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 2.7×10^7 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.

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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:

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