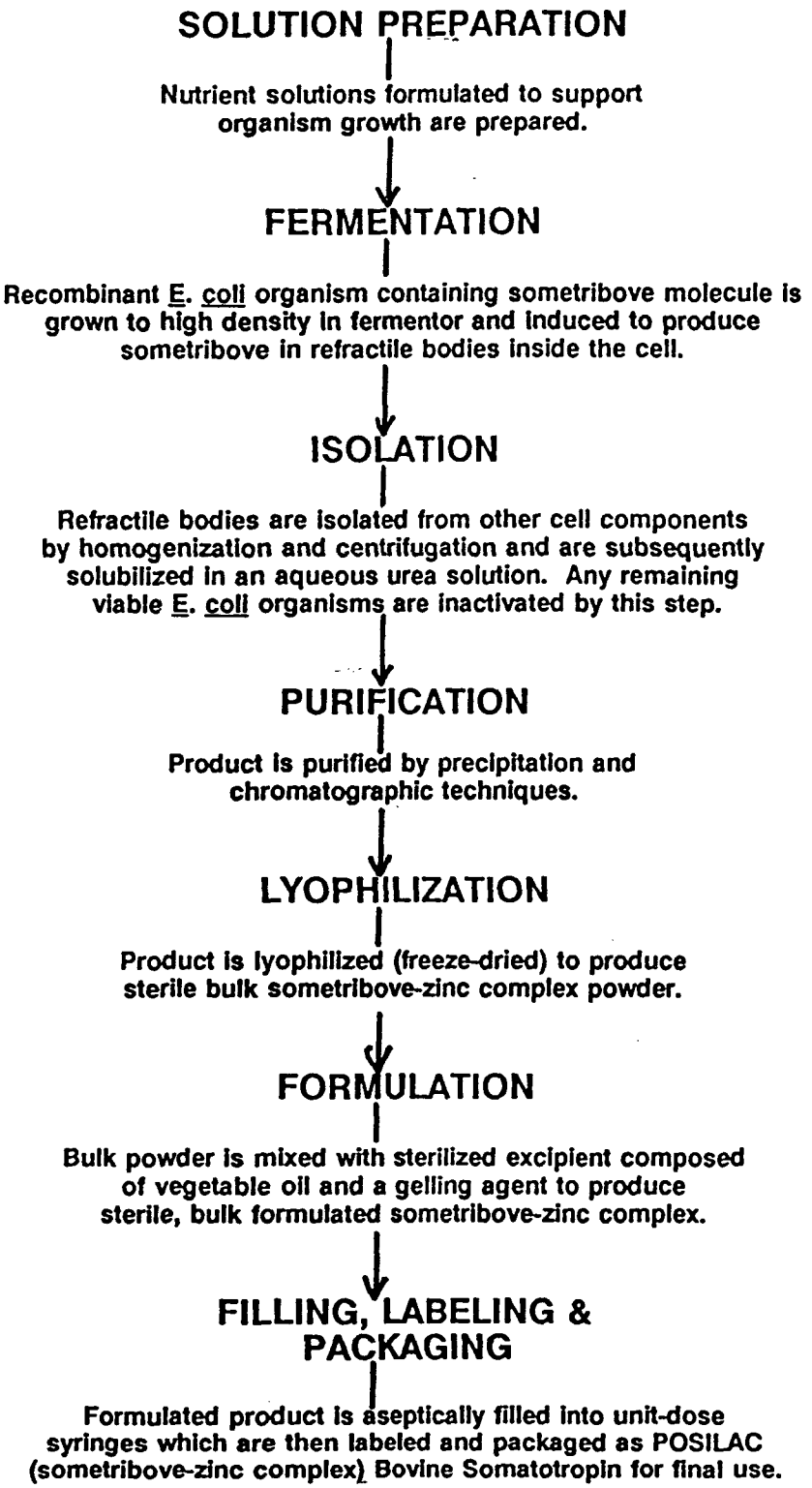
Figure 1 is a flow diagram of the sometribove-zinc complex production process.

Finished product, securely packaged, will be shipped to and stored in distribution warehouses until shipment to distributors/customers. Ultimately, sometribove will be used throughout the world at dairy facilities which, generally, are located in predominantly rural areas.

* Registered trademark, Solvay Duphar B.V.

Figure 1

# POSILAC® (SOMETRIBOVE-ZINC COMPLEX) BOVINE SOMATOTROPIN PRODUCTION FLOW DIAGRAM

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5. Identification of chemical substances that are the subject of the proposed action:

Nomenclature:

British Approved Name (BAN): sometribove

International Non-Proprietary Name (INN): sometribove

U.S. Adopted Name (USAN): sometribove

Chemical Name(s): 1-L-methionine-127-L-leucinesomatotropin (ox).

1-L-methionine-127-L-leucinegrowth hormone (ox).

N-L-methionylgrowth hormone (ox).

methionyl bovine growth hormone.

Other names: MBS - methionyl bovine somatotropin.

ZnMBS - methionyl bovine somatotropin, zinc.

BST - bovine somatotropin.

bGH; BGH - bovine growth hormone.

rBGH - recombinant bovine growth hormone.

CP-115099 - sometribove-zinc complex.

CP-104301 - sometribove without zinc.

Proprietary Name: POSILAC®

CAS Reg. No: 102744-97-8

Molecular Weight: 21,872 + 1 to 3 zinc atoms

Molecular Formula $C_{978}H_{1537}N_{265}O_{286}S_9 \bullet Zn$ (1 to 3)

Structural Formula: See Figure 2

Sometribove is a recombinant analogue of bovine pituitary somatotropin (BPS). BPS exists naturally as four molecular variants, differing only by two specific amino acid additions or substitutions.[1] Sometribove is the amino-terminal methionylated analogue of the molecular variant having LEU at position 127 and lacking ALA at the amino terminal.[2]

---

[1]Known molecular variants of bovine pituitary somatotropin*:

| 1 | 2 | 3 | | 127 | | 191 |
|---|---|---|---|---|---|---|
| ALA | PHE | PRO | . . . . . | LEU. | . . . . . | PHE |
| ALA | PHE | PRO | . . . . . | VAL. | . . . . . | PHE |
| | PHE | PRO | . . . . . | LEU. | . . . . . | PHE |
| | PHE | PRO | . . . . . | VAL. | . . . . . | PHE |

[2] Sometribove

| 1 | 2 | 3 | | 127 | | 191 |
|---|---|---|---|---|---|---|
| MET | PHE | PRO | . . . . . | LEU. | . . . . . | PHE |

* In some references PHE is designated as position 1 and LEU as 126.

ALA or MET would be designated "-1" in these instances.

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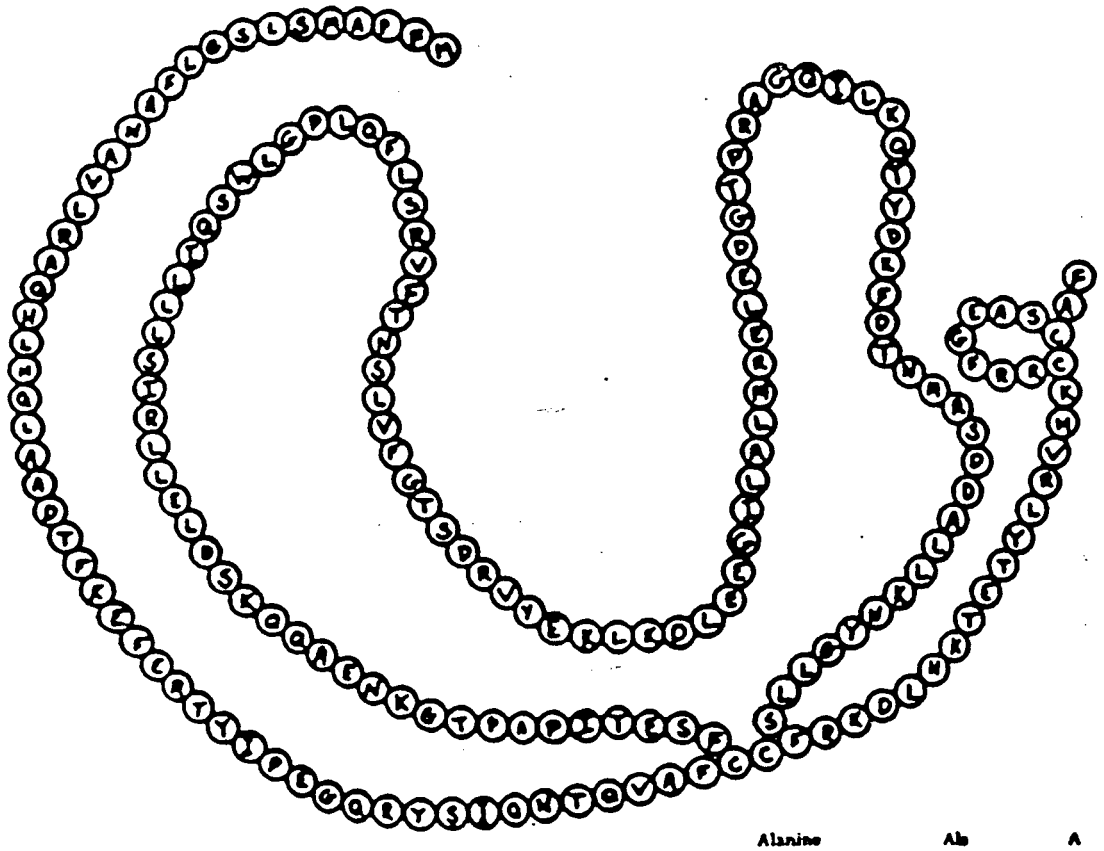
Figure 2. Sometribove Structural Formula



| | | |
|---|---|---|
| Alanine | Ala | A |
| Arginine | Arg | R |
| Asparagine | Asn | N |
| Aspartic acid | Asp | D |
| Asn and/or Asp | Asx | B |
| Cysteine | Cys | C |
| Glutamine | Gln | Q |
| Glutamic acid | Glu | E |
| Gln and/or Glu | Glx | Z |
| Glycine | Gly | G |
| Histidine | His | H |
| Isoleucine | Ile | I |
| Leucine | Leu | L |
| Lysine | Lys | K |
| Methionine | Met | M |
| Phenylalanine | Phe | F |
| Proline | Pro | P |
| Serine | Ser | S |
| Threonine | Thr | T |
| Tryptophan | Trp | W |
| Tyrosine | Tyr | Y |
| Valine | Val | V |

Physical Description/Additives: Sometribove is a white to off-white powder. The molecule complexes with 1 to 3 zinc atoms during the production process. The bulk drug substance must contain at least 92 to 104% w/w sometribove monomer by assay to be acceptable for formulation. Following formulation with pharmaceutical-grade excipient the product is a white to off-white viscous prolonged-release suspension containing 500 mg sometribove-zinc complex per dose.

Impurities: Potential impurities in sometribove may be derived from the host bacterium, fermentation media, solvents used for purification, and from chemicals or equipment used during the processing.

Since the process used to produce sometribove utilizes the procaryotic E. coli cell, adventitious agents (animal viruses, viroids, and oncogenes) which are obligate intracellular constituents only of eucaryotic cells are of minimal concern. Although fermentation media supplements derived from animal sources could be considered to be potential sources of introduction of these agents, they are heat-sterilized using validated methods so the potential for survival if accidentally introduced is minimal. The validated sterilization methods incorporate the concept of demonstrating an "over-kill" steam-sterilization process

with a minimum capability of reducing a microbial contamination level of six logs ($1 \times 10^6$) to zero viable microorganisms. The validation utilized both microbiological challenges and thermal data collection/analysis to confirm sterilization conditions. Some materials such as acids and bases used for pH adjustment are sterilized by filtration rather than heat. Others, e.g., trace metals, are heat-sterilized prior to addition to the fermentor.

Since the fermentation process employed in the production of sometribove is designed and optimized for the growth of procaryotic organisms, contamination by other procaryotes is possible. This could result in loss of the organism used to produce the biomolecule or introduction of a contaminant that might co-purify with the product. The use of a validated sterilization process prevents the introduction of unwanted procaryotes or their viruses. Any organism introduced after fermentation would not survive due to the biocidal activity of the solutions used during purification or would be removed during the final sterile filtration of the product prior to lyophilization.

Salts, trace metals, and carbon and nitrogen sources are generally water soluble and are removed during harvesting of the refractile bodies from the fermentation broth. Tetracycline is added to the fermentation broth to maintain

the presence of the plasmid in the bacterial cell. It is removed during isolation and washing of the refractile bodies. Because Biochemie manufactures penicillin products, bulk, lyophilized sometribove has been routinely analyzed for the presence of penicillin. None has been detected to date.

DNA is present for the E. coli strain used in this process both as chromosomal and plasmid DNA and is removed with other residual E. coli components during purification steps.

The purification process for sometribove effectively removes any contaminant E. coli protein.

Endotoxins are removed at several steps in the purification with final removal by a bind/release column.

Analyses of material produced at the commercial scale have demonstrated that the process effectively eliminates impurities and microbial contaminants.

6. Introduction of substances into the environment:

## Introduction of Substances into the Environment as a Result of Manufacture

### BIOCHEMIE

Sometribove will be manufactured at Biochemie for Monsanto.

Fermentation of sometribove will take place in a Biochemie facility of modern design, constructed from reinforced concrete, fireproofed, with reinforced concrete or quarry tile floors and glazed brick curtain walls where appropriate. Dedicated fermentors will be utilized for sometribove fermentations. The area around the fermentors complies with "National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules" (NIH Guidelines) for Biosafety Level 1 - Large Scale (BL1-LS) operations (Federal Register, Vol. 51, No. 88, May 7, 1986) (see Attachment 2). Procedures to minimize the probability of an accidental release of the recombinant organism are described in following sections.

Further processing of sometribove at Biochemie will take place in a separate building at Biochemie. This facility was

constructed on a concrete slab with insulated steel sides and roof and modularized interior concrete fire walls. A specially-designed stainless-steel tank wagon will transport the contents of a production fermentor from the fermentation hall to this production facility for further processing.

Emissions from the production facilities at Kundl, Austria, include gaseous emissions from fermentation, liquid emissions from fermentation, liquid emissions from the solutions used during the protein purification process, the purging of moisture and nitrogen during formulation, and waste water from the cleaning of pumps and filling equipment. Solid wastes include filter cartridges from solution preparation, precipitation sludge, resins and filter cakes, miscellaneous filters and raw materials' packaging, and rejected filling and packaging losses.

Fermentation

Fermentation is carried out under aerobic conditions with aeration controlled by valves and metering systems.

To minimize the probability of an accidental release of the recombinant organism the fermentors are pressure tested annually by the "Techn. Überwachungsverein" (Association for Technical Control). Biochemie personnel check fermentor tanks routinely to ensure that valves and cooling coils are

not leaking and that stirring gear bearings are properly adjusted. A plastic protector is installed around the flanges of the fermentor sampling valve and around the oxygen respirator pH probes to allow collection of any leaking material in a safety tub for disposal via the fermentation biowaste inactivation system.

Gaseous emissions are passed through a 0.2-micron air sterilization filter. The air discharge per fermentation batch is approximately 2000-3000 $m^3$ per hour and is composed of 0 to 16% oxygen, 0 to 5% carbon dioxide, odoriferous compounds and the remainder as normal constituents of atmospheric air. Because of the odoriferous compounds the resulting off-gas has a characteristic fermentation odor but is undetectable at or around the fermentation building. The venting of off-gas from fermentation is not subject to regulatory permits at Biochemie.

Biochemie operates two biowaste inactivation systems. One system, described below, services the fermentation plant. The other, which will be described in the Isolation section, services the sometribove-zinc complex isolation/purification facility.

The volume of discharged liquid waste from Biochemie's fermentation is estimated at 2 $m^3$/batch. This contains

residual fermentor broth, wash water, dilute caustic solution used for cleaning, and steam-sterilization condensate. The total volume is processed through a validated biowaste inactivation system before it leaves the fermentation plant. Following inactivation it is discharged to Biochemie's waste treatment facilities.

The fermentation plant biowaste inactivation system is located within the fermentation building. As described previously, at the completion of the fermentation process, vessel contents are pumped out of the fermentor tank into the tank wagon for transport to the isolation/purification facility. The small volume of broth remaining in the fermentor and tank wagon transfer line is rinsed to a sump tank. The contents of this sump tank are pumped, on level control, to one of two alternating biowaste inactivation batch tanks. Waste to be inactivated consists of viable rDNA E. coli from the fermentor broth and cells remaining in the cleaning rinse water. Following the fermentor rinse, the fermentor and drain valve are steam-sterilized and then rinsed with caustic solution. In addition, the transfer line from the fermentor to the sump tank and the sump tank which feeds the inactivation batch tanks are decontaminated by steam condensate and caustic rinse contributing more liquid to the inactivation system.

The biowaste inactivation batch tanks use direct steam injection to heat the waste liquids to sterilizing temperatures (100°C) for a prescribed length of time. The contents of the tanks are then cooled by pumping them through a heat exchanger after which they are discharged to Biochemie's waste water treatment facilities.

The fermentation equipment and biowaste inactivation system were inspected and verified as having been installed according to approved drawings and specifications. The installation inspection also verified that tanks, heat exchangers, and associated piping passed pressure leak tests and that calibration of all critical control instruments was acceptable. Data were systematically collected and evaluated as described below to confirm the validation of the equipment and the inactivation process.

The fermentation biowaste inactivation process was validated by a series of three thermocouple studies in which the temperature profile within each tank was monitored. The thermocouple studies documented that all locations within the biowaste inactivation tanks received sufficient heat to achieve considerably more than the minimum requirement of a 25-log reduction of viable E. coli.

The microbiological effectiveness of the inactivation process was validated through a series of microbiological studies. A series of 10 microbiological validation studies were conducted to evaluate the waste material from both tanks. The studies were conducted by filling the tanks with the maximum volume of untreated waste. Both the pre-sterilization rDNA E. coli population in the untreated waste and the post-sterilization population were monitored using eosin-methylene blue (lactose) agar with tetracycline to select for the tetracycline-resistant rDNA E. coli host. The testing laboratory verified the growth promotion characteristics of the media used during these studies. No viable rDNA E. coli cells were recovered from any of the post-sterilization samples analyzed. This heat treatment represented a significant reduction relative to the number of viable rDNA E. coli cells present in the untreated waste.

Pressure hold integrity testing of the vent filters servicing the waste sterilization tanks was also performed as part of the validation program. In addition, the system exhaust gas was sampled for the presence of rDNA E. coli during routine operations. No rDNA E. coli were recovered from any of the post-filter samples analyzed.

Biochemie also operates two waste water treatment plants in compliance with permits issued by regional authorities.

Treated effluents and cooling water are mixed and discharged to the River Inn. Current facilities are more than adequate to handle this liquid load.

The Biochemie fermentation equipment and plant are designed to minimize the possibility of release of rDNA E. coli. However, procedures and equipment are in place to ensure proper management of such an unlikely release.

In the event of a leak or minor spills during fermentor inoculation, fermentation, harvesting of fermentor broth, or transfer of the broth to the transfer wagon, the affected area is treated with a 0.5% peracetic acid solution, allowed to react for 10 to 15 minutes, and then flushed with water. The rinse water is collected in the biowaste collection tank and further decontaminated in the fermentation plant biowaste inactivation system.

The main fermentor is fitted with a weight control system which will trigger an alarm in the event of a sudden reduction of fermentor weight. Leaks from the fermentor, from a flange connection, for example, are collected in a pan under the fermentor and then routed to the biowaste inactivation system. In the event of a leak, repair of the damage to the system is undertaken immediately. After the

leak is repaired, the affected areas are decontaminated with a 0.5% peracetic acid solution and rinsed with water. Rinse water is disposed of through the biowaste inactivation system. If the damage cannot be immediately repaired, the fermentor contents are either decontaminated by the biowaste inactivation system (capacity of 4 $m^3$/hr) or harvested into the tank wagon and held at the tank loading station for analysis (1 to 2 hours maximum hold time). Analysis determines if further processing is practical. If further processing is practical, the tank wagon proceeds, as usual, to the isolation/purification facility. If further processing is not practical, the contents of the tank wagon are pumped back into an empty tank in the fermentation plant before being sent to the fermentation biowaste inactivation system.

Operators and supervisors must participate annually in training on the procedures described here. Training consists of reviewing each procedure and checking the equipment associated with each operation. Each participant must sign a form that is kept on file at Biochemie to document that they have participated in the training; that they understand the procedures and equipment; and that they are obligated to follow procedures in detail.

The fermentation plant is designed to contain the entire contents of the main fermentor in the event of a catastrophic accident. The biowaste inactivation system holding tanks, the heat-inactivation tanks, and fermentor operating sump are adequate to contain the entire fermentor contents. The holding tank and the operating sump are also fitted with sensors which will detect and alarm in the event of a system failure.

Procedures are in place for the proper inactivation and disposal of recombinant organisms. In the event of leaks or spills during fermentor inoculation, fermentation, or harvesting of the fermentor broth to the tank wagon the leak or spill area is disinfected with 0.5% peracetic acid solution followed by a water rinse. The waste water is collected in the holding tank and inactivation tanks for disposal through the fermentation biowaste inactivation system. Condensate from all vapor barriers that come into contact with the product is also collected and passed through the heating vessels. Leaking pumps are rinsed with water, steamed through, and then rinsed again with water.

Except for the waste water inactivation tanks all vessels that come into contact with the fermentor broth are vented through 0.2-micron sterilization filters. [The absence of vent filters for the waste water inactivation tanks was

approved by Biochemie's Institutional Biosafety Committee (IBC).] Vent filters are integrity-tested prior to installation and periodically thereafter per the filter manufacturer's recommendations. The filters are tested on an automated integrity test system for verification of acceptable pressure hold and forward flow to meet filter manufacturer's specifications. Additionally, the filter outlet gas is periodically monitored for the presence of recombinant organisms.

Before a filter casing may be opened to change filters it must be steam-sterilized. (The condensate is disposed of through the heating vessels).

Before vessels used for fermentation, intermediate storage, or culture broth can be opened they must be cleaned with water via spray heads, steam-sterilized, and rinsed again with water.

Operators must wear rubber gloves when involved in operations such as sampling which could result in product contact. Gloves are collected after use and autoclaved before disposal. Rubber boots are mandatory in operations involving the manipulation and/or disposal of broth quantities greater than laboratory scale (less than 10 liters). Boots are sprayed with a 0.5% peracetic acid

solution and rinsed with water after product broth contact. Work clothes which come in contact with product broth must be changed immediately. Protective overalls and glasses are required for handling the chemical sterilant.

The fermentation biocontainment system is designed to minimize the release of viable rDNA E. coli to the environment by filtering all fermentor exhaust gases and by thermal sterilization of liquid wastes. Fermentor exhaust gases and waste inactivation tanks are monitored routinely for the presence of the organism. The post-filtration exhaust gases from fermentation are monitored monthly either by filtering the gas through a 0.2-micron filter or by passing the gas through a fluid medium which is subsequently passed through a 0.2-micron filter. For either method, the filters are implanted on eosin-methylene blue agar that contains tetracycline (EMBT) and incubated to detect the presence of rDNA E. coli. The post-heat-treated fermentation waste stream is monitored monthly either by filtration or direct plate counts. Waste stream samples are filtered through 0.2-micron membranes which are implanted onto nutrient agar. The plates are then overlaid with EMBT media and observed for rDNA E. coli growth. If any rDNA E. coli were recovered the plant supervisor and biosafety officer must be notified and the observation is documented and

investigated. At that point, the need for corrective action is evaluated by the Institutional Biosafety Committee.

Tank Wagon

The tank wagon used to transport the fermentation broth is a baffled, heavy-duty, stainless-steel vessel equipped with a 0.2-micron sterilization filter in the tank breathing vent. The tank wagon is designed so that the tank sits in an inclined position to facilitate complete unloading. The tank is also equipped with clean-in-place (CIP) spray balls and a means to introduce steam for sterilization. During loading and unloading the tank wagon is parked in a sloped area where, in the case of a spill or leak, the contents would be contained and ultimately pumped to either the fermentation or the isolation area biowaste inactivation systems.

Procedures are in place to prevent any spill from entering the sewer system and to disinfect any tools, clothing, soil, etc., that might come in contact with fermentor broth in the event of a spill or leak during transport. Operators and supervisors must participate annually in training that includes: checking and explaining the contents of the emergency equipment carried on the transfer wagon; use of sewage system sealing cushions to prevent spills from reaching the sewer system; use of sawdust to collect spilled

broth; and the collection of contaminated sawdust for sterilization. Workers are also trained in decontamination procedures which utilize 0.5% peracetic acid solution, and in the use of appropriate clothing which includes gloves and safety goggles for all operations as well as rubber boots and apron and full visor helmet when peracetic acid is used. Training is documented by means of a signature log.

The speed of the tank wagon during transport is limited to 10 km/hour. The route to the processing facility is confined to Biochemie property with the exception of an approximate 10-meter traverse of a lightly travelled public road (Ing. Hermann Lindner-Strasse a.k.a. Alte Bundesstrasse). Transits can only take place during daytime hours. No rail traffic is allowed within the Biochemie property limits during the tank wagon transfer.

The driver, who is the only occupant of the tractor that pulls the tank wagon, must check the road worthiness of the tank wagon, examine it for leaks or damage, and confirm that the filling and emptying valves are tightly sealed prior to transport.

Two other people accompany the tank wagon on foot, one behind and one in front. They are in radio contact with the

driver and stop traffic when the tank wagon crosses the public road.

Fermentor broth leaks from the tank wagon must be reported and repaired prior to transit. If the filled tank wagon cannot be repaired the contents are pumped back into the fermentor. The emptied tank wagon is cleaned, steam-sterilized, and then made available for repair. If the leak is in a restricted, accessible location on the outside of the tank wagon the area is sprayed with disinfectant and water-washed before repair.

The tank wagon carries buckets, drain- and leak-sealing inflatable cushions, sawdust, and plastic bags to handle small leaks or spills which may occur in transit. In the event of a larger incident, 0.5% peracetic acid solution, spraying devices, plastic bags, sawdust, and protective clothing are available in the processing facility.

If a leak is detected during transit the tank wagon either returns to the fermentation building or continues to the processing facility to be emptied. The Biochemie Fire Department is notified if such an incident occurs.

Sawdust is used to absorb leaked or spilled material. The broth/sawdust is swept into plastic bags and the surfaces

from which it was collected are sprayed with disinfectant. After 10 minutes the area is rinsed with water. Employees are required to wear protective clothing and helmets with visors during cleaning operations. The contaminated sawdust is transferred to the heating tanks in the fermentation building and, after sterilization, disposed of through the waste water purification plant.

In the event of soil contamination, the area involved is sprayed with 0.5% peracetic acid solution. After one hour, soil samples from varying soil depths are taken for analysis. If live organisms are observed, the soil in the spill area is treated a second time with peracetic acid and resampled after one hour. If analysis after the second peracetic acid application shows the presence of living organisms, the affected soil is removed to a depth predetermined by analysis of core samples. Depending on the quantity removed, the contaminated soil is either autoclaved or incinerated.

Isolation

The isolation area for the manufacture of sometribove consists of homogenization, centrifugation, and urea solubilization steps. Isolation waste streams are subjected to a validated, continuous-flow biowaste inactivation system before being discharged to the waste treatment plant.

The isolation area biowaste inactivation system is located within the containment area of the isolation and purification plant. Waste streams generated during isolation of bovine somatotropin from the rDNA E. coli fermentor broth are directed to a sump tank from which they are then pumped to a collection vessel. All viable rDNA E. coli in the waste stream are destroyed by passing the waste liquid through the continuous biowaste inactivation system. The treatment conditions require the waste stream temperature to be raised to greater than 90°C and held at that temperature for not less than 60 seconds.

The continuous biowaste inactivation system was inspected and verified as having been constructed and installed in accordance with manufacturer/contractor specifications.

During the installation inspection all aspects of the biowaste inactivation system including the system piping, heat exchangers, tanks, and tank jackets were leak-tested. The system vent filter was integrity-tested as described previously.

All critical instruments were calibrated and the reliability of the automated control system (PROVOX*) was verified

* Registered trademark, Fisher Controls International, Inc.

through validation. Validation of the control hardware and software system included installation inspection of the hardware to verify the equipment was installed in an acceptable environment (temperature, relative humidity) and that all field instruments were connected to the proper channel within the PRŌVOX system.

Operation of the system was validated through rigorous testing and review of the control hardware and software against design specifications. Software code was manually checked against process specifications. This exercise was followed by single-stepping all programs to verify correct process sequencing. This included simulation of field errors and out-of-limit process conditions to ensure proper management of any out-of-limit condition. Finally, correct control software performance was verified in the automatic mode. Control system validation was completed before any rDNA E. coli were introduced into the system.

Correct performance of the biowaste inactivation system recirculation vs. feed forward to outside the containment area control interlocks was validated. Reliable function of both the PRŌVOX automated interlock and the independent hard-wire interlock was verified through repetitive testing during the validation program. The tests were conducted by reducing steam injection into the waste stream thereby

lowering the process stream temperatures and monitoring the temperature at which the waste was redirected back to the holding tank.

The duration of the high temperature exposure is regulated by control of flow rate through the system heat exchangers. Proper control of the system flow rate monitoring system and its interaction with the feed-forward vs. recirculation mode interlock were validated through field testing of the control logic. Testing was performed to document that waste streams are recirculated back through the hold tanks and not fed forward out of the containment area when the flow rates exceeded the maximum allowable rate and hence the time held at the control temperature would be less than that required.

The ability of the biowaste inactivation system to destroy rDNA E. coli in isolation waste streams was validated through microbiological testing. The pre-sterilization rDNA E. coli population in the untreated waste and the post-sterilization population were monitored using eosin-methylene blue (lactose) agar with tetracycline to select for the tetracycline-resistant rDNA E. coli host. The testing laboratory verified the growth promotion characteristics of the media used during these studies. No rDNA E. coli were recovered from any of the post-sterilization samples analyzed.

The integrity of the vent filters servicing the isolation area biowaste inactivation system also was verified as part of the validation program by pressure hold integrity testing and by microbial monitoring of the exhaust gas. The vent system exhaust gases were also sampled for the presence of rDNA E. coli during routine operations. No rDNA E. coli were recovered from any of the post-filter samples analyzed.

The liquid waste from the fermentation broth contains nutrients such as sugar, ammonia, sulfates, phosphates, buffering compounds, minerals, and vitamins required to support the growth of rDNA E. coli and which are found in standard fermentations for items such as beer and antibiotics. Viable recombinant E. coli organisms contained in either the broth or in other streams are inactivated by validated methods described above. The biowaste inactivation system is routinely sampled and analyzed for the presence of rDNA E. coli. Air samples in the biocontainment area and the area immediately adjacent are monitored for the presence of rDNA E. coli. Surfaces are tested for the organism and exhaust gases from the tank wagon and isolation-area filters are analyzed for rDNA E. coli. If any sample fails to meet acceptance criteria the plant supervisor and biosafety officer must be notified. The observation is documented and investigated, and then the

need for corrective action is evaluated by the Institutional Biosafety Committee.

The entire biocontainment area (isolation/solubilization) of the processing facility is a closed system providing minimal opportunity for operators to come into contact with the product-containing recombinant organism. Nevertheless, procedures are in place to minimize the probability of an accidental release and for the proper handling of the recombinant organism:

Equipment: Before any equipment in this area may be opened for any reason the viable rDNA *E. coli* must be reduced to a minimum by means of hot caustic solution wash and/or steam followed by water rinses.

Exhaust Air System: Tanks vent to a central line with a 0.2-micron sterilization filter to prevent organisms from entering the environment.

Cleaning: All product-carrying lines are cleaned by use of the clean-in-place (CIP) system which circulates a heated sodium hydroxide solution. This is followed by a deionized water rinse. The biocontainment CIP system includes the tank wagon, homogenizers, centrifuges, and several tanks.

Waste Water: All waste water from the CIP system and floor drains is routed to a collecting tank and then to the continuous biowaste sterilization system.

Clothing: Safety glasses must be worn in the biocontainment area. Rubber gloves must be worn for operations such as sampling where product contact might be possible. Used gloves are collected in a separate container and are autoclaved before disposal. Rubber boots must be worn if larger quantities (>10 liters) of product must be manipulated or disposed of. Boots must be sprayed with disinfectant solution (0.5% peracetic acid solution) then rinsed with water if they come in contact with product. Work clothing must be changed immediately if it comes in contact with product. Before laundering, the clothing is immersed in a heated disinfectant solution.

Laboratory Procedures: Fermentation samples are prepared and contained within a basin on the laboratory bench. The basin is disinfected after each preparation.

Work surfaces must be disinfected after each step, as spills are disinfected should they occur.

Liquid and solid wastes are collected in containers provided specifically for collection purposes. Containers of liquid and solid waste are sealed and autoclaved prior to disposal.

Mouth pipetting is forbidden. Disposable pipet tips are used, collected in bags, and autoclaved prior to disposal.

Eating, drinking, and smoking in the laboratory work area are prohibited.

Rubber gloves must be worn when handling articles that might be contaminated with the organism.

While containment procedures are expected to greatly reduce the quantity of cells released as a part of routine operations and limit the opportunity for human error and equipment failure that might result in a large accidental release it is possible, or even probable, that small numbers of the recombinant organism are released into the environment as a consequence of the production of sometribove.

In the event that viable recombinant E. coli are released to the environment, studies have shown this strain dies rapidly in water. Genetic studies indicate that even under ideal conditions the recombinant plasmid cannot be transferred to

another bacteria. Study results are discussed in greater detail in Section 7.

Purification

The purification area, where sometribove is separated and purified from other fermentation components, contains no viable recombinant organisms as they are destroyed in the isolation area. Effective rDNA E. coli kill at an earlier step in the process has been validated. The purification process steps emit no gaseous components.

A variety of solutions are used in the purification area. After separating the product from the solutions, the waste streams are either recovered, disposed of as a nitrogen source for waste treatment, or sewered.

All solid wastes are categorized, segregated, and disposed of according to Austrian law either in a landfill or by incineration. Trace-back is possible.

Formulation

The finished protein product is formulated using a pharmaceutically accepted excipient composed of a vegetable oil and gelling agent. Liquid waste products are discharged to Biochemie's waste water treatment facility and the process sewer. During formulation gaseous emissions include

water vapor and nitrogen. Solid waste product is disposed of by incineration.

Filling and Packaging

There will be no gaseous emissions at Biochemie as a result of the filling/packaging operations. Liquid emissions will include waste water from the cleaning of pumps and filling equipment. Solid wastes would include rejected filling and packaging losses.

Waste water from cleaning operations is expected to contain small amounts of product (<0.5 kg/day) and cleaning agents. Effluents (<100 liters/day) from cleaning operations flow to Biochemie's waste water treatment plant with ultimate discharge to the River Inn. Waste packaging material is rendered unusable and is disposed of in a landfill. Waste product and rejected syringes are incinerated.

Occupational Safety of the Workers/Medical Surveillance

The process developed for the production of sometribove is in compliance with the NIH Guidelines for BL1-LS operations (Attachment 2).

Appropriate hygienic practices (i.e., including but not limited to appropriate protective clothing, gloves, respiratory and eye protection, etc., procedures for handling materials of hazardous nature or in the biocontainment area, etc.) are followed in the handling of sometribove and associated chemicals to avoid exposure. If exposure were to occur, inhalation and dermal contact are expected to be the primary routes of occupational exposure to sometribove.

Sometribove is a protein and, as such, does not possess any unusual toxicologic properties which would require special handling procedures different from other proteins. Contact with the product should be avoided. Human experience indicates that repeated skin contact with product dust has infrequently produced allergic dermatitis in workers. It is well established that frequent skin contact with foreign proteins, like sometribove-zinc complex, may cause dermatitis in susceptible individuals who become sensitized to the protein (see Attachment 3). Exposure to high airborne concentrations of sometribove-zinc complex dust, associated with research and development activities conducted in the United States, has produced respiratory symptoms such as cough, sneezing, inflammation of the mucous membranes of the nose (rhinitis), and, in one individual with long-term exposure, an asthmatic reaction.

Because the final product is a sterile injectable, most of the product manufacture takes place within a closed system that affords little opportunity for worker exposure to or contact with the product itself. In those areas where product exposure might be possible workers must, in any case, wear appropriate protective clothing. An example is the aseptic areas where gloves and suits covering the body and masks covering the nose and mouth must be worn.

The following safety measures have been established to provide assurance that workers are protected during the manufacture of sometribove:

The gene for methionyl bovine somatotropin is cloned into a debilitated derivative of an E. coli plasmid. This recombinant plasmid is inserted and propagated in an E. coli K-12 host strain. This strain of E. coli K-12 is classified as a Class 1 agent as outlined in Appendix B-I-A in the NIH Guidelines: it is not considered pathogenic to man or other animals. Survival data indicate that the organism can be killed effectively by heating, drying, and disinfectants. As recommended in NIH Guidelines Appendix C-11, this strain of E. coli K-12 does not contain conjugation-proficient plasmids or generalized transducing phages. A copy of the NIH Guidelines is included as Attachment 2.

Since the volume of the culture that is used is greater than 10 liters, Biosafety Level 1-Large Scale (BL1-LS) is used for sometribove production. The NIH Guidelines require that the local Institutional Biosafety Committee be notified before initiation of a project. The project has undergone local IBC review and approval. The minutes of the first two meetings of the Biochemie IBC establishing a charter and defining the original membership are included in Attachment 4. Current (as of August, 1991) IBC members are also listed.

The facility and operations have complied with the relevant NIH Guideline recommendations in Appendix K for a BL1-LS facility and operation. The following safety features and procedures have also been established at the facility:

i) The areas around process tanks and fermentors are designed to contain a spill in the unlikely event that a complete and sudden failure might occur.

ii) Workers are required to undergo a comprehensive training program which includes an explanation of the process, the organism involved, the containment employed in the facility, Good Manufacturing Practices (GMP), and safety considerations.

iii) All operators are required to wear company-provided and -laundered uniforms and company-provided shoes while working in the facility.

iv) An environmental monitoring program is in effect. This program provides for an evaluation of the air handling capabilities of the facility and a microbial analysis of the organisms present in the facility including assays for the recombinant organism. Fermentation off-gases and the biowaste inactivation system treatment waste stream are routinely monitored for the recombinant organism.

v) Documentation of training and safety is kept.

vi) Emergency medical care including the services of a staff physician and nurse is available on site.

vii) Workers at Biochemie are required to submit to an initial medical examination. Included in the examination is a work history, general health questionnaire (with special attention to immunological diseases), urinalysis, and general physical examination. Follow-up examinations are administered every six to 36 months as deemed necessary by the physician.

A blood sample is drawn at the initial examination and the serum is aliquotted and frozen. The initial sample is to be retained for ten years. Each year following the initial sampling, new samples are taken and stored for at least three years.

viii) Special physical examinations are provided, as deemed necessary by the physician, and may include a complete blood count, sedimentation rate, serum analyses, chest X-ray, pulmonary function test, and other testing as required by the physician.

ix) All accidents and spills which occur during working hours must be reported and the company physician notified. Additionally, every non-trivial (severe or long-term) illness must be reported to the physician and the physician must be advised of medical treatments.

x) Steam lines are insulated where the risk of personnel contact exists.

xi) Every employee is advised of safety regulations before beginning a job. This safety training is repeated annually.

xii) The Austrian State of Tirol has a Department of Working Inspector which reports to the Governor.

Occupational illnesses and injuries must be reported to the Working Inspector and to Biochemie's insurance company. The Working Inspector must also be consulted when a new process is introduced to the plant. Biochemie is inspected by the Working Inspector at least once a year.

Austria observes emissions norms (TA Luft norms) established by Germany as do several other countries near Germany. Harmonization of norms within the EEC is under discussion and it is likely that the TA Luft norms will form the basis for future EEC emissions norms. The Austrian operating permit for the manufacture of sometribove at Biochemie is silent on allowable emissions. In the event of complaints or inquiries (there have been none) TA Luft norms would be used for evaluation. An allowable noise limit of 45 decibels (dbA) has been established by Austrian authorities for the Biochemie plant boundary at night. Sometribove operations do not exceed this limit.

Permits

Biochemie holds a permit issued by the Minister for Public Health and Public Services, Republic of Austria, for the production of bovine somatotropin. An additional permit from the same organization to cover expanded production and filling and packaging operations was issued in 1990. Biochemie also holds permits issued by the Tirolean State

Government for the existing waste water disposal plant and discharge to the River Inn and for expansion of that facility. A permit issued by the local administrative district of Kufstein also allows operation of the new formulation area and syringe filling and labeling operations. Applicable Biochemie permits are included in Attachment 1.

SOLVAY DUPHAR

Sometribove may also be filled into the final container and packaged at Solvay Duphar for Monsanto.

Sterile bulk formulated product is transported to Solvay Duphar from Biochemie in specially-designed sealed stainless-steel transfer containers each capable of holding one sublot of product.

There are no gaseous emissions at Solvay Duphar as a result of the filling operations. Liquid emissions include waste water from the cleaning of pumps and filling equipment. Solid wastes include rejected filling and packaging losses.

Waste water from cleaning operations contains small amounts of product (<0.5 kg/day) and cleaning agents. Effluents (<100 liters/day) from cleaning operations are discharged to

the River Ijssel. Waste packaging materials are rendered unusable and disposed of in a landfill. Waste product and rejected syringes are incinerated.

Solvay Duphar holds permits to allow the discharge of effluents to the River Ijssel and to incinerate pharmaceutical and chemical wastes as well as a general operating permit, the latter issued by the Netherlands' Secretary of State for Welfare, Human Health and Culture.

Both Biochemie and Solvay Duphar are currently in compliance with applicable emissions requirements. Approval of the New Animal Drug Application for sometribove-zinc complex (POSILAC) will have no effect on continuing compliance with current requirements.

MATERIAL SAFETY DATA SHEETS (MSDS)

Material Safety Data Sheets for the lyophilized bulk product, sometribove-zinc complex, and for the final formulated product, POSILAC® (sometribove-zinc complex) bovine somatotropin, are included as Attachment 5.

Introduction of Substances into the Environment as a Result of Use

POSILAC (sometribove-zinc complex) bovine somatotropin, after parenteral administration, can potentially enter the environment by the following routes: 1) via exhaled gases, 2) via the milk, 3) via feces, and 4) via the urine.

Sometribove has a large molecular weight (22,000 daltons) and a negligible vapor pressure. It would not be exhaled until it is broken down into $CO_2$, $H_2O$, and the other small molecular weight gaseous substances into which all proteins are ultimately degraded. Although these substances are of environmental significance, the incremental amounts released from dairy cows due to use of POSILAC will be insignificant.

In the case of milk, assays of individual milk samples from control cows have indicated that bovine somatotropin is present at levels approximately equal to the detection limit of the assay (0.3 ppb). This concentration is in general agreement with a publication by Marcek et al., 1989, who reported that the BST content of milk from untreated cattle ranges from 0.42 to 0.64 ppb. When cattle were treated with POSILAC at 1.2 times the recommended bi-weekly dosage rate of 500 mg per cow there was no increase in the BST content

of their milk. However, when cows were treated with sometribove-zinc complex at six times the recommended dosage rate, somatotropin levels in milk were significantly elevated to about 3 ppb. The elevation was noted midway through the two-week injection cycle but not at the end of the cycle. Thus, the milk levels of somatotropin approximately reflected blood levels which are higher in the middle of the injection cycle than at the end. These data indicate that, used according to label instructions, POSILAC will not elevate somatotropin levels in milk and that even in the case of a gross (six-fold) overdose, somatotropin levels in milk will be elevated only to about 3 ppb. This conclusion is also supported by the study of Marcek and co-workers referenced above. These authors noted that treatment of cattle with a daily dose of 430 mg (nominally equivalent to a bi-weekly dose of 6020 mg) increased the BST concentration in milk only to 2.56 to 3.28 ppb.

Introduction of sometribove into the environment via the feces is extremely unlikely for two reasons: 1) biliary secretion into the small intestine is unlikely for a large hydrophilic molecule such as sometribove and has in fact not been reported for any protein hormone; and 2) even if sometribove entered the gastrointestinal tract, it would be degraded very rapidly by the various proteases within the tract. <u>In vitro</u> studies presented in Section 7 demonstrate

that sometribove has a mean half-life in feces of only 1.4 hours. Thus, any sometribove which entered the gastrointestinal tract would be degraded appreciably even before the material left the cow. For these reasons, excretion of sometribove via the feces will be negligible.

Based on the human clinical literature, excretion of sometribove via the urine of dairy cows appears to be a likely possibility. For example, Hourd and Edwards (1989) showed increased urinary output of human somatotropin following a bolus injection of somatotropin which elevated serum levels nearly 100-fold, and Albini et al. (1989) reported a correlation, based on a relatively narrow range of values, between serum and urinary hormone levels in healthy children.

The following study, which is the subject of a proprietary Monsanto Technical Report, was done to test whether dairy cows, like humans, excrete immunoassayable somatotropin in their urine and to determine if the amount excreted is affected by treatment with POSILAC. Six cattle were treated every two weeks with sometribove-zinc complex as per label instructions for a total of three treatment cycles. Four cows served as controls. The following measurements were made during the third two-week treatment cycle: 1) daily milk production; 2) daily urine output (the urine was

obtained via a chronic indwelling catheter);
3) concentration of immunoassayable somatotropin in urine; and 4) concentration of immunoassayable somatotropin in blood based on daily bleeding.

The results demonstrated that POSILAC increased milk production by 4.3 kg per day and elevated somatotropin levels in the blood about three-fold. These changes are within the ranges defined by previous experiments measuring POSILAC's effects on milk production and on somatotropin levels in blood. In spite of the elevated blood levels, the concentration of somatotropin in the urine was unchanged. Both the control cows and the treated cows had a mean concentration of 2.7 ng/ml. POSILAC treatment increased the volume of urine excreted. Treated cows excreted a mean volume of 22.6 L per day while control cows excreted a mean volume of 18.5 L per day. Although this difference was significant ($p < .05$), the amount of urine excreted by treated cows was well within the range reported in the literature of 17 to 45 ml/kg per day, which for 600 kg cows, as were used in this experiment, corresponds to 10.2 L to 27 L per day (Gans and Mercer, 1977). In spite of the increased volume of urine, the total amount of somatotropin excreted by treated cows was not significantly different from that excreted by control cows ($p > .17$). Based on these data, it is concluded that treatment with POSILAC affects

neither the concentration nor the total amount of somatotropin excreted in urine. This conclusion appears to be at variance with the human clinical data reported above. However, it should be noted that in those cases in which urinary output of somatotropin was convincingly increased, the corresponding blood levels were elevated considerably more than the two- to three-fold commonly seen in cows treated with sometribove. There are, in fact, no human clinical data which demonstrate that a two- to three-fold elevation in circulating somatotropin levels results in an increased urinary output of somatotropin.

It was shown in this same study that most of the immunoreactive bovine somatotropin in cow urine is partially degraded. When urine from either treated or control animals was filtered through a membrane which permitted passage of proteins with molecular weights of less than 10,000 daltons, approximately 70% of the immunoreactive material passed through the filter. Since the molecular weight of intact bovine somatotropin is approximately 22,000 daltons, these data indicate that the majority of immunoreactive BST in cow urine is partially degraded.

Introduction of Substances into the Environment as a Result of Disposal

Substances potentially might enter the environment as a result of the disposal of unused product or through the disposal of expended syringes still containing small quantities of POSILAC bovine somatotropin. To mitigate these possibilities, the following instructions are provided in the package insert:

> "Used syringes and needles and syringes containing POSILAC should be placed in a leak-resistant, puncture-resistant container for disposal in accordance with applicable Federal, state, and local regulations."

In addition, Monsanto has entered into an agreement with Browning-Ferris Industries (BFI) to provide dairy farmers with a complete sharps waste management program. Monsanto will provide customers with sharps mail-back kits consisting of appropriate collection containers and packaging which will comply with U.S. Postal regulations and allow dairymen to mail spent POSILAC syringes and needles to a BFI medical waste treatment facility where the containers and contents will be destroyed by incineration or by autoclaving and shredding.

BFI will provide the appropriate manifest and shipping documents to assist the customer with compliance with applicable regulations.

Receipt of a filled container sent by a customer to BFI for destruction will automatically result in dispatch of a replacement kit to that customer so that collection containers will always be available for the safe disposal of used sharps.

This program will be provided without charge to the customer and is intended only for disposal of POSILAC syringes and needles. Other veterinary medical wastes and/or hazardous materials are not to be included in the containers sent for disposal.

At this writing, several states (Maine, Missouri, South Carolina, and Virginia) do not permit the U.S. Postal Service to transport medical waste claiming that only state-registered trash haulers may transport waste material and that the Postal Service is not so licensed. Federal regulations, however, permit the mailing of used sharps and other used medical devices upon compliance with restrictions designed to achieve safe transmission (Federal Register, Vol. 57, No. 126, June 30, 1992). The Postal Service has concluded that the mail-in service is an appropriate use

of the postal system and that any state seeking to preclude prospective shippers from having sharps transported by mail because the Postal Service is not licensed in that state would put that state in the position of interfering with the operation of the national postal system. The U.S. Postal Service believes that Federal law, in this instance, preempts state regulations.

7. Fate of emitted substances:

Fate of Substances Emitted as a Result of Manufacture

As noted in Section 6, the fermentation and isolation operations at Biochemie have been designed, engineered, and validated to minimize the possibility of release of viable rDNA E. coli. Nevertheless, procedures, also described in Section 6, are in place to handle a spill or accidental release should either occur.

Monsanto undertook a study to determine the environmental impact of an accidental release into water of the recombinant E. coli K-12 strain used in the manufacture of sometribove-zinc complex. The report of this study (Attachment 6) describes the fate of E. coli K-12 strains W3110G [pBGH1], LBB269, and LBB269 [pBGH1] in microcosms containing Missouri River water. In April, 1992, this study was inspected by FDA and was found to have been conducted in compliance with current Good Laboratory Practice regulations (cGLPs).

The primary objective of the study was to determine the environmental impact of an accidental release into water of the recombinant Escherichia coli K-12 strain W3110G [pBGH1], the microorganism used in the production of sometribove. A number of systems have been designed at the manufacturing

facility to prevent the escape of this organism. Despite these safeguards, it is important to determine the fate of W3110G [pBGH1] in environments outside the production plant. If this recombinant organism were released from containment into the environment, one possible area of contamination would be surface waters such as streams or lakes.

Although water contains modest levels of nutrients, it also contains a variety of microorganisms that are adapted to survival in such an environment. This competitive environment is not suitable for E. coli K-12 strains. The study report documents the fate of W3110G [pBGH1] and its nalidixic acid-resistant derivative LBB269 ± pBGH1 inoculated into samples of non-sterile water obtained from the Missouri River near where it passes the Monsanto Research Center site at Chesterfield, Missouri. Strain LBB269 was made in order to facilitate tracking of a plasmid-free E. coli strain in an environment populated with other species of microorganisms. Strain LBB269 was transformed with pBGH1 to yield a nearly isogenic pair of strains, differing only in that one carried pBGH1 while the other did not. Thus, the effect of the plasmid pBGH1 on the survival of the LBB269 strain could also be examined in this environment.

Water samples were inoculated with strain W3110G [pBGH1], LBB269, and LBB269 [pBGH1], and incubated at 26°±2°C. The number of viable colony-forming units (cfu) was measured in each microcosm as a function of time. For each of these E. coli strains, the cfu declined from an initial level of about $1X10^7$ cfu per ml to less than $1.5X10^2$ cfu in eight days. The presence of the plasmid pBGH1 in LBB269 did not affect the survival of that strain, and there were no significant differences between strains W3110G [pBGH1] and the nalidixic acid-marked strain LBB269 [pBGH1]. The loss of viable cfu for each of these strains demonstrated that these E. coli strains did not survive in an environmental source of water such as the Missouri River in detectable numbers for longer than eight days.

The advent of modern recombinant DNA techniques has resulted in the increased use of engineered organisms for the production of proteins and other types of products. Most commonly, such systems utilize E. coli K-12 strains containing recombinant plasmids derived from pBR322. The plasmid pBR322 is one of the most widely used cloning vehicles because of the extensive body of information on its structure and function. pBR322 consists of three distinct segments: (i) a gene that encodes for tetracycline resistance, (ii) a gene that encodes for ampicillin resistance, and (iii) an origin of DNA replication.

Typically, commercial production of proteins from recombinant E. coli strains involves large-scale fermentation of the production strain. The prospect of up to $1X10^{17}$ (or more) recombinant E. coli cells being inadvertently released into the environment during some type of catastrophic industrial accident has generated considerable interest in the determination of the likely consequences of such an event. Interest has centered on whether recombinant E. coli can survive in natural environments and whether or not the plasmid it contains can be transferred to indigenous inhabitants of these environments. Attachment 7 is a review of the scientific literature prepared by Drs. G. Bogosian and J.F. Kane for this environmental assessment and subsequently published (Advances in Applied Microbiology, Vol. 36, 1991) that deals with the survival of E. coli K-12 strains containing recombinant plasmids derived from pBR322 and the potential to transfer such plasmids to other organisms in the environment. Studies involving transfer from other species of organisms or of other types of plasmids are included when considered pertinent. The environments covered in the review include soil, water, sewage, and the mammalian intestinal tract. Studies reviewed in this report demonstrate that strains of E. coli K-12 did not persist in non-sterile water, soil, sewage, or the conventional mammalian intestinal tract. The studies indicated maximum survival

times of approximately 15 days in water, 20 days in soil, and about 10 days in sewage. It was observed that strains of E. coli were unable to adhere to mammalian intestinal cells and did not colonize the conventional mammalian intestinal tract; they were cleared in three to six days from conventional human and mouse intestines. E. coli K-12 strains were able to colonize germ-free or antibiotic-treated rats and mice, but not antibiotic-treated humans. The presence of conjugative and nonconjugative plasmids was observed to impart an additional disadvantage to E. coli K-12 strains in these environments, particularly in the mammalian intestinal tract.

For the conjugational transfer of pBR322, or a derivative of pBR322, several conditions must be met. The pBR322 plasmid must have intact *bom* and *nic* sites; a derivative such as pBR327, which lacks these two sites, is unable to be transferred. The pBR322 plasmid must be in a donor strain which also contains both a conjugative plasmid and a plasmid which can provide the four *mob* gene products necessary for transfer; in the absence of the *mob* gene products, a few studies have demonstrated the low-frequency transfer of portions of pBR322 plasmids as cointegrates with conjugative R factors. The donor strain must be able to not only survive, but actually to multiply, in the environment in which the transfer is to occur; conjugation does not occur

in the absence of growth. The donor strain must retain the pBR322 plasmid; in the absence of selective pressure, pBR322 is rapidly lost from host E. coli K-12 strains. The conjugative transfer of pBR322 or derivatives of pBR322 from strains of E. coli K-12 to indigenous inhabitants of water, soil, sewage, or the mammalian intestinal tract, in their natural environment, has never been demonstrated. Given the stringent conditions required, coupled with the limitations of pBR322 and E. coli K-12 strains, this is not an unexpected result. Particularly clear is the observation that E. coli K-12 strains do not survive in these environments. These results are reassuring from the point of view of using recombinant E. coli K-12 strains in large-scale fermentations. Negative results such as these do not mean, however, that there is absolute certainty that transfer of the plasmid to indigenous inhabitants of the release site will not occur in the event of an accidental release of a recombinant E. coli K-12 strain.

The Bogosian/Kane review addresses conjugation as a means of transferring genetic information more extensively than it addresses transduction or transformation. In a subsequent review (Attachment 8) Dr. J.F. Kane notes that conjugation is by far the best studied means of transferring genetic information. Transformation is viewed as a less likely route because the process is selective to both donor DNA and

recipient. The DNA of bacterial systems would not have DNA homologous to that of the gene for bovine somatotropin. Likewise, transduction was not considered a probable pathway for transfer of genetic information since this mechanism is mediated by certain phages and several factors limiting the frequency of transduction in the natural environment: 1) phages will have some limits in available host cells including one or only a few species of bacteria; 2) bacteria have DNA restriction modification systems which may protect them from infecting phages; 3) dilution of phages in the environment may reduce to undetectable levels the probability of transduction; and 4) phages in the environment have only a finite lifetime. In addition, it should be noted that for all of the gene transfer studies discussed in the review article as well as the Monsanto gene transfer study discussed below, detection methods were employed which could measure gene transfer regardless of mechanism.

Finally, while it is acknowledged that gene transfer is a relatively rare event dependent upon the probability of the various components of the gene transfer system encountering each other, Monsanto carried out a study of the potential for gene transfer from E. coli strain W3110G [pBGH1] to indigenous bacteria in Missouri River water. The purpose of the study was to determine if gene transfer between E. coli

strain W3110G [pBGH1], the microorganism used in the production of sometribove, and the indigenous microbial populations found in the Missouri River occurs in a laboratory microcosm. The test for such an occurrence involves the detection of DNA containing the BST structural gene in the indigenous microbes isolated from Missouri River water that had been inoculated with E. coli strain W3110G [pBGH1]. DNA from the indigenous microbes is examined for the BST structural gene using the polymerase chain reaction (PCR) assay. The presence of a DNA fragment with the size and restriction pattern of the BST structural gene is taken as evidence of gene transfer from the production organism to indigenous microbes. This study also was inspected by FDA in April, 1992, and was found to have been conducted in compliance with cGLPs.

In published accounts of studies simulating an accidental release of genetically engineered E. coli strain K-12 into the environment there are no reports of the transfer of DNA from the recombinant organism to indigenous microbial populations (see Attachment 7). Even in laboratory studies under optimum conditions the frequency with which a non-conjugative plasmid is transferred is on the order of $1X10^{-6}$ or less. Therefore, to assess gene transfer from E. coli W3110G [pBGH1] to indigenous microorganisms present in Missouri River water, it was necessary to amplify the number

of potential recipients. It has been established that viable colony-forming units (cfu) of E. coli W3110G [pBGH1] fall below the limit of detection after about eight days in a microcosm containing Missouri River water. Therefore, the indigenous microbes free of E. coli W3110G [pBGH1] can be isolated by plating aliquots from the microcosm on a solid medium after the E. coli W3110G [pBHG1] have dropped below the detectable limit. The indigenous colonies are scraped from the surface of the plate and plasmid DNA is extracted for analysis. By growing the cells on the solid medium, the putative recipient(s) containing DNA with the BST structural gene reach large numbers by forming colonies on the agar. Furthermore, extracting the DNA and subjecting it to PCR further amplifies the specific DNA fragment with the BST structural gene.

The study report (Attachment 9) describes experiments designed to measure the transfer of the plasmid pBGH1, which carries the gene for bovine somatotropin, from E. coli K-12 strain W3110G [pBGH1] to indigenous microorganisms present in microcosms containing Missouri River water. Water samples were inoculated with strains W3110G [pBGH1] and LBB269 and incubated at 26°± 2°C. These are the same microcosms used for the water study described previously.

After 21 days of incubation the number of viable colony forming units of E. coli K-12 strains W3110G [pBHG1] and LBB269 was reduced from an initial level of about $1X10^7$ cfu per ml to less than 1 cfu per ml. This represents over a billion-fold drop in viable colony forming units of the E. coli strains. At this time indigenous microbes resistant to both ampicillin and tetracycline were isolated from 100 ml of water from each of the microcosms inoculated with either strain W3110G [pBGH1] or LBB269. Plasmid DNA was isolated from these organisms and examined for sequences containing the gene for bovine somatotropin from pBGH1, using the polymerase chain reaction (PCR) assay.

Strain LBB269 is a nalidixic acid-resistant derivative of W3110G which was used as a plasmidless control strain in these studies.

The microcosm containing LBB269 [pBGH1] that was used in the water study was not used in the PCR assays because this strain is not used in the commercial production of sometribove. Nevertheless, this microcosm served an important function in the design of the study. The possibility of false positive results was a primary concern with a technique so sensitive as the PCR assay. The microcosm containing LBB269 [pBGH1] was used to ensure that the number of viable E. coli cells had fallen below the

level of 1 cfu in 100 ml of water. Data from the water viability study described previously indicated that cells were disappearing at similar rates for both LBB269 [pBGH1] and W3110G [pBGH1]. On days 15 and 21 the microcosm containing LBB269 [pBGH1] was sampled to ensure that no E. coli could be seen in 100 ml of water. This also allowed the microcosm containing W3110G [pBGH1] to be saved for the gene transfer study.

The microcosm inoculated with LBB269 was used as the negative control for this study so there was no need to use the uninolulated flasks of Missouri River water that were used in the water viability study for this purpose. The uninoculated microcosm was used, however, for the PCR experiment in the determination of assay sensitivity.

The sensitivity of the PCR method was determined by adding known numbers of W3110G [pBGH1] to known numbers of indigenous microorganisms resistant to ampicillin and tetracycline. The estimated sensitivity of this method was such that it could detect 1 cfu of E. coli per $1.5X10^7$ cfu of indigenous microorganisms.

On day 21 of the study the three flasks of Missouri River water that had been inoculated with W3110G [pBGH1] contained an average of $2.7X10^5$ cfu of indigenous microorganisms per

ml. For each sample in this study, all of the organisms in 100 ml of water, $2.7X10^7$ cfu, were examined by a two-step screening process involving first isolating microorganisms resistant to ampicillin and tetracycline and then isolating DNA from those microorganisms and subjecting it to a PCR assay.

Indigenous microrganisms resistant to both ampicillin and tetracycline were isolated as follows. On day 21, samples (100 ml total volume) were filtered through Whatman filter paper to remove large particulate matter. The filtrate was subequently filtered through 0.45-$\mu$m membranes to retain the indigenous microbes. These filters were placed on EMB media containing nalidixic acid or tetracycline + ampicillin to score for the inoculated strains of E. coli. After incubation the filters were examined for E. coli. The number of antibiotic-resistant indigenous microbes were estimated on day 21 of the study to be 6 to 8 cfu per ml of water. No E. coli colonies were present.

As expected, the day 0 sample from the microcosm inoculated with E. coli K-12 strain W3110G [pBGH1] gave a positive PCR response and the day 0 sample from the microcosm inoculated with E. coli strain LBB269 gave a negative PCR response. All of the day 21 samples containing indigenous microbes isolated from microcosms that were inoculated with either

W3110G [pBGH1] or LBB269 were negative in the PCR assay indicating that the target sequence from pBGH1 was not present in any of these indigenous microorganisms. These results indicate that pBGH1 had not been transferred from W3110G [pBGH1] to indigenous microbial inhabitants of the Missouri River water microcosms during this study.

The four filters used to generate each of the samples in the experimental PCR assays only contained a total of 800 to 1000 colonies. This is far fewer than the limit of sensitivity for this assay, as determined above. The negative result of the gene transfer study performed on these samples indicates that the plasmid pBGH1, or the portion of pBGH1 including the BST structural gene, was not transferred from the E. coli K-12 strain W3110G [pBGH1] to indigenous microorganisms in a microcosm of Missouri River water.

It cannot be said absolutely that gene transfer events involving W3110G [pBGH1] can never occur. Nevertheless, using a two-step gene transfer assay which could detect a single positive recipient cell among 27 million cells, this study was unable to demonstrate gene transfer from the recombinant E. coli to indigenous microbes. The absence of gene transfer in this study demonstrates that such an event

occurred, if at all, at a frequency of less than 1 in $2.7X10^7$ in these microcosms.

Fate of Substances Emitted as a Result of Use and/or Disposal

As described in Section 6, bovine somatotropin enters the environment via the urine of dairy cattle, albeit in a concentration and total amount which are not increased by treatment with POSILAC bovine somatotropin. BST can also enter the environment via milk but at a concentration which is approximately 10-fold less than that in urine. And, as was true for urine, the concentration of BST in milk is not increased by treatment with POSILAC. Finally, there is the opportunity for BST to enter the environment as a result of the improper disposal of unused product or expended administration devices. Whether via urine, via milk, or as a result of improper disposal, BST can potentially enter both aquatic and terrestrial environmental compartments, and thus, the following analyses will emphasize these two compartments. The atmospheric environmental compartment is not of concern because sometribove is a large molecule with negligible vapor pressure. It will enter the atmosphere only after it has been degraded into $H_2O$, $CO_2$ and the other simple volatile molecules into which all proteins are ultimately degraded.

Microorganisms and their associated proteases are ubiquitous in both the terrestrial and aquatic compartments. Since sometribove has no special characteristics which might be expected to render it resistant to proteolytic attack, it can be predicted that any sometribove entering the aquatic and terrestrial ecosystems will be degraded into small peptides and free amino acids by proteases present in the particular ecosystem.

As discussed in Attachment 10, the endopeptidases trypsin, chymotrypsin, and pepsin provide examples of the types of proteolytic attack to which sometribove might be subjected. In the case of trypsin, which preferentially cleaves on the carboxyl side of arginine and lysine, there are 24 potential cleavage points within sometribove. In the case of chymotrypsin which preferentially cleaves on the carboxyl side of large hydrophobic amino acids such as tryptophan, tyrosine, and phenylalanine, there are 19 potential cleavage sites; and in the case of pepsin which preferentially cleaves on the carboxyl side of phenylalanine, tryptophan, leucine, and methionine, there are 44 potential cleavage sites. In addition to these endopeptidases, sometribove (and peptides derived from sometribove) would be subject to hydrolysis by exopeptidases which cleave one amino acid at a time from either the carboxyl or amino terminal end.

As noted above and as discussed in Attachment 10, most of the proteases to which sometribove would be exposed in the environment would be associated with microorganisms. Among the many microbial proteases to which sometribove might be exposed are subtilisn, from Bacillus subtilis, which cleaves on the carboxyl side of aspartate, glutamate, glycine, and valine; thermolysin, from Bacillus subtilis, which cleaves the amino side of isoleucine, valine, leucine phenylalanine, tyrosine, methionine, and alanine; V-8 protease, from Staphylococcus aureus, which cleaves on the carboxyl side of aspartate and glutamate; and pronase, a mixture of proteases from Streptomyces griseus, which has both endo- and exopeptidase activities.

Based on these considerations, it can be predicted that sometribove will be relatively unstable in both aquatic and terrestrial environmental compartments and that the degree of instability will generally reflect the abundance of microorganisms and their proteases. The series of experiments described below confirm these predictions.

The experiments were designed to measure the stability of sometribove when incubated in the presence of material obtained from various aquatic and terrestrial environmental compartments. Feces, representing a dung pat, and pasture soil are two terrestrial environments which could come into

contact with sometribove. Oxidation lagoon water from the waste treatment facility at Monsanto's dairy research center and pond water represent two extremely different types of aquatic environment which could be exposed to sometribove. Stability was determined by incubating sometribove that had been radiolabeled with iodine-125 with each of these materials. At various times, samples were taken from the reaction mixtures and were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) which separates proteins based on their molecular weight. After electrophoresis was completed and the proteins separated, the gel was sliced and each slice (containing proteins of only a limited range of molecular weights) was analyzed for its content of radiolabel. In the absence of any degradation, virtually all label was detected in the region of the gel where one would expect to find intact sometribove, which has a molecular weight of 22,000 daltons. When degradation occurred, the radiolabel disappeared from this region of the gel and the rate of disappearance was used to evaluate the half-lives of sometribove under the various conditions. The radiolabel lost from the intact sometribove region of the gel either appeared as new bands in the reduced-molecular weight regions of the gel or was lost from the gel entirely. Since proteins smaller than 10,000 daltons are lost from SDS-PAGE gels during

electrophoresis, loss of radiolabel from the gels is indicative of digestion to small fragments.

Table 1 below summarizes the results of these studies. The observations that sometribove degraded most rapidly in feces, less rapidly in lagoon water and soil, and least rapidly in pond water, are consistent with the proposal that the instability of sometribove in an environmental niche is approximately proportional to the abundance of micro-organisms in that niche.

**TABLE 1**

| Incubation Matrix | Half-Life of Sometribove (Hours) (Mean + SE) |
|---|---|
| Feces | 1.4 ± 0.7 |
| Pond Water | 18.3 ± 1.5 |
| Lagoon Water | 4.0 ± 1.0 |
| Pasture Soil | 4.7 ± 2.4 |

In the case of feces, the mean half-life was only 1.4 hours. As discussed in Section 6, physiological considerations indicate that sometribove is very unlikely to be excreted in the feces. The half-life data above indicate that even in the unlikely event that small quantities of sometribove are excreted via the feces, it would be degraded shortly after, or possibly even before the feces have left the cow. Of the four environmental samples tested, sometribove was most stable in the presence of pond water (mean half-life of 18.3

hours). Even in this case, 9.2 half-lives would elapse in a one-week period and thus, at the end of the week less than 0.2% of the sometribove present at the start of the week would still be present.

These considerations indicate that sometribove, by virtue of its sensitivity to proteases, is unstable in the environment. As a result, it will neither persist nor accumulate in either the terrestrial or the aquatic environmental compartments.

8. Environmental effects of released substances:

Potential Effects of Substances Released as a Result of Manufacturing

As discussed in Sections 6 and 7, the fermentation and isolation operations at the manufacturing facility in Austria have been designed, engineered, and validated to minimize the possibility of release of viable rDNA E. coli and procedures are in place to handle a spill or accidental release should either occur. Typically, however, commercial production of rDNA-derived proteins such as sometribove involves large-scale fermentation of the production organism and the consequences of a catastrophic accident must be considered. Considerable interest has been generated as to whether recombinant E. coli can survive in natural environments and whether or not the plasmid might be transferred to indigenous organisms in the environment.

The E. coli K-12 strain has been widely employed in research by microbiologists since the early 1950s and is considered by scientists to be the most well-understood organism in the world. In fact, the National Institutes of Health "Guidelines for Research Involving Recombinant DNA Molecules" (Federal Register, Vol. 51, No. 88, May 7, 1986) (Attachment 2) state the following in Appendix C-II on page 16969:

"Experiments which use E. coli K-12 host-vector systems.....are exempt from these guidelines provided that: (i) the E. coli host shall not contain conjugation proficient plasmids or generalized transducing phages; and (ii) lambda or lambdoid or Ff bacteriophages or nonconjugative plasmids [2] shall be used as vectors."

and in Appendix C-VI on page 16970, footnote 2:

"A subset of non-conjugative plasmid vectors are also poorly mobilized [e.g., pBR322, pBR313]. Where practical, these vectors should be employed."

A review of scientific literature summarized in Section 7 demonstates that strains of E. coli K-12 did not persist in non-sterile water, soil, sewage, or the conventional mammalian intestinal tract. The studies reviewed indicated maximum survival times of approximately 15 days in water, 20 days in soil, and about 10 days in sewage. Strains of E. coli were unable to adhere to mammalian intestinal cells and did not colonize the conventional mammalian intestinal tract, clearing in three to six days from human and mouse intestines.

Also, as described in Section 7, a Monsanto study conducted in compliance with cGLPs looked at the potential effects of an accidental release into water of the recombinant E. coli K-12 strain W3110G [pBGH1] which is a derivative of pBR322 and is used to manufacture sometribove. The results of the study reenforced literature reports that E. coli K-12 strains will not persist if released into non-sterile water. It was determined by the Monsanto study that populations of E. coli K-12 strains W3110G [pBGH1], LBB269, and LBB269 [pBGH1] inoculated into non-sterile Missouri River water declined by over 10,000-fold in eight days at 26°C and over a billion-fold in 15 days. There was no significant difference in rate terms among these three strains. These results are interpreted to mean that the E. coli K-12 production strain will probably not survive in detectable numbers for longer than eight days in environmental sources of water such as the Missouri River.

The literature review described in Section 7 also examined the possibility of conjugative transfer of genetic material from E. coli strains containing recombinant plasmids derived from pBR322. For such a genetic transfer to occur, the donor strain must be able not only to survive but to multiply in the environment in which the transfer is to occur since conjugation does not occur in the absence of growth. The donor strain must be able to retain the pBR322 plasmid. In

the absence of selective pressure, however, pBR322 is rapidly lost from the host E. coli K-12 strains. The conjugative transfer of pBR322 or its derivatives from strains of E. coli K-12 to indigenous inhabitants of water, soil, sewage, or the mammalian intestinal tract in their natural environment has not been demonstrated.

In addition to conjugation as a means of transferring genetic information the possibility of genetic transfer via transformation or transduction was also reviewed in Section 7. These routes are considered to be much less likely means for transferring genetic material than would conjugation because of the specific circumstances necessary for successful transfer.

Finally, a second Monsanto GLP study summarized in Section 7 was designed and carried out to determine if genetic transfer between the sometribove production organism and indigenous microbial populations in Missouri River water occurs in a laboratory microcosm. The study involved detection of DNA containing the BST structural gene in indigenous microbes isolated from Missouri River water inoculated with E. coli strain W3110G [pBGH1] using a polymerase chain reaction (PCR) assay. Using this method which could detect a single positive recipient among 27 million cells this study was unable to demonstrate gene

transfer from the recombinant E. coli K-12 strain W3110G [pBGH1] to indigenous microbes. While it cannot be said absolutely that gene transfer involving this organism can never occur the study demonstrates that such an event occurred, if at all, at a frequency of less than 1 in $2.7X10^7$ of these microcosms.

In summary, in the unlikely event of a release of the recombinant E. coli K-12 strain to the environment it has been established that the organism can persist for a relatively short length of time and it is highly unlikely that it might transfer the plasmid containing the BST structural gene to indigenous organisms.

Potential Effects of Substances Released as a Result of Use and/or Disposal

As shown in Section 7, any sometribove entering the environment via the milk or urine of dairy cows or as a result of improper disposal will be unstable and thus will be unlikely to have any detectable impact on the environment. The following analysis supports the environmental safety of POSILAC bovine somatotropin by demonstrating that BST has growth-stimulating effects in only a few species and that even in those species in which it is active, it is active only at doses far greater than any likely to be achieved in the environment. It should

also be noted that in those species in which BST has been tested and had an effect, most times it was administered by the parenteral route. That this route was almost invariably chosen doubtlessly reflects the fact that most investigators have not expected BST to be efficiently absorbed if given by any route other than parenteral.

In the case of humans, bovine somatotropin has been found to be inactive even when given via the parenteral route at doses in excess of 5 mg/kg (Bennett et al., 1950; Kinsell et al., 1954; Bondy, 1954).

For primates other than man, BST has also been found to be inactive even when infused at doses as high as 20 mg/kg (Knobil and Greep, 1959). BST is active in rats and, indeed, this species serves as the basis of several laboratory assays of BST bioactivity. As an example, Seaman and co-workers (1988) showed that daily injection of BST into hypophysectomized female rats at daily doses of between 150 and 600 mcg/kg stimulated body weight gain. These authors also showed that oral doses of up to 40 mg/kg per day were without effect. These data indicate that BST injected is more than 260-fold more active than BST given orally and thereby support the choice, by most investigators, of parenteral administration.

In the case of domestic chickens, King and Scanes (1986) found that BST injected daily into hypophysectomized chickens at a dose of 0.5 mg/kg per day for 18 days stimulated increase in body weight gain and in bone growth.

In a more recent study, Buonomo and Baile (1988) treated female broiler chickens with BST at daily doses of 0.5 and 2.5 mg/kg, administered subcutaneously. Growth was stimulated by both doses during the first week of treatment, but the effect was attenuated during the second week of treatment, apparently because of the development of an immune response directed against the heterologous somatotropin.

For green sea turtles, injection of 3.52 mg per turtle (average weight of 32.05 kg.) every three weeks stimulated growth as measured by weight gain and increased carapace length (Owens et al., 1979).

In the case of carp, injection weekly with doses of 12.5 to 100 mg/kg stimulated growth as measured by increases in both length and weight. (Adelman, 1977).

For American eels, BST has been found to be orally active. When fed to elvers at doses of 2 or 10 ppm in the diet, BST increased weight gain and improved food conversion (Degani

and Gallagher, 1985). Based on the body weight and feed conversion data provided by these authors, it can be calculated that 2 ppm corresponds to a dose of approximately 0.95 mg/kg per 10 days or 0.667 mg/kg per week.

The effects of BST in coho salmon have been the subject of considerable research. In 1975, Higgs and co-workers found that BST stimulated growth of yearlings when given by intraperitoneal injection three times per week at a total dose of 10 or 100 mg/kg per week. These results were confirmed by this group in 1976. (Higgs et al.)

Markert et al. (1977) also noted that BST improved feed conversion as well as growth in coho salmon when given at a weekly dose of 10 mg/kg. These results were confirmed by Higgs et al., 1977.

In 1978, Higgs and co-workers found that the lowest effective dose of BST for stimulation of growth of yearling salmon was 1.0 mg/kg weekly, given by intramuscular injection. The next lowest dose tested, 0.32 mg/kg, did not have a significant effect.

In 1986, Sheridan reported the effects of BST on coho salmon parr and smolts. This investigator found that a parenteral dose of 1.5 mg/kg per 12 days (equivalent to 0.88 mg/kg per

week) stimulated lipid mobilization in parr but not in smolts.

In 1988, Down and co-workers found that recombinant BST at doses as low as 0.5 mg/kg per week given as a bi-weekly dose of 1.0 mg/kg stimulated growth of yearling salmon as measured by increases in length or in weight.

These data permit addressing the potential effect of BST on aquatic ecosystems and terrestial ecosystems. The atmospheric environmental compartment is excluded from this analysis since, as mentioned in Section 7, BST is nonvolatile and will not enter the atmosphere until it has been degraded to $CO_2$, $H_2O$, and the other small molecular weight volatile compounds into which all proteins ultimately degrade.

In the case of the terrestrial environmental compartment, rats appear to be the most sensitive of all terrestrial animals with daily parenteral doses as low as 15 mcg per rat administered daily reported to stimulate growth of hypophysectomized animals. (Seaman et al., 1988). For a 100-gram rat this corresponded to a dose at 150 mcg/kg. As shown in Section 6, the concentration of immunoreactive BST in the urine of treated cattle is slightly less than 3 ng/ml. For comparison, the dose of 150 mcg/kg daily

corresponds to a daily dose of 50 liters of dairy cattle urine per kg. To put this dose into perspective, a rat would have to efficiently absorb the BST from an amount of urine equal to 50 times its body weight each day; a situation which is extremely unlikely. It should be noted that this scenario is very conservative for several reasons. The dose chosen, 150 mcg/kg, is based on activity in animals that were hypophysectomized. In fact, intact animals are much less sensitive to BST since they have their own endogenous growth hormone. As shown in Section 6, most of the immunoreactive BST in urine is degraded and unlikely to be active. Thus, 50 L of urine probably contains considerably less than 150 mcg active BST. As shown in Section 7, proteases capable of degrading BST are ubiquitous in the environment and BST in urine would be expected to degrade rapidly after entering the environment. Accordingly, the 50 L/kg dose described above would correspond to 50 L of very fresh urine. Furthermore, BST is not absorbed when given by the oral route, which is a likely route of exposure in the environment. As noted above, it is at least 260-fold more active parenterally than orally. Thus, 50 L of cow urine per kg parenterally would correspond to at least 1300 L of urine per kg orally per day.

These calculations indicate that the consequences of urinary BST in the terrestrial ecosystem will be negligible because

(i) the amount of BST in urine is clearly too low to cause endocrine effects, and (ii) it is generally unstable.

Finally, it must be remembered that even in the extremely unlikely event that urinary BST has environmental effects, these effects would not be increased by BST treatment, since as shown in Section 6, BST treatment does not increase urinary output of BST.

These general conclusions are also true for the aquatic environmental compartment. The lowest dose reported in the literature to be active in an aquatic animal is 0.11 mg/kg, injected every three weeks, which was reported to stimulate growth of green sea turtles (Owens et al., 1979). When calculated on a per week basis, this dose corresponds to a weekly dose of slightly more than 36 mcg/kg. As shown in Section 6, the concentration of BST in the urine of dairy cows is slightly less than 3 ng/ml. A dose of 36 mcg/kg weekly would correspond to a weekly dose of approximately 12 L/kg cow urine. Thus, a turtle weighing on the average of 32 kg would have to accumulate the BST from 384 L of cattle urine each week. This situation would be extremely unlikely. It should be noted that this scenario is very conservative for many of the same reasons noted above for the terrestial scenario. Most of the immunoreactive BST in urine is degraded; BST is less active orally than

parenterally; and BST is likely to be degraded by proteolytic enzymes after it enters the environment. And again, it must be remembered that even in the unlikely event that urinary BST has effects in the aquatic environment, these effects would not be increased by POSILAC treatment since POSILAC treatment does not increase urinary output of BST.

Handler Safety

Although the chance of BST causing an endocrine response in humans is nil, BST is a protein foreign to humans and thus could cause an allergenic response in certain individuals.

Since POSILAC is non-dusting, the only likely route of exposure is by direct contact. The following paragraph from the warnings section of the package insert mitigates the possibility of an allergenic reaction to POSILAC bovine somatotropin:

> "Avoid prolonged or repeated contact of POSILAC with eyes and skin. POSILAC is a protein. Frequent skin contact with proteins in general may produce an allergic skin reaction in some people. Always wash hands and skin exposed to POSILAC with soap and water after handling. Clothing soiled with the product should be laundered before reuse."

9. Use of resources and energy:

Potential Changes in Land Use

Treatment of dairy cows with BST increases their daily milk production. As a result, treated cows require more feed, and may, in some cases, require feed of enhanced nutritional value. It has been speculated that these changes in feed usage by the dairy industry may result in increases in the acreage devoted to some crops and decreases in the acreage devoted to other crops. These changes in turn may affect the use of various agricultural chemicals, including insecticides, herbicides and fertilizers, and thus may lead to additional amounts of these chemicals being introduced into the environment or reductions in use. It is theoretically possible that increased crop production due to changes in feed usage resulting from use of BST by dairy farmers could also require that additional land be tilled. The following analysis shows that approval of BST is unlikely to have any significant effect on land use and will have no significant environmental impact.

The starting point for this analysis is a report published by the Economic Research Service of the U.S. Department of

Agriculture (USDA) on the potential effects of introduction of BST on the U.S. dairy industry (Fallert et al., 1987).

The environmental impact of BST will depend on the price of BST and on levels of government milk price supports, since these factors will influence the rate of adoption of BST by dairy farmers. For purposes of their analysis, Fallert and co-workers assumed that BST would be priced at $0.24 per day for each cow. The work of Kalter and co-workers at Cornell University (1984) suggests that this pricing is likely to be low. In the Kalter study, the price of BST was estimated to range from $4.23/gram to $1.93/gram depending on the scale of production. Since POSILAC (sometribove-zinc complex) bovine somatotropin contains 0.5 grams of BST in each bi-weekly dose, these prices correspond to $1.51 per cow per day to $0.068 per cow per day respectively. These estimates allow for a real after-tax rate of return-on-equity capital of 10% and for a real before-tax rate of interest on debt capital of 6.67% with a 75/25 debt-to-equity ratio. However, these price estimates do not allow for the cost of purification of bovine somatotropin, the cost of formulation into a prolonged-release system, the cost of distribution, and the cost of marketing. Nor do these estimates allow for markups throughout the distribution chain. Due to the costs not included in the Kalter price estimate, the $0.24 per cow per day chosen by Fallert in 1987 is likely to be low and,

in fact, tends to be lower than the price estimates used by other economists. This was noted by Blayney and Fallert (Blayney and Fallert, 1990). Thus, the 1987 Fallert report may overestimate the potential adoption rate of BST. Since the potential environmental effects of the introduction of BST depend largely on the adoption rate of BST by dairy farmers, the conclusions in the 1987 Fallert report may be conservative based on the BST pricing assumption used in the analysis.

The 1987 Fallert study predicted that the effects of BST will depend on the flexibility of government price support systems, with higher levels favoring greater numbers of cows, higher BST adoption rates, and greater potential for effects on the dairy industry due to BST. To account for the impact of government milk price supports, the 1987 Fallert paper analyzed four government milk price support levels:

> Scenario I assumes a support price of $10.10 per hundred weight (CWT) from 1990 (the projected introduction date of BST in the report) through 1996 (the final year analyzed by the study). Under the Food Security Act of 1985 the support price dropped from $10.60 per CWT to $10.10 per CWT on January 1, 1990. (The 1985 Act mandated a drop in the support price of

$0.50 per CWT on January 1 each year from 1988 through 1990 if government milk purchases were forecast to exceed 5 billion pounds in the coming years. If government milk purchases were forecast at less than 2.5 billion pounds, the support price was to be raised by $0.50 per CWT.)

Scenario II assumes a support price of $9.60 per CWT in 1990. This figure is the statutory price reduction limit of the 1985 Act. No further changes in the support price are assumed through 1996.

Scenario III assumes a support price of $9.60 per CWT in 1990 and extends the provisions of the 1985 Act by permitting two further $0.50 annual reductions in the support price to $9.10 in 1991 and $8.60 in 1992.

Scenario IV assumes a support price of $11.10 per CWT through 1996 regardless of supply and demand conditions or government purchases.

Even at the highest price support levels assumed in the report (Scenario IV), Fallert predicts that the effects of introduction of BST are likely to be relatively minor and will not fundamentally change structural trends already underway in the dairy industry.

Adoption of BST, when viewed at the national level under Fallert's assumptions, is simply one additional factor affecting the 30-year trend toward increased milk production per cow.

In 1990, Blayney and Fallert updated the 1987 Fallert study in response to a request by Senator Patrick Leahy, Chairman, Senate Committee on Agriculture, Nutrition, and Forestry (Blayney and Fallert, 1990). The updated report states that Scenarios I and III used in the 1987 Fallert report are the most applicable as potential bases for analysis under current conditions. The authors concluded that:

> "The reevaluation of the 1987 study and the review and analysis undertaken in response to Senator Leahy's request indicate that most of the previously listed general trends, results, and implications of the 1987 study remain valid today."

Thus, the 1987 Fallert study remains a valid starting point for analyzing the potential effects of BST on land use.

Preckel and his co-workers at Purdue used the 1987 Fallert study to investigate the potential environmental effects of the use of BST, including its effects on crop production,

use of agricultural chemicals, and manure production (Preckel et al., 1988). The Preckel study is appended to this environmental assessment (Attachment 11).

The 1988 Preckel study analyzed potential effects of BST for Scenarios I and III in the 1987 Fallert report. Scenario II was not analyzed since it is bracketed by Scenarios I and III and, thus, its effects can be interpolated. Scenario IV, with its very high support level of $11.10 per CWT, was viewed as unrealistic by Preckel and co-workers and was not analysed in the 1988 study. (Note, however, that Scenario IV, which is still viewed as being very unrealistic, was analyzed by Preckel in 1990 as an addendum to the 1988 study. The 1990 addendum is included as Attachment 12 and will be discussed in greater detail below.)

As noted by Blayney and Fallert, the choices which Preckel and co-workers made in 1988 turned out to be very appropriate. The 1990 Farm Bill which will be in effect until 1995 calls for a price support level of $10.10 per CWT, which is the government milk price support level in Scenario I.

At the national level, the 1988 Preckel study found that the effect of BST adoption on crop production was likely to be negligible. By 1996, production of the primary dairy feed

ingredients (alfalfa, corn, and soybeans) was projected to increase due to BST by 0.3% under Scenario I and to decrease by 0.1% under Scenario III. Preckel further predicted that any associated changes in agricultural chemical use would also be negligible.

With respect to states with large dairy sectors, Preckel's analysis suggested that the effects might be more pronounced. Three states, California, New York, and Wisconsin, were chosen for detailed analysis. In 1986, these three states accounted for 35% of the national dairy herd and 38% of milk production. For these analyses, the diet for cattle treated with BST was assumed to be equivalent to that used for BST clinical trials conducted by Monsanto in the same or a comparable region.

Based on a 200-day BST treatment period, changes predicted in the three states of acreage committed to alfalfa, corn grain, soybeans, and corn silage due to introduction of BST were usually quite small. Therefore, predicted changes in application of agricultural chemicals were also quite small - generally less than 1 or 2%. Indeed, under Preckel's analysis of Scenario III, BST was predicted to cause an overall reduction in the use of agricultural chemicals.

There were two exceptions under Scenarios I and III in which Preckel found BST potentially to cause changes in agricultural chemical usage which exceeded 1 or 2%. These were Wisconsin soybean acreage and California corn acreage. The model used in the Preckel study assumes that any new crop land needed to produce additional feed needed as a result of introduction of BST would be concentrated in the same state where demand for the additional feed is located. This would tend to concentrate any potential effects due to increased demand for feed in those states. However, in both of these cases, it is extremely likely that any increased demand for feed rations will be satisfied by interstate shipments rather than by increased feed crop production in these states. This is because, as Preckel acknowledged, Wisconsin exports about 16% of the corn produced in that state, Wisconsin lacks significant soybean crush facilities, and California, according to 1985 surveys, has historically been a net corn importer. Thus, any additional land needed to produce additional feed as a result of use of BST would be likely to be in other states rather than concentrated in Wisconsin or California.

As discussed by Furchtgott-Roth in a study appended to this assessment (Attachment 13), because the Preckel study was designed to provide a "worst case" analysis of the effects of introduction of BST based on the Fallert assumptions, the

conclusions in this study are very conservative. Furchtgott-Roth's analysis of the Preckel study further supports the conclusion that BST will have no significant environmental impact. The potential effects noted in the Preckel study, though small and insignificant, are based on conservative assumptions intentionally adopted in order to provide a "worst-case" analysis of the potential environmental impact of adoption of BST. In his analysis, Furchtgott-Roth points out that if the assumptions used as the basis for the Preckel study are made less conservative and more realistic, the potential environmental effects of adoption of BST are even smaller and that other economic factors are more important than BST in determining land usage for livestock feed production.

Furchtgott-Roth identifies a number of factors which reduce or eliminate the potential environmental effects identified in the Preckel study.

Preckel assumes that feed rations remain constant and does not consider demand elasticity for feed ingredients. Thus, the study does not account for substitution possibilities. In fact, dairy farmers are likely to substitute feed ingredients in order to minimize their production costs. Neither does the Preckel study consider that not all corn, alfalfa, and soybeans are used for livestock feed. In fact,

in 1988, livestock accounted for no more than 55% of corn and no more than 70% of soybean production. Thus, Preckel's percent changes for these feedstuffs are overstated because the study does not take into account use other than livestock feed. The Preckel study also does not consider the possibility that additional demand for feed grains by the dairy industry due to the introduction of BST would be satisfied at least in part by diversion of these grains from other uses (e.g., from export), rather than by production of additional feed grain. Nor does the study consider the trend to increasing yields per acre so that any increased demand for feed grains due to the introduction of BST is likely to be dwarfed by increases in productivity.

The Preckel study was intentionally not designed to consider the shift of agricultural land needed for feed grain cultivation from production of another crop. Preckel assumes, instead, that fallow land is shifted to feed grain production and, thus, that any chemical needed for additional feed grain production causes a net increase in the use of agricultural chemicals. Land shifted from another crop would probably affect the composition of chemicals, but the change in the total amount of chemical applied would likely be less than predicted in the Preckel study.

In spite of the very conservative assumptions intentionally adopted for purposes of the Preckel study, the study concludes that BST will have little, if any, environmental impact. Furchtgott-Roth points out that when more realistic assumptions are considered, the potential effects of BST are indeed likely to be negligible or nonexistent.

Thus, when viewed under the assumptions used in the 1988 Preckel and 1987 Fallert studies, adoption of BST is not likely to lead to any significant shift in agricultural land use, even on an individual state basis, nor is it likely that BST would have any measurable secondary, tertiary, or higher level impacts on such items as agricultural chemical usage, surface or ground water quality, non-target species, or soil tillage.

In 1990, Preckel and co-workers expanded their 1988 study to include Fallert's Scenario IV, which calls for a price support level of $11.10 per CWT. The 1990 report is included as Attachment 12. The $11.10 per CWT assumption in Scenario IV is $1.00 per CWT more than specified by the 1990 Farm Bill. This rather extreme case was analyzed to examine the effect of introduction of BST under conditions clearly intended to maximize any potential effects.

Unlike the other three scenarios, Scenario IV predicts that BST will increase the number of dairy cows in the national herd, apparently because BST will allow an increased number of marginal producers to stay in business. However, in spite of the increased numbers of cows, the effects of BST on crop production and on manure disposal will not be significant. Preckel and co-workers conclude the following in the 1990 addendum:

> "In summary, USDA Scenario IV causes much larger changes in feed requirements than either Scenario I or III. However, when viewed in the greater scheme of national or even regional row crop production, the changes amount to no more than 1-2% of 1986 production levels.....Thus, we conclude that the environmental effects of the introduction of BST, even under the somewhat unrealistic high price support assumptions of USDA Scenario IV, will be negligible."

That BST is very unlikely to have any significant effect on land usage is further supported by a study from the United States Department of Agriculture (Kuchler and McClelland, 1989). These authors analyzed not only the effects of the introduction of BST on the dairy industry, but also the effects of use of BST in beef production and PST (porcine somatotropin) in pork production. The following summary

refers to the combined effects of introduction of these proteins:

> "Widespread adoption of the hormones alone is unlikely to much affect the number, size, and location of producers of major crops. Some shifts in the location of the various crops could occur and some regions may produce relatively larger quantities of soybeans, but total acreage in any region is relatively insensitive to these changes in feed demand."

Potential Effects on Manure Production

The effects of introduction of bovine somatotropin on manure production are complex in that administration of BST causes an increase in manure production per cow but a decrease in the amount of manure which results from the production of a given amount of milk. The 1988 Preckel study analyzed the likely impact of BST on manure production in 1996 in California, New York, and Wisconsin under Fallert's Scenarios I and III. In all three states, use of BST caused a slight increase of less than 2% in manure production under Scenario I and a slight decrease of about the same amount under Scenario III. It is important to recognize that with or without introduction of BST the amount of manure produced under either scenario is predicted to be less than was

produced in 1986. Thus, under both scenarios the estimated effects on manure production will be insignificant.

The 1987 Fallert report noted that the historical trend to fewer but larger dairy farms is likely to continue regardless of introduction of BST and that farms which adopt BST will have increased manure production per cow. Preckel, in the 1988 study, concludes,

> "....if dairy farms remain spread out over dairying regions as they currently are, then there will be sufficient farm land in the vicinity of the larger dairy farms to allow safe, proper application of manure."

In the 1990 addendum to the 1988 Preckel study, Preckel expanded the analysis of manure production, discussing the impact of introduction of BST on manure production in California, New York, and Wisconsin under Fallert's Scenario IV. As noted above, Scenario IV entails a support level of $11.10 per CWT which is $1.00 above the level in the 1990 Farm Bill. In all three states, introduction of BST was projected to increase manure output in 1996 but the magnitude of the increases was less than 7% compared to 1996 levels if BST were not introduced. In both Wisconsin and New York, while introduction of BST increases manure

production in 1996, manure production is predicted to be less than in 1986. In California, manure output after introduction of BST was projected to be greater in 1996 than in 1986, but the magnitude of the increase is not significant (1.1%).

That the effect of the introduction of BST on manure production will be insignificant is also supported by the analysis of greenhouse gas emissions by G. F. Hartnell, which is appended to this assessment as Attachment 14 and is summarized below.

Potential Effects of POSILAC on Emissions of the Greenhouse Gasses Carbon Dioxide and Methane

Carbon dioxide and methane have been implicated in contributing to the phenomenon of global warming commonly called the "greenhouse effect". On a per molecule basis, the contribution of methane is about 20-fold greater than the contribution of carbon dioxide (Byers, 1990). Since the molecular weight of carbon dioxide is 2.75-fold greater than that of methane, the contribution of methane is about 55-fold greater than that of carbon dioxide when calculated on a weight basis.

Dairy cattle contribute to emissions of both gasses. Methane is emitted from cattle as a result of microbial

activity in their rumen and intestine and it is also produced when manure from dairy cattle is degraded anaerobically. Carbon dioxide is emitted when dairy cattle respire and when their manure is degraded aerobically. Carbon dioxide is also produced when fossil fuels are burned to provide energy for the dairy industry.

The analysis of POSILAC's potential effects on greenhouse gas emissions that follows is comprised of three sections: 1) calculation of the potential effects of POSILAC use on emissions by the dairy industry in the U.S.; 2) calculation of greenhouse gas emissions due to the manufacture and transport of POSILAC; and 3) calculation of the net effects of POSILAC use and manufacturing and transport on greenhouse gas emissions. The results indicate that use of POSILAC will either slightly increase or slightly decrease emissions depending on whether the increased milk yield resulting from POSILAC permits a reduction in the number of dairy cows in the national herd. In either case the magnitude of the changes will be extremely small and insignificant compared to total worldwide emissions of carbon dioxide and methane.

Potential Effects of POSILAC Use on Emissions

In a report appended to this assessment (Attachment 14), Dr. G.F. Hartnell of Monsanto analyzes the potential effects of POSILAC use on methane and carbon dioxide emissions by

the U.S. dairy industry. The analysis divides carbon dioxide emissions into two components: carbon dioxide from feedstuffs (i.e., from cattle feed via respiration or via aerobic degradation of manure); and carbon dioxide from fossil fuels burned to produce the energy needed to grow and transport feedstuffs for dairy cattle. Carbon dioxide from fossil fuels is of special concern since it is derived from carbon that has been sequestered for an extremely long time and thus its emission causes a net increase in atmospheric carbon dioxide. In contrast, carbon dioxide from feedstuffs is derived from carbon which was only recently fixed by green plants. Thus, it only replaces carbon dioxide which was recently removed from the atmosphere and it does not cause a net increase in carbon dioxide levels. It should be noted that Hartnell's analysis of carbon dioxide emissions from fossil fuel combustion is conservative since it assumes that all energy inputs are derived from combustion of coal which produces more carbon dioxide per energy yielded than do other common sources of energy including, in particular, petroleum hydrocarbons.

Hartnell analyzes the possible effects of bovine somatotropin under two base-case scenarios: For Scenario 1, Hartnell assumes that U.S. milk production will stay constant at 66 billion kg per year, which is the 1988 production level. Under this scenario, POSILAC use reduces

the number of cattle in the national herd from 10 million, the 1988 population, to 9 million; for Scenario 2, Hartnell assumes that the number of dairy cattle in the U.S. will stay constant at 10 million and that use of POSILAC bovine somatotropin will increase national milk production from 66 billion kg per year to 73.3 billion kg. In order to magnify any potential effects of POSILAC, Hartnell assumes for both scenarios that all cows eligible for treatment with POSILAC will be treated. It is further assumed for both base-case scenarios that all carbon from manure is converted to methane and that none is converted to carbon dioxide. From the standpoint of the greenhouse effect, this represents the worst case for the following two reasons, both of which were discussed above: 1) methane is a more potent greenhouse gas than carbon dioxide; and 2) carbon dioxide from feedstuffs has little or no net impact on atmospheric carbon dioxide levels.

The results of Hartnell's analysis, summarized in Table 2, indicate that usage of POSILAC will either slightly increase or slightly decrease emissions, depending on whether BST usage causes the number of dairy cattle in the national herd to decrease (Scenario 1) or not (Scenario 2). For example, under Scenario 1 use of POSILAC will reduce methane emissions by 0.15 million metric tons annually while under Scenario 2 its use will increase methane emissions by 0.22

## TABLE 2: POTENTIAL NET EFFECT OF BST ON EMISSIONS OF GREENHOUSE GASSES

## (ALL EMISSIONS IN MILLIONS OF METRIC TONS PER YEAR)

| Scenario | Usage | | | Manufacturing/Transport | Net Difference |
|---|---|---|---|---|---|
| | With BST | Without BST | Differences | | |
| Scenario 1 | | | | | |
| Number of Cows | $9x10^6$ | $10x10^6$ | $1.0x10^6$ | – | – |
| Methane | 3.38 | 3.53 | - 0.15 | 0 | - 0.15 |
| Carbon Dioxide/Biological | 30.4 | 32.6 | - 2.2 | 0.010 | - 2.19 |
| Carbon Dioxide/Fossil Fuels | 44.7 | 48 | - 3.3 | 0.35 | - 2.95 |
| Scenario 2 | | | | | |
| Number of Cows | $10x10^6$ | $10x10^6$ | 0 | – | – |
| Methane | 3.75 | 3.53 | 0.22 | 0 | 0.22 |
| Carbon Dioxide/Biological | 33.8 | 32.6 | 1.2 | 0.011 | 1.21 |
| Carbon Dioxide/Fossil Fuels | 49.5 | 48 | 1.5 | 0.39 | 1.89 |

million metric tons. These correspond to changes of less than 7% compared to methane emissions without POSILAC. Johnson and Ward (1992) using assumptions similar to those used in Scenario 1 calculated a decrease in methane emissions of 0.14 million metric tons. In a similar fashion, changes in carbon dioxide emissions, whether from fossil fuels or from feedstuffs will be either slightly decreased (Scenario 1) or slightly increased (Scenario 2) but in no case will the change in carbon dioxide emissions exceed 7% of the corresponding emission without POSILAC.

In addition to the two base case scenarios shown in Table 2, Hartnell also changed several parameters within the two scenarios and repeated his analysis. Among the parameters changed were the response of dairy cattle to POSILAC, the amount of dietary energy converted to methane, and the proportion of carbon dioxide and methane produced from manure. The results indicate that none of these changes appreciably alters the effects of POSILAC on carbon dioxide and methane emissions under Scenarios 1 or 2.

Emissions Due to Manufacture and Transport

In a report appended to this assessment as Attachment 15, Dr. G.H. Irwin of Monsanto analyzes the effects of POSILAC manufacture and transport on emissions of the greenhouse gasses carbon dioxide and methane for Hartnell's Scenarios 1

and 2. As was done by Hartnell, Irwin divides carbon dioxide emissions into those derived from fossil fuels and those derived from biological sources, since the latter emissions are comprised of carbon only recently fixed from the atmosphere. The major conclusions from the Irwin analysis are as follows:

1) Incremental carbon dioxide and methane emissions due to manufacture of raw materials consumed during production of POSILAC bovine somatotropin will be negligible. POSILAC production consumes only very common raw materials and the incremental increase in consumption due to POSILAC approval in the U.S. will be insignificant.

2) As shown in Table 3 of the Irwin analysis, the annual amount of carbon dioxide resulting from combustion of fossil fuels to provide energy for production of POSILAC will be 0.15 million metric tons (Scenario 1) or 0.17 million metric tons (Scenario 2). These estimates are conservatively high since it was assumed that the total energy needs of manufacturing are met by combustion of coal. As noted above, coal emits more carbon dioxide per energy yielded than do other common sources of energy. As shown in Table 4 of the Irwin analysis, the annual amount of carbon dioxide emitted as a result of the combustion of fossil fuels to

transport POSILAC will be 0.20 million metric tons (Scenario 1) or 0.22 million metric tons (Scenario 2).

3) As shown in Table 3 of the Irwin analysis, the amount of methane and biologically derived carbon dioxide emitted as a result of manufacture and transport depends on the extent to which the manufacturing site waste treatment facility is aerated. If one assumes that the waste treatment facility is completely anaerobic and that all waste carbon is converted to methane, then annual production of methane will be 0.0014 million metric tons (Scenario 1) or 0.0015 million metric tons (Scenario 2). It should be noted that these numbers are less than 1% of the changes in methane emissions resulting from POSILAC usage which were calculated by Hartnell to decrease by 0.15 million metric tons (Scenario 1) or increase by 0.22 million metric tons (Scenario 2). In actual fact, the waste treatment facility is heavily aerated and it is more realistic to assume that all waste carbon is converted to carbon dioxide. Under this assumption, there will be no methane produced and the total amount of biologically derived carbon dioxide emitted each year from waste disposal will be 0.0029 million metric tons (Scenario 1) or 0.0032 million metric tons (Scenario 2). Both of these numbers are less than 1% of the changes in feedstuff-derived carbon dioxide emissions resulting from POSILAC usage which were calculated by Hartnell to decrease by 2.2

million metric tons (Scenario 1) or increase by 1.2 million metric tons (Scenario 2).

Under the assumption that all waste carbon is converted to carbon dioxide, waste treatment will result in emissions of a small mount of carbon dioxide derived from fossil fuels. This results from degradation of waste urea which, as discussed by Irwin, is typically synthesized from ammonia and from carbon dioxide isolated from the flue gasses which result when fossil fuels are burned to produce energy. The total amount of carbon dioxide derived from fossil fuels liberated during waste treatment will be 0.0009 million metric tons (Scenario 1) or 0.001 million metric tons (Scenario 2). Under either scenario it is less than 1% of the amount of carbon dioxide emitted when fossil fuels are burned to provide energy for manufacturing.

In the calculations shown in Table 2 net effects on emissions are determined by summing emissions due to manufacturing and transport, as calculated by Irwin, with changes in emissions due to use, as calculated by Hartnell. In these calculations, it is assumed that all waste carbon from manufacturing is converted to carbon dioxide and none to methane, which is realistic in view of the design and practice of the waste treatment facility. However, as noted above, emissions due to treatment of manufacturing waste are

less than 1% of the changes in emissions due to usage. Thus, whether waste treatment emissions are all methane or all carbon dioxide or some mixture of carbon dioxide and methane, has little effect on the magnitude of carbon dioxide and methane emissions when the net effects of usage and manufacturing and transport are calculated.

Potential Net Effects on Emissions

For both Scenarios 1 and 2, the net effects of POSILAC on greenhouse gas emissions can be determined by summing the emissions due to its manufacture and transport with the changes in emissions due to its usage. The results shown in Table 2 indicate that under Scenario 1 the net effects of POSILAC will be to decrease emissions while under Scenario 2 the net effects will be to increase emissions. It should be noted that for methane as well as for carbon dioxide, whether derived from feedstuffs or from fossil fuel, the changes in emissions due to POSILAC do not exceed 7% of the corresponding emissions without POSILAC.

The changes in emissions shown in Table 2 can be put into perspective by comparing them to worldwide annual emissions. In the case of methane, the net changes due to POSILAC are an annual decrease of 0.15 million metric tons (Scenario 1) or an annual increase of 0.22 million metric tons (Scenario 2). For comparison, worldwide annual emissions have been

estimated to be 540 million metric tons (Johnson et al., 1991) or 550 million metric tons (Byers, 1990). Among the more important contributors to worldwide methane emissions are natural swamps, variously estimated to contribute 116 million metric tons (Byers, 1990) or 115 to 345 million metric tons annually (D.E. Johnson by Muirhead, 1990); and rice paddies, variously estimated to contribute 116 million metric tons (Byers, 1990) or 60 to 170 million metric tons annually (D.E. Johnson by Muirhead, 1990). When compared to these emissions, any changes in methane emissions which could be caused by POSILAC are negligible.

In the case of carbon dioxide from fossil fuels, Table 2 indicates that the annual net changes due to POSILAC will be a decrease of 2.95 million metric tons (Scenario 1) or an increase of 1.89 million metric tons (Scenario 2). For comparison, annual worldwide emissions due to combustion of fossil fuels has been estimated to be 18,330 to 25,670 million metric tons (Hileman, 1990). It has been estimated that 3465 million metric tons of hard coal were consumed in 1988 (United Nations, 1990). If one assumes that coal is comprised solely of carbon and that the carbon is fully oxidized, it can be calculated that this corresponds to emission of 12,710 million metric tons of carbon dioxide. Compared to these numbers, any effects of POSILAC on

emissions of carbon dioxide derived from fossil fuels will be negligible.

In the case of carbon dioxide derived from feedstuffs and other biological materials, Table 2 indicates that POSILAC will either reduce annual emissions by 2.2 million metric tons (Scenario 1) or will increase annual emissions by 1.2 million metric tons (Scenario 2). For comparison, biomass burning has been estimated to result in annual emissions of 12,760 million metric tons of carbon dioxide (Hileman, 1990), which is considerably greater than any potential change due to POSILAC.

In summary, POSILAC could have small effects on carbon dioxide and methane emissions but the effects are likely to be negligible and without environmental significance. If the increased milk production caused by use of POSILAC results in a reduction in the number of cattle in the national herd, POSILAC will reduce emissions. If introduction of POSILAC does not cause a decrease in the number of cows in the national herd but rather increases national milk production, use of POSILAC will increase emissions. In either case, the magnitudes of the changes are so small compared to worldwide emissions that they are unlikely to be of environmental significance.

Waste Disposal

POSILAC (sometribove-zinc complex) bovine somatotropin will be marketed in ready-to-use, disposable syringes, each of which contains sufficient formulation to treat one dairy cow. A sterile, disposable needle is provided with each syringe and the recommended treatment interval is two weeks.

To reduce the possibility of contaminating the environment with POSILAC, or with hypodermic syringes and needles, Monsanto has contracted with Browning Ferris Industries as described in Section 6 to provide a complete sharps waste management program at no extra cost to customers. In addition, the following disposal instructions are included in the package insert:

> "Used syringes and needles and syringes containing POSILAC should be placed in a leak-resistant, puncture-resistant container for disposal in accordance with applicable Federal, state, and local regulations."

One estimate of the number of POSILAC syringes which may be used by the average dairy farmer can be calculated by reference to Fallert and co-workers (1988, 1990). According to these authors the average dairy farm in 1996 will have 67 cows (Scenario II) or fewer (Scenarios I, II, and IV). One can predict that, on the average, only 75% of these animals

(51 cows) will be milking. POSILAC is intended for use only in the final eight months of an average 10-month lactation cycle. Thus, of the 51 cows that are milking, only 80% (41 cows) will be eligible for treatment with POSILAC bovine somatotropin.

Accordingly, the average farm will produce 41 expended syringes and needles and associated waste over an average two-week period. This would add only a small increment to the waste already produced by an average dairy farm.

Effects on Endangered or Threatened Species

In view of the specificity of bovine somatotropin, its instability in the environment, and the fact that sometribove does not increase urinary output of BST, any effects on any species, including those endangered or threatened are very unlikely.

There will be no effects from the approval of POSILAC bovine somatotropin on property listed or eligible for listing in the National Register of Historic Places.

10. Mitigation measures:

The measures taken to avoid or mitigate potential adverse environmental impacts associated with approval of POSILAC have been described in detail in Sections 6 and 8.

In particular, Section 6 describes the procedures in place at the manufacturing sites which ensure that the production organism is contained and that the various waste products are disposed of in an appropriate fashion. Section 6 also describes the arrangement with Browning Ferris Industries to provide customers with the means to safely dispose of used needles, syringes, and unwanted product. In addition, label instructions will remind the dairy producer to dispose of waste appropriately.

Section 8 describes the label instructions intended to minimize the possibility of an allergenic reaction to bovine somatotropin and thereby assure handler safety.

11. Alternative to the proposed action:

No significant environmental impacts are anticipated as a result of the approval of POSILAC (sometribove-zinc complex) bovine somatotropin as a new animal drug. Nevertheless, the following two alternatives were considered:

Imposition of Additional Controls on Production and Use

The FDA could impose controls on the production and use of POSILAC other than or in addition to those which form the basis for this approval. Among these might be additional labeling requirements. However, imposition of additional regulatory controls will not provide additional environmental protection. The production facility in Austria is operated under the highest standards to ensure that the organism used to produce BST is contained and that waste material is properly handled to prevent harm to the environment. Furthermore, as discussed in Sections 7 and 8 of this environmental assessment, even if the recombinant organism were to be released, it is harmless and poses no threat to the environment. With respect to use of POSILAC, a sharps waste management program is in place to provide for safe disposal of injection devices and unwanted product. Additionally, labeling encourages appropriate disposal procedures. As discussed in Section 6, use of POSILAC does not increase the amount of bovine somatotropin which cattle

excrete into the environment. Even if the product was inadvertently released into the environment, which is very unlikely due to the fact that it will be distributed prepacked in syringes, it will not cause harm. The product contains no recombinant organisms, and sometribove, the active ingredient, is a protein which biodegrades rapidly in the environment and, as discussed in Section 8, is harmless to organisms, including endangered species, which may come in contact with it. As explained in Section 9, there is no reasonable basis to conclude that use of POSILAC (sometribove-zinc complex) bovine somatotropin will have any measurable environmental impact as a result of changes in land use or other agricultural practices. It is even possible, depending on a variety of economic factors which are very likely to actually occur, that POSILAC usage will reduce agricultural by-products that may be harmful to the environment.

These considerations demonstrate that the labeling and other conditions which form the basis of this approval are adequate to protect the environment.

No Action

Not approving POSILAC bovine somatotropin for commercial use will not provide additional environmental protection for the same reasons as discussed above.

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13. Certification:

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of the firm or agency responsible for preparation of the environmental assessment.

Date: December 8, 1992

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