FREEDOM OF INFORMATION SUMMARY

POSILAC® **(sterile sometribove zinc suspension)**

For Increasing Production of Marketable Milk in Lactating Dairy Cows

Sponsored by The Animal Sciences Division of Monsanto Company

® Registered trademark of Monsanto Company

1. General Information

NADA Number: 140-872

Sponsor:

Animal Sciences Division
of Monsanto Company
800 N. Lindbergh Blvd.
St. Louis, MO 63167

Generic Name: Recombinant DNA-derived methionyl bovine somatotropin

Trade Name: POSILAC® (sterile sometribove zinc suspension)(® Registered trademark of Monsanto Company)

Marketing Status: Over-the-counter (OTC)

Date Stamped: November 5, 1993

2. Indications for Use: For increased production of marketable milk in lactating dairy cows.

3. Dosage Form:

Sterile, prolonged-release injectable formulation in single-dose syringes each containing 500 mg sometribove zinc.

Routes of Administration:

Subcutaneous injection in the postscapular region (behind the shoulders) or ischiorectal fossa (depression on either side of the tailhead).

Recommended Dosage:

One syringe every 14 days beginning during the 9th week after calving and continuing until the end of lactation.

4. Overview of Studies Evaluated to Provide Pivotal Effectiveness and Animal Safety Data

Throughout the Freedom of Information (FOI) Summary, the term "sometribove" is used to represent the formulated drug product, sterile sometribove zinc suspension.

The general presentation of the results from the Chronic Animal Toxicity Study and full-lactation clinical studies consists of a discussion of study design, data analysis, pathology results (Chronic Animal Toxicity Study), and production results for each individual study

in Sections 5.a and 6.b - 6.e. Results of other variables, such as reproduction, cow health, and mastitis, were pooled across most of these studies for evaluation and are provided in Sections 6.f - 6.m.

The following table indicates the pivotal studies evaluated to determine effectiveness and animal safety of sometribove, and the specific data derived from each study.

TABLE A

OVERVIEW OF STUDIES EVALUATED TO PROVIDE PIVOTAL EFFECTIVENESS AND ANIMAL SAFETY DATA

Study and ID Number*

Multi-location SC Dose Response Study:

Arizona #87-023:
Efficacy
Lameness
Injection Site Reactions
Nutrient Intake, Body Weight and Condition
Reproduction
Mastitis
Cow Health **
Offspring

Cornell #87-034:
Efficacy
Lameness
Injection Site Reactions
Nutrient Intake, Body Weight and Condition
Reproduction
Mastitis
Cow Health **
Offspring

Florida #87-029:
Efficacy
Lameness
Nutrient Intake, Body Weight and Condition
Reproduction
Mastitis
Cow Health **
Offspring

Utah #87-024:
Lameness
Injection Site Reactions
Nutrient Intake, Body Weight and Condition
Reproduction
Cow Health **
Offspring

Tailhead vs. Postscapular:

Arizona #89-075:
Efficacy

Idaho #88-129:
Efficacy

14-Day Drug Tolerance Study #86-011:
Pathology
Injection Site Reactions
Nutrient Intake, Body Weight and Condition
Cow Health **
Blood Variables
Body Temp.

Multi-lactation Chronic Animal Toxicity Study #85-010:
Pathology
Lameness
Nutrient Intake, Body Weight and Condition
Reproduction
Mastitis
Cow Health **
Offspring
Circulating Anti-Somatotropin Binding
Blood Variables
Body Temp.
Urinalyis

IM/SC Bridging #86-032:
Lameness
Injection Site Reactions
Nutrient Intake, Body Weight and Condition
Reproduction
Mastitis
Cow Health **
Circulating Anti-Somatotropin Binding
Blood Variables
Body Temp.
Urinalyis

IM-Dose Titration Study #86-023:
Lameness
Nutrient Intake, Body Weight and Condition
Reproduction
Mastitis
Cow Health **
Offspring
Blood Variables
Body Temp.
Urinalyis

Multi-location IM Single Dose Study:

Arizona #85-039:
Lameness
Nutrient Intake, Body Weight and Condition
Reproduction
Mastitis
Cow Health **
Offspring
Circulating Anti-Somatotropin Binding
Blood Variables

Cornell #85-038:
Lameness
Nutrient Intake, Body Weight and Condition
Reproduction
Mastitis
Cow Health **
Offspring
Circulating Anti-Somatotropin Binding
Blood Variables

Dardenne #85-021:
Lameness
Nutrient Intake, Body Weight and Condition
Reproduction
Mastitis
Cow Health **
Offspring
Circulating Anti-Somatotropin Binding
Blood Variables
Urinalyis

Utah #86-003:
Lameness
Nutrient Intake, Body Weight and Condition
Reproduction
Cow Health **
Offspring
Circulating Anti-Somatotropin Binding
Blood Variables

Lameness Study #92-007:
Lameness

Injection Site Reaction Field Study #91-072:
Injection Site Reactions

Non-Clinical Injection Site Reaction Study #91-068:
Injection Site Reactions

Injection Site Reactions in Jersey Cows:

Vermont #86-031:
Injection Site Reactions

Arizona #89-075:
Injection Site Reactions

Preclinical #92-003:
Injection Site Reactions

Carcass Evaluation Study #89-049:
Injection Site Reactions

*****Complete Study ID Numbers provided in text.

******Includes data from daily health observations and routine physical examinations, except for the Multi-location SC Dose Response Study, where routine physical examinations were not performed.

SC = Subcutaneous
IM = Intramuscular

5. Effectiveness

The indication for use of this product is: "For increased production of marketable milk in lactating dairy cows."

Increased production refers to an increase in **weight** of milk produced. Dairy producers in the U.S. measure (and describe) milk production per cow or per farm in weight units, not volume. Thus, in the pivotal effectiveness and animal safety studies, weight of milk produced (not volume) was measured.

The term "marketable" milk refers to the milk that the dairy producer can legally sell. (The term "salable" milk is used interchangeably with "marketable" milk in this section of the FOI Summary.) Milk produced the first few days after a cow has a calf, also called colostrum, is not marketable as its composition is considerably different from true milk. Also, milk produced from cows after receiving certain therapeutic drugs cannot be sold legally if the milk contains residues of the drug above the FDA-established tolerance. In determining the effectiveness of sometribove, milk produced by a cow during the "withdrawal period" for any drug she had been administered therapeutically was excluded. (In the case of extra-label drug use, specific drug withdrawal periods were specified by the firm for purposes of the calculation, with CVM's concurrence.) Thus, on any day that a cow's milk should have been discarded because of a withdrawal period, the cow's production of "marketable/salable" milk was considered to be **zero**. Therefore, the evaluation of the effectiveness of the drug was based on production of marketable milk.

The evaluation of the effectiveness of sometribove was also based upon production of salable "3.5 % fat-corrected milk" or "FCM." The content of fat in milk typically varies among cows from about 2.0 to 6 % and is affected by factors such as the breed of the cow, stage of lactation, and its diet. The current pricing system in the U.S. generally favors milk of higher fat and other "solids" content, such as protein, because more cheese and other manufactured dairy products can be produced from the same unit of milk if solids content is higher. (For example, producers may get a bonus for milk of higher fat or solids content.) A long-standing practice in the dairy industry and the field of dairy science is to express milk production in terms of how much milk would have been produced if it was standardized to 3.5 % fat content (i.e., FCM). Thus, a cow that produced 100 lbs of milk with 2.5 % fat content would be calculated as having produced only about 84 lbs of FCM, whereas another cow producing 100 lbs of milk with 5 % fat content would be said to have produced 124 lbs of FCM. The actual equation for the calculation of FCM is provided on page 12.

Other calculation procedures are discussed in Section 5.a of the FOI Summary, including standardization to a specific length of lactation. All of these steps were taken to remove any potential biases in the calculation of the effectiveness of sometribove and also to evaluate milk production as it typically is evaluated in the dairy industry.

a. Multi-location SC Dose Response Clinical Study (4 Dose-SC)

Clinical studies were conducted at four separate locations (University of Arizona, Cornell University, University of Florida, and Utah State University) to determine the effective dose range of sometribove when administered subcutaneously (SC) commencing 60 ± 3 days postpartum and continuing until the end of lactation. The same study protocol was

employed at each location with minor variations to allow for differences in management practices.

Investigators: J. T. Huber, Ph.D.
University of Arizona
Tucson, AZ 85721

D. E. Bauman, Ph.D.
Cornell University
Ithaca, NY 14853

H. H. Head, Ph.D.
University of Florida
Gainesville, FL 32611

R. C. Lamb, Ph.D.
Utah State University
Logan, UT 84322

Materials and Methods:

One hundred nine primiparous (first calf heifer) and 145 multiparous (mature) Holstein cows were randomly assigned within the two parity groupings to treatments of 0 (excipient), 250, 500, or 750 mg of sometribove every 14 days. Animals calved on location to allow acclimation prior to study initiation. At approximately 40 days postpartum, each cow was given a general physical examination by a veterinarian. Selection criteria for study cows included acceptable body and udder conformation, disposition, and body condition, adequate milk production and general health, vaccinations for IBR, PI3, BVD, and leptospirosis, and a negative test for brucellosis and tuberculosis. An additional requirement at Cornell was a negative test for Johne's Disease (bovine paratuberculosis).

The 0 mg dose consisted of the formulation ingredients (excipient) minus the active ingredient. Sometribove was administered via subcutaneous injection in one of four postscapular sites: right upper, right lower, left upper, or left lower. Injections were rotated among these four sites so that eight weeks elapsed prior to reinjection in a particular site, except at Florida, where due to a protocol misinterpretation each site was used twice consecutively before rotating to the next site. Treatments were administered using 16-gauge 1-inch hypodermic needles. Within each study location, cows were injected on the same day of the week, and all cows receiving treatment on a given day were injected in the same postscapular site.

Treatment was initiated at 60 ± 3 days postpartum and continued at 14-day intervals throughout lactation. In pregnant animals, treatment ceased at least 74 days prior to expected calving date to allow for a dry period of approximately 60 days or when average daily milk production decreased below 8.2 kg/day (unless low milk production was due to temporary ill health), whichever occurred first. Open (non-pregnant) animals

continued on treatment for 25 treatment cycles (approximately 400-day lactation), or when average daily milk production decreased below 8.2 kg/day (unless low milk production was due to temporary ill health), whichever occurred first. In all cows, injections ceased as closely as possible to 14 days prior to dry off.

The distribution of cows started on treatment at each study location is provided below:

Table 1.

Number of Cows Started on Treatment

Location and Study No.	Parity*	0 mg	250 mg	500 mg	750 mg	Totals
Arizona 100-ARI-COW-LK-87-023	P/M	4/12	4/12	5/10	4/12	17/46
Cornell 100-COR-COW-VKM-87-034	P/M	6/6	6/7	5/6	6/6	23/25
Florida 100-UFL-COW-WAS-87-029	P/M	9/8	9/8	10/9	9/9	37/34
Utah 100-UTA-COW-LK-87-024	P/M	8/10	8/10	8/9	8/11	32/40
POOLED	P/M	27/36	27/37	28/34	27/38	109/145

*P=Primiparous; M=Multiparous

Within each parity group (primiparous and multiparous), blocks consisting of random permutations of the four treatments were generated for each location prior to the start of the study. Cows were assigned to blocks in order of calving dates. Within a block the maximum range in average daily milk production per cow during weeks 4 through 6 of lactation was ¾ to 9.1 kg/day (with the exception of one multiparous block at 15.8 kg/day). The range in the first day of treatment for the first and last cows entering a block was no greater than 56 calendar days.

All cows were milked twice daily at approximately 12-hour intervals except at Florida where intervals were 11 and 13 hours. All locations followed the same general milking procedures. Udders were washed and dried with individual paper towels. In addition, at Arizona and Utah, teats were predipped with an approved iodine teat dip and dried. Milk was forestripped onto the floor from each quarter to detect abnormal milk before milking machines were attached. The appearance of mastitic/abnormal milk was recorded. After milking was complete, teats were dipped with an approved iodine teat dip. Weight of milk yield was recorded for each cow at each milking throughout lactation. Individual cows' milkings that were discarded (withheld from human consumption) because the animal was administered med ion that had a milk withdrawal period were identified.

Milk fat, protein, and lactose percentages and somatic cell counts were determined from milk samples collected weekly from each cow on consecutive PM and AM milkings throughout lactation. Consistent days of the week were used at each location. Across the four locations, samples were collected three to four and 10 to 11 days post-injection during the treatment period.

Content of milk minerals (ash, calcium, and phosphorus) was determined on samples collected at consecutive PM and AM milkings from each cow during treatment weeks -2, -1, 3, 15, and 27. Consistent days of the week were used at each location. Across the four locations, samples were collected three to six days post-injection during the specified treatment weeks.

Diets at each location were formulated to meet or exceed nutritional requirements recommended by the National Research Council (NRC; 1978). All cows were fed total mixed rations (TMR) *ad libitum* with sufficient feed offered to allow for approximately 5 % refusal. Amount of feed offered and refused was recorded daily throughout lactation.

Feeding practices were according to established management at each location. At all locations, cows were fed a high energy diet from freshening to at least 90 days postpartum. Thereafter, cows were switched to lower energy density diets depending on level of milk production. Because the dietary formulations for different levels of milk production were based on typical levels of *ad libitum* intake, it was also necessary to consider body condition in making dietary shifts. At the discretion of the herd manager in consultation with the investigator, the timing of a cow's shift to a lower energy density diet may have been modified. If actual feed consumption was such that a cow was underconditioned for that stage of lactation, she may have remained on the higher energy density diet for a longer period than that indicated by her level of milk production.

At each location, diets and components were sampled weekly and composited monthly. Analyses of these samples were performed at appropriate testing facilities.

Cows were weighed weekly throughout the entire lactation. Within a study location, weights were taken at approximately the same time of day on a consistent day of the week such that during the treatment period, across the 4 locations, weighings occurred on days three to five and 10 to 12 post-injection. Body condition was scored at each weighing using a scale of 1 to 5 (1 = excessive underconditioning; 5 = excessive overconditioning) in quarter point increments (Wildman et al., 1982, Journal of Dairy Science 65:495).

The incidence of subclinical mastitis was evaluated from microbiological cultures of duplicate milk samples collected from each quarter of each cow once during the two-week pretreatment period, at 56-day intervals thereafter throughout lactation, and at dry-off. All cows at a location were sampled on the same day relative to injection.

For cases of clinical mastitis, duplicate samples were to be collected for microbiological culture before antibiotic treatment, if any, was administered. Adherence to this provision varied from location to location, and some discretion regarding treatment regimen was allowed at each location. However, the Utah location rarely treated clinical mastitis, resulting in unacceptably long cases. The lack of any attempt to control clinical mastitis did not reflect accepted mastitis management practices and could have resulted in

mastitis organisms being shed from infected quarters to other quarters and/or cows, therefore confounding attempts to evaluate the effect of sometribove on mastitis incidence at this location. Mastitis data from the Utah location were therefore eliminated from analysis. Cows received intramammary dry cow treatment in each quarter after their last milking of the lactation (end of sometribove treatment period) at Arizona, Cornell, and Florida locations.

General herd health programs were followed at all locations. Current guidelines (National Institutes of Health), entitled "Guide for the Care and Use of Laboratory Animals," were followed. Cows were observed daily for signs of disease, injuries, or other disorders. Minor disorders were treated by farm personnel according to standard animal care procedures. A veterinarian was consulted when necessary. Cows were treated with approved medications which were used according to label direction unless the herd veterinarian or designee deemed the extra-label use of medication necessary. All health related observations and medications administered were documented. All available practical means were taken to diagnose the cause of any illness or injury. Cows received a general physical examination by a veterinarian during the last injection cycle. Sick or injured animals were removed from study if deemed necessary by the attending veterinarian and the investigator or herd manager. Necropsies were usually performed on clinical trial animals that died or were euthanized while on study. Any gross lesions observed at necropsy were collected for histopathological examination. All aborted fetuses of at least 100 days gestation and stillborn calves were examined externally and internally for malformations. If possible, the cause of abortion was determined.

Estrus detection was according to existing location practices and was based on visual observation and tail head marking. All observed heats were recorded and scored as described in Section 6.i on Reproduction.

Prostaglandins were not used as a reproductive aid in normal cycling cows until after 120 days postpartum. Prostaglandin use was allowed to treat pyometra or cystic ovarian disease at any time if prescribed by a veterinarian.

Cows received a veterinary examination to evaluate reproductive health prior to initiation of treatment. All breeding was by artificial insemination commencing at the first observed estrus after treatment initiation. Pregnancy was confirmed by rectal palpation at least 30 days after breeding. Breeding was continued through 400 (305 at Cornell) days postpartum. Sire selection was according to location practices.

Injection sites were observed for any swellings or adverse reactions three and ten days post injection and scored (see Section 6.g for description of the scoring system). If a visible swelling remained, scoring continued weekly until the swelling disappeared, or up to 8 weeks, whichever occurred first.

All calves born after the initial lactation of treatment were weighed after birth. Birth weight, sex of the calf, and a calving ease score (as described in Section 6.i on Reproduction) were recorded, in addition to any unusual observations (surgical intervention or stillbirth) noted during calving. After birth, the navel was treated with tincture of iodine. All calves were fed 4 to 6 quarts of colostrum within 24 hours of birth. At Florida, calves may have been allowed to nurse for up to 24 hours. Calves were then

fed whole milk until weaned after at least 35 days of age. Free choice feeding of calf-starter began 2 days after birth except at Cornell, where calf-starter was begun 5 days after birth. All calves were given a general physical examination, usually within 72 hours of birth. Female calves were weighed during the second and fourth weeks of life.

Data Handling:

All cows started on treatment were included in the analysis of clinical health, mastitis (excluding Utah, see below), and injection site reactions (excluding Florida, see Section 6.g) for the period of time in which they were on study. Thus, every incident that occurred in these categories during the study was included in the evaluation of the effect of the drug. However, the evaluation of effectiveness, reproduction, nutrient intake, and body weight and condition generally involved the calculation of average values over the treatment period. Therefore, to be included in these latter analyses, cows had to complete greater than two-thirds of the 252-day standard treatment period (i.e., > or = 169 days of treatment) to provide sufficient data to accurately estimate effects over an entire treatment period. Reasons for not completing > or = 169 days of treatment included low production, health problems, administered an incorrect dose, and incorrect blocking. Also, a block had to include at least two cows so that an estimate of within block variation was possible. The following cows did not complete at least 169 days on treatment:

Table 2.

Dose	Parity*	Study Location	Cow ID	Days on Treatment	Reason
0 mg	P	Florida	0010	154	¾ 2/3 treatment period due to low production
	M	Arizona	8540	14	Incomplete block
250 mg	M	Arizona	74	70	Lameness
			1134	42	Misinjected (Wrong dose)
		Cornell	3951	6	Incomplete Block
500 mg	P	Arizona	210	140	Incomplete Block
	M	Cornell	3449	68	Toxic mastitis
		Florida	2942	168	¾ 2/3 treatment period due to low production
750 mg	P	Florida	0004	154	¾ 2/3 treatment period due to low production
		Utah	306	22	Died due to Right Displaced Abomasum
	M	Arizona	133	112	Incomplete Block
			2071	14	Misinjected (Wrong Dose)
		Utah	6096	37	Multiparous Cow Assigned to Primiparous Cow Block

*P=Primiparous; M=Multiparous

Daily milk yield for each cow was the sum of two milkings per treatment day and was set to missing if the yield at either milking was not recorded due to mechanical or human error. An average daily AM milk yield for each cow was calculated for each treatment week from all of her non-missing daily AM milk weights that week. The same calculation was performed for all non-missing PM milk weights to obtain an average daily PM milk yield per week for each cow. Each cow's average daily AM and PM milk yields per week were summed to determine her **average daily milk yield** for the week.

An **average daily 3.5 % fat-corrected milk (FCM) yield** for each week was calculated for each cow as follows.

1. Each cow's average AM milk fat percent value and average daily AM milk yield during a week were used in the following equation to determine her average daily AM FCM yield for that week:

AM FCM (kg/d) = AM Milk (kg/d) X (0.43237 + (.16218 X AM fat %))

2. A cow's average daily PM FCM yield for the week was calculated using the same equation and the animal's average PM milk fat percent value and average daily PM milk yield during the week.

3. A cow's average daily AM and PM FCM yields for the week were summed to obtain her average daily FCM yield for that week.

4. Any milk fat percent value greater than 8 % was designated as missing because such a high value would generally represent a laboratory error. During the pretreatment period, missing weekly AM or PM milk fat percent values (e.g., sample not collected or laboratory error) were estimated from the average of the adjacent weeks' AM or PM fat percent values, respectively. However, during the treatment period, weekly milk fat percent values tended to be cyclic. Thus, if a cow's AM or PM milk fat percent value was missing during an **even** week of the treatment period, the average of her adjacent **even** weeks' respective AM or PM milk fat values was used as a replacement for purposes of calculating a FCM value for the week. Missing values during odd numbered treatment weeks were similarly estimated.

The actual period of time in which cows remained on treatment varied depending on conception date, milk yield, and health conditions. Thus, an **average daily standardized FCM (SFCM) yield** per cow was calculated, adjusting to a 252-day treatment period (i.e., 36 weeks) according to the following procedures.

1. For cows on treatment > or = 252 days, the average daily FCM yields during each of the first 36 weeks of treatment were averaged.

2. For cows dried off at < 252 days due to low milk production, an average daily FCM yield of 0 kg/day was assigned to the weeks following the last injection cycle through 36 weeks of treatment. The average daily FCM yields for each of the 36 weeks were then averaged.

3. For cows dried off at < 252 days to allow a 60-day dry period (i.e., early conception) or because of non-treatment related removal from study, data were extended beyond the last complete injection cycle by linear regression. Individual cow regressions were

estimated from the last six completed injection cycles, excluding cycles for which milk production was judged to be atypical for the general trend. Average daily FCM yield for the cycles was regressed on median treatment day of the cycle. The resulting equation for each cow was used to estimate her production during each week after the last completed injection cycle through 36 weeks of treatment. The average daily FCM yields for each of the 36 weeks were then averaged.

Average daily salable standardized FCM (SSFCM) yield was calculated for each cow. Each cow's average daily standardized FCM (SFCM) yield was multiplied by 252 days to obtain her total standardized FCM yield. The sum of all FCM discarded during the treatment period was subtracted from the total standardized FCM yield. The result was then divided by 252 days.

For the purposes of evaluating the effect of treatment on **milk composition** (milk fat, lactose, and protein percent and somatic cell counts), each week's AM and PM milk component values for each cow were weighted by the daily AM and PM milk yields within the week to calculate a weekly milk component value. Any PM or AM fat percent value greater than 8 % again was set to missing. If any of a cow's AM and/or PM milk component values were missing, her week's average milk component value was designated as missing so that, for the evaluation of effects on milk composition, only actual data (i.e., no estimated values) were used in the analysis. Somatic cell count data were converted to log base 10 prior to any statistical analysis.

Milk mineral values (ash, calcium, and phosphorus) were calculated for each cow during each sampled week by averaging replicates for each sampling, and then averaging values for consecutive milkings sampled during that week.

The amount of feed consumed by each cow was calculated for each day by subtracting the feed refused from the feed offered. The consumed value was set to missing if any offered or refused value was missing.

Results of laboratory analysis of TMR and feed ingredient samples were used to calculate feed composition for each day that a particular TMR (also hay at Utah) was fed. Estimated composition for days with missing composition data was the average of values for two adjacent periods.

Net energy of lactation (NE) for TMRs was calculated from NE of the components based on the proportion of each in the total diet. Values for NE for grains and by-products were those documented by NRC (1978). Values for NE for forages were those reported by the feed analysis laboratory for that sample. Dry matter content and all other nutrient values for each sample were also those reported in the feed composition analysis.

Amount consumed and composition for each day were combined to calculate dry matter, net energy, protein, calcium, and phosphorus intakes. These intakes were calculated separately for each feedstuff consumed and summed to obtain total nutrient intake per cow per day. Total daily intake was set to missing on any day that an individual feed consumed was missing.

Average daily dry matter, net energy, protein, calcium, and phosphorus intakes per cow per treatment week were calculated as the mean of non-missing daily intakes.

Energy, protein, calcium, and phosphorus balances were calculated for each cow as follows:

1. The requirements for these nutrients for milk production were calculated from average AM and PM milk yield per treatment week and weekly AM and PM fat test separately, then summed for the average daily requirement per treatment week. The equations for these requirements are as follows (derived from 1978 NRC):

NE Required for Milk Production (Mcal/d):

< or = 3.00 % fat: milk (kg/d) * ((fat (%)*.044)+.158)*2.205
> or = 3.01 and < or = 4.00 % fat: milk (kg/d) * ((fat (%)*.046)+.152)*2.205
> or = 4.01 and < or = 4.50 % fat: milk (kg/d) * ((fat (%)*.036)+.192)*2.205
> or = 4.51 and < or = 8.00 % fat: milk (kg/d) * ((fat (%)*.046)+.147)*2.205

Protein Required for Milk Production (g/d):

< 4.5 % fat: milk (kg/d) * ((fat (%)*10)+47)
> or = 4.5 and <5 % fat: milk (kg/d) * ((fat (%)*12)+38)
> or = 5 and < or = 8 % fat: milk (kg/d) * ((fat (%)*10)+48)

Calcium Required for Milk Production (g/d):

< or = 8 % fat: milk (kg/d) * ((fat (%)*.2)+1.9)

Phosphorus Required for Milk Production (g/d):

< 5 % fat: milk (kg/d) * ((fat (%)*.1)+1.4)
> or = 5 and <5.5 % fat: milk (kg/d) * ((fat (%)*.2)+0.9)
> or = 5.5 and < or = 8 % fat: milk (kg/d) * ((fat (%)*.1)+1.45)

2. The daily maintenance requirement was calculated as (1978 NRC):

net energy (Mcal/d) = .08 * (body weight)^3/4
protein (g/d) = 3.61 * (body weight)^3/4 + 50.06
calcium (g/d) = 0.185 * (body weight)^3/4 -1.256
phosphorus (g/d) = 0.137 * (body weight)^3/4 + .411

where body weight was in kilograms and weekly weights were estimated by interpolation between actual weekly weights.

3. Total nutrient requirement for milk and maintenance was the sum of the two individual parts. Nutrient balance was calculated on a weekly basis as the net nutrient intake minus the sum of the nutrient requirements for milk and maintenance.

Statistical Analyses:

Because primiparous cows are generally still growing and have a lactation curve shaped differently from multiparous cows, analyses of all effectiveness data and animal safety data (excluding injection site reaction and body temperature data) were performed separately for the two parity groups. Statistical analyses were conducted using the Statistical Analysis System (SAS). Data were analyzed using analysis of variance. The pooled analyses used the linear model $Y(i,j,k) = U + T(i) + S(j) + T(s,i,j) + B(k)(S(j)) + b*PRE(i,j,k) + e(i,j,k)$, where U = overall mean, T(i) = treatment effect, S (j) = location

effect, T(s,i,j) = treatment by location interaction, B(k)(S (j)) = block (within location) effect, PRE(i,j,k) = covariate, b = regression coefficient for PRE(i,j,k), and e(i,j,k) = residual. The treatment by location interaction, when significant ($P < 0.25$), was used as the error term to test the significance of the treatment effect. Treatment effects were deemed to be significant when $P < 0.05$ for effectiveness data and $P < 0.10$ for safety data. Each cow's average 14-day pretreatment value for each variable was expressed as the deviation from the location and parity mean and included as a covariate in the analysis for that variable to reduce experimental variation. Variables not covariately adjusted (e.g., somatic cell counts) are so indicated where results are tabulated.

Analyses of effectiveness data were first performed on average daily values computed up to 18 completed cycles (252 days of treatment). Variables were first subjected to tests for normality for information only; no transformations were made for non-normal data. Bartlett's test for homogeneity of variance across locations was also performed. For pretreatment effectiveness data, Bartlett's test was based on a model that did not include treatment or the treatment by location interaction. For treatment period data, the full model was used for Bartlett's test. Significant heterogeneity ($P<0.005$) required that analyses be weighted using the reciprocal of the residual variance for each location.

Nutritional parameters, body weight, and body condition score data were also analyzed as daily averages calculated over four 56-day periods of treatment and for the four periods combined (224 days). Pretreatment values were used to reduce experimental variation in each analysis, and each was weighted if the 252 day average for that variable had significant heterogeneity of variance across locations. Treatment effects were determined for each period separately. Although not analyzed by repeated measures techniques, these analyses allowed for comparison of treatment effects during different time intervals of the entire lactation.

Linear-plateau modelling (Anderson and Nelson, 1975 and 1984) was used to determine the effective dose range of sometribove. The relationship examined was between the dose administered and average daily SSFCM and adjusted for the effects of location, block, and pretreatment production (deviations from within location by parity mean used as a covariate). Five types of models were used with notation following Anderson and Nelson (1975) and dose = 0, 1, 2, 3 for the 0, 250, 500, 750 mg/14 day treatments, respectively:

1. Model II - a straight line where response = [[beta(0)]] + [[beta(1)]] *dose + [[epsilon]].

2. Model III - a linear plateau model where response = [[beta(0)]] + [[beta(1)]] *dose + [[epsilon]]. Two submodels were tested, III-1 with plateau beginning at dose 1 (250 mg/14 days) and III-2 with plateau beginning at dose 2 (500 mg/14 days).

3. Model IV - A linear plateau model where response = [[beta(0)]] + [[beta(1)]] *dose, + [[beta(2)]] *dose + [[epsilon]]. Two submodels were tested, IV-1 with plateau beginning after dose 1 (250 mg/14 days) and IV-2 with plateau beginning after dose 2 (500 mg/14 days).

4. Model V - two sloping lines where response = [[beta(0)]] + [[beta(1)]] *dose, + [[beta(2)]] *dose + [[epsilon]]. Two submodels were tested, V-1 with lines intersecting at dose 1 (250 mg/14 days) and V-2 with lines intersecting at dose 2 (500 mg/14 days).

5. Quad - a quadratic model where response = [[beta(0)]] + [[beta(1)]] *dose + [[beta(2)]] *dose* + [[epsilon]].

Four models were excellent candidates to fit the results for each parity separately. These are the linear, quadratic, IV-2, and V-1. All had R^2 values greater than 0.96. However, the quadratic model was not appropriate since all coefficients in the model were not significantly different from zero, and the model degenerates to the linear model. For model V-1, the slopes of the two line segments do not differ, leaving a linear model. For model IV-2, the breakpoint to a plateau can be calculated to occur above the highest administered dose. This violates the assumption that the plateau occurs before the highest administered dose, and causes the model to degenerate to the linear model. Therefore, the linear model was chosen as the best candidate for an appropriate dose titration over this dose range. The linear model mean squared error also best approximates the ANOVA mean squared error.

Results:

Data from the four locations were intended to be pooled to evaluate the measure of effectiveness, salable standardized FCM (SSFCM). However, the SSFCM data from the Utah location were excluded from this analysis. As discussed previously, most mastitis cases at the Utah location were not treated, thus, it was not possible to quantitate the amount of milk that would have been discarded due to mastitis therapy. Also, the incidence and duration of mastitis cases may have been affected by the lack of treatment, which would have also affected the calculation of SSFCM. Thus, the Utah data were excluded from the analysis of effectiveness in increasing the production of SSFCM.

Average SSFCM yields by parity for the remaining three study locations are provided in the following table:

Table 3.

	Sometribove Dosage (mg/14 days)			
Study	**0**	**250**	**500**	**750**
Primiparous				
Arizona	26.2**a** ± 1.79*****	28.6**a** ± 1.72	28.3**a** ± 1.74	32.8**b** ± 1.75
Cornell	25.2**a** ± 0.80	27.5**ab** ± 0.78	27.4**ab** ± 0.88	29.9**b** ± 0.82
Florida	19.1**a** ± 1.16	21.4**ab** ± 1.06	23.4**bc** ± 1.00	26.2**c** ± 1.16
Multiparous				
Arizona	25.4**a** ± 1.85	28.6**a** ± 1.88	26.9**a** ± 1.94	31.4**b** ± 1.90
Cornell	27.6**a** ± 1.16	30.9**ab** ± 1.16	33.4**b** ± 1.31	33.9**b** ± 1.22
Florida	19.3**a** ± 1.25	25.0**b** ± 1.32	24.6**b** ± 1.23	26.8**b** ± 1.13

a,b,c Means with unlike letters (a,b,or c) are significantly different ($P < 0.05$).

***** Results represent least-squares means (kg/day) ± standard errors of the mean.

As shown in the following table, statistical analysis of the SSFCM data pooled across the three locations showed a linear response in SSFCM in both parity groups. The response at 250 mg per biweekly administration was significantly greater than in controls, and the response was linear through the highest dose tested, 750 mg per biweekly administration. The dose requested by the sponsor, 500 mg administered every 14 days, was within the range of effectiveness and therefore approvable for use.

Table 4.

The effect of sometribove administered at 0, 250, 500, and 750 mg/14 days on salable standardized 3.5 % fat-corrected milk production in primiparous and multiparous cows; pooled analysis of the three locations.

		------Sometribove Dosage (mg/14 days)------			
Parameter*		**0 mg**	**250 mg**	**500 mg**	**750 mg**
		--------**Mean SSFCM Production (kg/day)**---------			
Primiparous Cows**	(N)	22.7 (18)	25.0 (19)	26.0 (19)	28.8
Multiparous Cows**	(N)	24.2 (25)	27.7 (24)	28.1 (23)	30.8

***Results are reported as least-squares means.
****Overall treatment effect and linear contrast were significant (P<.0001).

The milk yield response in the pooled analysis of all four locations exhibited a cyclic pattern for all sometribove doses throughout each 14-day injection cycle, peaking on approximately day 8 and having the lowest response at the beginning and end of each cycle. As seen in Table 5, the difference between the daily milk yield at the lowest point of response and the peak of the cycle was approximately 10-12 percent.

Table 5.

Milk yield cyclicity of the 500 mg/14 day sometribove group pooled 4-location study

Day of Cycle	Controls Pooled	500 mg/14 days Pooled	500 mg/14 days Primiparous	500 days Multiparous
	----------Average Daily Yield Actual Milk (kg/d)----------			
1	25.74	27.57	26.27	28.68
2	25.98	28.64	27.40	29.69
3	25.74	28.97	27.48	30.25
4	25.56	29.30	27.93	30.48
5	25.60	29.15	27.66	30.43
6	25.59	29.91	28.34	31.27
7	25.59	30.11	28.68	31.34
8	25.49	30.49*	28.97*	31.79*
9	25.49	30.23	28.77	31.45
10	25.21	29.80	28.32	31.07
11	25.15	29.25	28.01	30.30
12	25.10	28.36	27.12	29.43
13	25.20	27.96	26.65	29.04
14	25.21	27.34	26.32	28.21

* Peak value

All four locations were included in an evaluation of the effects of sometribove treatment on milk composition through 252 days of treatment. As shown in Tables 6 and 7, there was no significant effect on overall milk composition in either parity group.

Table 6.

	Sometribove Dosage (mg/14 days)			
Variable*	**0**	**250**	**500**	**750**
Number of Primiparous Cows	26	27	27	25
Fat, %	3.66	3.57	3.58	3.62
Protein, %	3.21	3.28	3.20	3.23
Lactose, %	4.94	4.94	4.97	4.97
Ash,** %	0.74	0.76	0.74	0.75
Calcium,** ppm	1081	1139	1071	1056
Phosphorus,** ppm	904	964	924	927

*Results reported as least squares means.
**Excludes one 250 mg cow missing pretreatment value to use as covariate.

Table 7.

	Sometribove Dosage (mg/14 days)			
Variable *	**0**	**250**	**500**	**750**
Number of Multiparous Cows	35	34	32	35
Fat,** %	3.59	3.49	3.52	3.48
Protein,*** %	3.22	3.28	3.24	3.20
Lactose,*** %	4.76	4.78	4.77	4.77
Ash,%	0.74	0.75	0.73	0.74
Calcium, ppm	1050	1048	1019	1020
Phosphorus, ppm	854	899	885	896

*Results reported as least squares means.
**Excludes two 0 mg and one 250 mg cows missing pretreatment value to use as covariate.
***Excludes one 0 mg and one 250 mg cows missing pretreatment value to use as covariate.

There was a small effect on cyclicity of fat, protein, and lactose content in milk.

Figure 1 - Effect of varying doses of sometribove on percent milk fat of primiparous cows.
Figure 2 - Effect of varying doses of sometribove on percent milk fat of multiparous cows

Figure 3 - Effect of varying doses of sometribove on percent milk protein of primiparous cows.
Figure 4 - Effect of varying doses of sometribove on percent milk protein of multiparous cows.

Figure 5 - Effect of varying doses of sometribove on percent milk lactose of primiparous cows.
Figure 4 - Effect of varying doses of sometribove on percent milk lactose of multiparous cows.

At the 500 mg dose, fat and protein percents were slightly increased for both parity groups (0.14 and 0.06 percentage units, respectively) and lactose percent was slightly decreased (0.02 percentage units) for primiparous cows in the second week compared to the first week of the treatment cycle. However, because cows within a herd are generally at various stages of lactation, and stage of lactation had a greater impact on milk composition than cyclicity due to treatment with sometribove, these minor effects would be expected to have no impact on average milk composition of bulk tank milk.

Dry matter intake increased for both primiparous and multiparous cows (see Table 44 in Section 6.h). Feed efficiency could not be evaluated in this study since the change in body condition over the treatment period was not similar across treatment groups for each parity for the cows treated at the 500 and 750 mg level. For further discussion of body condition see Section 6.h. Mastitis data are reviewed in Section 6.j. Reproduction data are discussed in Section 6.i. Health data are discussed in Section 6.k. Data on offspring are discussed in Section 6.l.

Conclusions:

The increase in marketable (salable) milk in response to sometribove in lactating dairy cows was linear in both parity groups. The response at 250 mg every 14 days was significantly greater than in controls, and the response was linear through the highest dose tested, 750 mg every 14 days. The dose requested by the sponsor, 500 mg administered every 14 days, was within the range of effectiveness and therefore approvable for use. The milk yield response exhibited a cyclic pattern during each 14-day injection cycle, with greatest response during the middle of each cycle; this effect is described in the product labeling so that dairy producers are aware of the pattern in milk yield during treatment. Administration of sometribove had no significant effect on the overall composition of milk.

Figures 1-6 go here

b. Tailhead Versus Postscapular Subcutaneous Administration Studies

Two field studies were conducted to evaluate the effectiveness of sometribove by SC administration in the ischiorectal fossa (depression on either side of the tailhead region,

subsequently referred to as tailhead) compared to the postscapular region in increasing the production of 3.5 % fat-corrected milk. Results from these studies, as well as histopathological data (see Section 6.g), were used to determine whether the tailhead was an acceptable SC route of administration for sometribove.

Study No. 100-AZF-COW-JAD-89-075

Investigators: D. Armstrong, Ph.D.
A. Burgos, M.S.
University of Arizona
Tucson, AZ 85721

Eighty-eight Jersey cows from a commercial dairy farm in Arizona were assigned to one of three treatment groups: control; 500 mg sometribove administered SC in the postscapular (PS) region every 14 days; or 500 mg sometribove administered SC in the tailhead (TH) region every 14 days. The distribution of cows started on treatment is provided below:

Table 8.

Parity	Control	500 mg-PS	500 mg-TH	Totals
Primiparous	9	8	8	25
Multiparous	31	16	16	63

Following a 14-day pretreatment period, a 14-week (98-day) treatment period was initiated in which controls were untreated and the sometribove treatment groups received a maximum of 7 injections. Treatment was initiated on the same calendar day for all animals, and cows ranged from 59 to 179 days postpartum on this date.

Cows were housed in an open-lot corral throughout the study and fed a total mixed ration which met or exceeded nutritional requirements recommended by NRC (1989).

The cows were milked twice a day. Daily milk yield per cow was measured once every week, with consecutive PM and AM milk weights recorded on days -10 and -3 relative to treatment initiation during the pretreatment period, and then on days 3 and 10 following each treatment injection. Milk was also sampled on these days, and daily PM and AM samples were composited and analyzed to estimate the average weekly milk fat percent value for each cow. Each cow's milk fat percent and average daily milk yield for the week were used to calculate an average daily FCM yield per week as described in Section 5.a.

Two primiparous cows (one in each sometribove treatment group) and 9 multiparous cows (six 500 mg-PS; three 500 mg-TH) missed one of the 7 injections during the

treatment period. Average daily FCM yield for these cows during these weeks of the treatment period was considered missing.

During even-numbered treatment weeks, milk fat percents or milk weights that were not recorded (human or mechanical error) were estimated from the average of the cow's adjacent even weeks' values; missing values during odd-number treatment weeks were similarly estimated. One multiparous control cow was excluded from analysis because her milk yields were not recorded for three consecutive weeks.

An average daily FCM yield during the treatment period was calculated for each cow from all treatment weeks in which injections were not missed. Results were analyzed using analysis of variance, fitting the model Y(i,j,k) = U + T(i) + B(j) + b*PRE(i,j,k) + e(i,j,k), where U = overall mean, T(i) = treatment effect, B(j) = block effect, PRE(i,j,k) = covariate (14-day pretreatment FCM yield), b = regression coefficient for covariate, and e(i,j,k) = residual. Results are provided in Table 9. There was no difference in FCM response in dairy cows administered sometribove subcutaneously in either the postscapular or tailhead region.

Table 9.

Effect of sometribove on 3.5 % fat-corrected milk (FCM) production (kg/d) during the treatment period.

		-------Sometribove-------	
	Control	**Postscapular**	**Tailhead**
Primiparous (N)	19.9**a**(9)	24.3**b**(8)	24.3**b**(8)
Multiparous (N)	21.9**a**(30)	27.4**b**(16)	27.5**b**(16

a,b Means with different letters (a or b) are significantly different (P

Study No. 100-IDA-COW-GAG-88-129

Investigator: G. A. Green, D.V.M.
Monsanto Company
St. Louis, MO 63198

Forty-eight Holstein cows from a commercial dairy farm in Idaho were assigned to one of three treatment groups: control; 500 mg sometribove administered SC in the PS region every 14 days; or 500 mg sometribove administered SC in the TH region every 14 days. The distribution of cows started on treatment is provided below:

Table 10.

Parity	Control	500 mg-PS	500 mg-TH	Totals
Primiparous	9	6	3	18
Multiparous	15	6	9	30

Following a 14-day pretreatment period, a 12-week (84-day) treatment period was initiated in which controls were untreated and the sometribove treatment groups received a total of 6 injections. Treatment was initiated on the same calendar day for all animals, and cows ranged from 65 to 175 days postpartum on this date.

Cows were housed in free-stall lots throughout the study and fed one of four total mixed rations balanced to meet or exceed nutritional requirements recommended by NRC (1989) depending on stage of lactation, parity, production, and body condition score.

The cows were milked three times a day. Weight of milk yield was recorded for each cow at each milking during the pretreatment and treatment period. Daily milk yield for each cow was the sum of the three milkings per day and was set to missing if the yield at any milking was not recorded. An average daily milk yield was calculated for each cow during each treatment week from all non-missing daily milk yields measured that week. Beginning the week prior to treatment initiation, each cow's milk was sampled once a week (days 2 and 9 post-injection) during the three milkings that day. The three samples were composited and analyzed to estimate the animal's average weekly milk fat percent value. Each cow's milk fat percent and average daily milk yield for the week were used to calculate an average daily FCM yield per week as described in Section 5.a.

During even-numbered treatment weeks when a cow's milk fat percent value was missing, the average of her adjacent even weeks' values was used as a replacement; missing values during odd-number treatment weeks were similarly estimated.

An average daily FCM yield during the treatment period was calculated for each cow from her weekly values. Results were analyzed using the model described for the Arizona Jersey study (#100-AZF-COW-JAD-89-075). One multiparous 500 mg-TH treated cow was diagnosed with lymphosarcoma on day 63 of treatment and was excluded from analysis. Results are provided in Table 11. There was no difference in FCM response in dairy cows administered sometribove subcutaneously in either the postscapular or tailhead region.

Table 11.

Effect of sometribove on 3.5 % fat-corrected milk (FCM) production (kg/d during the treatment period.

		--------Sometribove--------	
	Control	**Postscapular**	**Tailhead**
Primiparous (N)	37.1**a**(9)	42.9**b**(6)	46.0**b**(3)
Multiparous (N)	41.0**a**(15)	44.2**ab**(6)	47.5**b**(8)

a,b Means with different letters (a or b) are significantly different (P

The results of these two studies demonstrated no difference in the FCM response in dairy cows to administration of sometribove when injected subcutaneously in either the postscapular or tailhead region.

6. Animal Safety

a. 14-Day Drug Tolerance Study (30X)

Study No. 100-DDC-COW-JLV-86-011

An acute drug tolerance study was conducted to evaluate the safety of extremely high doses (up to 30X) of sometribove in lactating cows. The study was conducted at Monsanto's Animal Research Center at Dardenne, MO.

Investigator: J. L. Vicini, Ph.D.
Monsanto Company
St. Louis, MO 63198

Clinical Veterinarian: W. J. Cole, DVM, Diplomate ACT.
Monsanto Company
St. Louis, MO 63198

Eight lactating (238-246 days postpartum), pregnant (102-190 days in gestation) multiparous Holstein cows were assigned randomly to receive five concurrent, independent 3000 mg doses of sometribove (a total of 15,000 mg) or saline (control) by SC administration on days 1 and 8 of the 14-day study. This represented 30 times the intended commercial use dose of 500 mg at one-half the intended dosing interval of 14

days. Cows were housed in an evaporatively cooled facility and maintained in individual box-stalls.

Cows were milked twice a day. Weight of milk yield was recorded for each cow at each milking beginning 7 days prior to first injection, and milk composition (fat, protein, and lactose percent and somatic cell count) was measured at AM and PM milkings from each cow on days -4, -2, 1, 2, 4, 6, 8, 10, 12, and 14. A total mixed ration formulated to meet the nutritional requirements recommended by NRC (1978) was fed. Samples of the diet were collected daily, composited weekly, and analyzed for nutrient content. Cows were acclimated to the facility and diet at least 30 days prior to first treatment. Daily feed intake was measured for each cow beginning day -7.

Animals were observed for health status at least twice daily. Heart, respiratory, and rumen motility rates and clinical signs were monitored at -1, 2, 4, and 6 hours relative to sometribove administration on days 1 and 8 and daily throughout the treatment period. Rectal body temperatures were measured twice daily. Animals received physical examinations before and at the end of the treatment period. Body weight was measured on day -7 and at the end of the study. Blood samples were collected for hematology and clinical chemistries on days -4, -2, 1, 8, and 14. Additional blood samples were collected for hormone and metabolite analyses on days -4, -2, 1, 2, 4, 6, 8, 10, 12, and 14 (samples on days 1 and 8 were taken at 0, 6, and 12 hrs postadministration). Results from blood analyses are presented in Section 6.m.2. All cows were necropsied on days 15 or 16. Tissues collected from all cows are listed in Table 12.

Table 12.

Tissues collected from all cows at necropsy.

Cow tissues:

eyes	injection sites	trachea
esophagus	sciatic nerve	skeletal muscle
skin	pituitary gland	thyroids
parathyroids	adrenals	pancreas
ovaries	uterus	thymus
lymph nodes	brain	pineal
spinal cord	lungs	mammary glands
liver	gall bladder	kidneys
urinary bladder	heart	aorta
bone/marrow	spleen	rumen
reticulum	omasum	abomasum
duodenum	jejunum	ileum
colon	cecum	
articular cartilage		

Fetal tissues:

brain	lungs	kidneys
heart	liver	spleen
thyroids	pituitary	adrenals
abomasum	ileum	skeletal muscle
sciatic nerve	thymus	lymph node
bone/marrow	spinal cord	placenta
gonads	trachea	esophagus
uterus		

Quantitative variables were analyzed by analysis of variance, using pretreatment responses, when appropriate, as covariates.

The only pathologic abnormalities detected either grossly or microscopically were injection site lesions. The lesions were limited to the subcutis in 12 of 16 sites examined. In the remaining 4 sites, the inflammation extended into the *M. cutaneous trunci* . All sites had granulomatous inflammation with macrophages, lymphocytes, and giant cells, as well as fibrous connective tissue. Necrosis was found centrally in these granulomata. The lesions graded from moderate to severe and were considered a direct effect of the test article. Injection site reactions resulting from sometribove at the proposed use level were evaluated in additional studies and are discussed in Section 6.g.

No health problems, such as lameness, digestive anomalies, metabolic disorders, tetany, or abortions were observed during the treatment period. Clinical mastitis was not

observed, and milk somatic cell counts were not suggestive of subclinical mastitis in treated cows (Table 13).

Milk and 3.5 % fat-corrected milk production were significantly increased in treated cows (Table 13). Milk fat percent was decreased during the first week of treatment but not during the second week nor when averaged over the entire treatment period. Milk protein percent was decreased during sometribove treatment, probably due to decreased energy and nitrogen balances as a result of increased milk production and a decrease in feed intake (see below). Milk lactose percent was not significantly affected by treatment.

Table 13.

The effect of sometribove administered as two weekly 30X doses on milk production and composition.

		---------Treatments----------	
Item*	**Week of study**	**Control (n=4)**	**Sometribove**
Milk Yield	-1	14.5	21.2
(kg/day)	1	16.3**a**	21.1**b**
	2	15.3**a**	23.1**b**
	Overall**	15.8**a**	22.1**b**
3.5 % Fat-	-1	15.0	21.1
Corrected	1	16.5**a**	21.1**b**
Milk	2	15.3**a**	24.9**b**
(kg/day)	Overall	15.9**a**	23.0**b**
Fat	-1	3.71	3.44
(%)	1	3.85**a**	3.42**b**
	2	3.72	3.85
	Overall	3.78	3.64
Protein	-1	3.44	3.31
(%)	1	3.45**a**	3.02**b**
	2	3.49**a**	3.17**b**
	Overall	3.47**a**	3.09**b**
Lactose	-1	4.81	4.98
(%)	1	4.87	5.05
	2	4.78	5.03
	Overall	4.83	5.04
Somatic	-1	223	123
Cell Count	1	237**a**	85**b**
(X 1000/ml)	2	140	88
	Overall	188**a**	87**b**

a,b Means within a row with different letter (a or b) are significantly different (P<.05).

***** Results during the treatment period are presented as least-squares means adjusted for pretreatment values.

****** Overall response during the treatment period.

A small, but measurable, increase in average rectal temperature was observed within six hours and peaked within two days of sometribove administration. However, group averages remained within the range of rectal temperatures typically observed in dairy cows (Table 14). Heart, respiratory, and ruminal contraction rates were within normal ranges for dairy cows and were not significantly affected by treatment.

Table 14.

The effect of sometribove administered as two weekly 30X doses on rectal temperature, heart rate, respiratory rate, and ruminal contraction rate.

Response*	Week of Study	Treatments Control (n=4)	Sometribove (n=4)
Rectal Temperature (°F)	1	101.7	102.4
	2	101.6**a**	102.8**b**
Heart Rate (Beats/min)	1	74	82
	2	72	88
Respiratory Rate (Respirations/min)	1	44	55
	2	45	58
Ruminal Contraction Rate (Contractions/min)	1	2	2
	2	2	2

a,b Means within a row with different letters are significantly different (P<.05).

***** Analysis uses mean of one measurement per day (pretreatment value on day 8).

Dry matter intake was depressed in treated cows (Table 15). The increase in milk production and decrease in intake resulted in a significantly decreased net energy balance in treated cows. Body weight was not significantly affected by treatment. (Subsequent long-term studies demonstrated that dry matter intake in sometribove-treated cows increases after several weeks of treatment; see Section 6.h.)

Table 15.

The effect of sometribove administered as two weekly 30X doses on dry matter intake, net energy intake, net energy balance, percent of dry matter intake to body weight, and body weight.

		-------Treatments-------	
Item*	**Week of study**	**Control (n=4)**	**Sometribove (n=4)**
Dry Matter Intake (kg/day)	-1	17.2	17.7
	1	18.3**a**	16.9**b**
	2	18.3**a**	16.5**b**
	Overall**	18.3**a**	16.7**b**
Net Energy Intake (Mcal/day)	-1	26.6	29.4
	1	29.3**a**	27.2**b**
	2	29.5**a**	26.6**b**
	Overall	29.4**a**	26.9**b**
Net Energy Balance (Mcal/day)	-1	5.5	4.6
	1	7.7**a**	1.8**b**
	2	9.0**a**	-1.5**b**
	Overall	8.4**a**	0.1**b**
Dry Matter Intake to Body Weight (%)	-1	2.50	2.74
	1	2.73**a**	2.54**b**
	2	2.74**a**	2.48**b**
	Overall	2.73**a**	2.51**b**
Body Weight*** (kg)	-1	677.0	637.2
	2	682.0	673.8

a,b Means within a row with different letters are significantly different (P<.05).

***Results during the treatment period are presented as least-squares means adjusted for pretreatment values.

****Overall response during treatment period.

*****Body weights were measured at the start of pretreatment and end of the treatment period.

Conclusions

Extremely high doses (up to 30X) of sometribove administered to pregnant lactating dairy cows could result in injection site lesions, an increase in body temperature, and a reduction in feed intake.

b. Multi-lactation Chronic Animal Toxicity Study (TAS)

Study No. 100-DDC-COW-PJE-85-010

A two-lactation chronic toxicity study was conducted to evaluate the safety of sometribove administered intramuscularly to 82 Holstein cows in their first, second, or third lactation. The study was conducted at Monsanto's Dardenne Dairy Center, Dardenne, MO.

Investigator:

P. J. Eppard, Ph.D.
Monsanto Company
St. Louis, MO 63198

Clinical Veterinarian:

W. J. Cole, DVM, Diplomate ACT.
Monsanto Company
St. Louis, MO 63198

Cows were housed in a tie-stall facility with artificial lighting and ventilation. All cows were purchased and calved on-location prior to treatment initiation to allow acclimation.

Cows were assigned to treatment in a randomized block design. Treatments were 1, 3, or 5 concurrent unit doses (unit dose = 600 mg, or 1.2X intended commercial dose) of sometribove or 5 unit doses of excipient administered intramuscularly. Treatments were every 14 days from 60 ± 3 days postpartum until a minimum of 74 days prepartum to allow a sufficient dry period or until average daily milk production declined below 5 kg/day. Open (non-pregnant) cows remained on treatment until 305 days of lactation or until average daily milk production was below 5 kg/day, whichever occurred first, at which time they were necropsied. Injections were alternated among 10 different sites in the semitendinosus, semimembranosus, and gluteus muscles.

The distribution of the 82 cows started on treatment during the first lactation of the study is provided in Table 16.

(Eds. note: The following table consists of 7 columns.)

Table 16.

Distribution of cows initiating treatment in Lactation 1 and necropsied at the end of the lactation.

Sometribove Dosage	-----Parity----- 1	2	3	Totals	-Animals Necropsied*- Pregnant	Nonpregnant
	---Number of animals---				---Number of animals---	
0 mg (0X)**	6	7	7	20	5	1
600 mg (1.2X)	7	7	7	21	3	9
1800 mg (3.6X)	7	6	7	20	3	7
3000 mg (6X)	7	7	7	21	2	9
Totals	27	27	28	82	13	26

* Necropsied at or near the end of lactation. Excludes two 1800 mg and one 3000 mg cows which were necropsied as found dead or moribund during the first lactation.

** Multiple of intended commercial dose of 500 mg/14 days.

Three cows were removed from the study prior to completing the first lactation treatment period:

Table 17.

Dose	Parity	Cow ID	Days on Treatment	Reason
1800 mg	1	85075	35	Pneumonia
	3	85117	23	Died; Mastitis
3000 mg	1	85005	136	Left Displaced Abomasum

All open cows and selected pregnant cows from the various treatment groups were euthanized at the end of the first lactation. Thirty-nine cows underwent complete necropsy (see Table 16); of these, twenty-six were not pregnant and thirteen were pregnant.

The forty remaining cows began the dry period following the first lactation of treatment. One 1800 mg cow (85078, 3rd parity) had toxic mastitis during the dry period and also aborted her calf about one month before expected calving. She was euthanized and necropsied.

Thirty-nine cows began the second lactation of study; one cow in the 600 mg treatment group and her second parity during the first lactation (85690) was euthanized and necropsied at day 38 of lactation for paratuberculosis. Thus, 38 cows started on treatment in the second lactation (Table 18).

(Eds. note: The following table consists of 7 columns.)

Table 18.

Distribution of cows started on treatment in Lactation 2 and remaining at the end of the lactation.

Sometribove Dosage	Parity 2	Parity 3	Parity 4	Totals	Animals Remaining* Nonpregnant	Animals Remaining* Third Lactation Post Calving
	-Number of Animals-			----Number of Animals----		
0 mg (0X)**	4	5	5	14	0	14
600 mg (1.2X)	2	3	3	8	3	3
1800 mg (3.6X)	2	3	2	7	1	6
3000 mg (6X)	1	5	3	9	2	7
Totals	9	16	13	38	6	30

* Excludes two 600 mg cows necropsied as found moribund during the second lactation.

** Multiple of intended commercial dose of 500 mg/14 days.

Two cows were removed from the study prior to completing the second lactation treatment period:

Table 19.

Dose	Parity (2nd Lactation)	Cow ID	days on Treatment	Reason
600 mg	3	85771	125	Mastitis; Left Displaced Abomasum
	4	85017	120	Pneumonia

Six cows remained open during the second lactation of treatment (Table 18). Thirty cows began the third lactation of study after giving birth. These cows were euthanized 2-3 weeks into lactation (Table 18). Four control and four 3000 mg cows that began the third

lactation underwent complete necropsy (gross anatomic pathology and histopathology). The remaining cows underwent postmortem evaluation.

Cows were milked twice daily at 12-hour intervals, and weight of milk yield was recorded for each cow at each milking throughout both lactations. The appearance of mastitic/abnormal milk was recorded. Milk samples were collected four times each week at consecutive PM, AM, PM, and AM milkings (days 5, 6, 12, and 13 post-injection) and analyzed for fat and protein percent and somatic cell count. Milk samples were collected at four consecutive milkings per week (days 4, 5, 11, and 12 post-injection) for analysis of lactose content. Milk ash, calcium, and phosphorus (both lactations) and magnesium and zinc (2nd lactation only) were measured at approximately monthly intervals.

Cows were fed diets formulated to meet or exceed nutritional requirements recommended by NRC (1978). Cows were fed twice daily, and amounts of feed offered and refused were recorded daily throughout the study. Samples of each diet were collected daily, composited weekly, and analyzed for nutrient content. Body weights were measured once weekly from parturition until dry-off for each cow.

Estrus detection was based on visual appraisal. In addition, commercial milk progesterone kits were used during the first lactation of study to aid in estrus detection. Breeding occurred from approximately 40 to 140 days postpartum in the first lactation and days 60 to 275 postpartum during the second lactation. Semen from two sires were randomly assigned for artificial insemination. When prescribed by the staff veterinarian, prostaglandins were utilized as a reproductive aid.

The general health status of all animals was evaluated daily throughout the study. Rectal temperatures were measured daily beginning two days prior to injection until treatment was discontinued. Veterinary physical examinations were conducted at approximately 40 and 180 days postpartum and during the last week of treatment in each lactation as well as at two weeks postpartum in the third lactation. Blood and urine samples were collected at physical examinations and analyzed. Any animal that became moribund or died on study was necropsied.

Blood was sampled during treatment weeks -2, -1, 3, and 7 and then every 8 weeks (year 1) or every 4 weeks (year 2). Whole blood was analyzed for hematology parameters. Serum or plasma samples were analyzed for clinical chemistries and hormones and metabolites, as well as anti-somatotropin binding.

All calves born on study were observed for 30 days following birth. The male calves born following the first lactation were then necropsied; the female calves from the first lactation continued on study through sexual maturity to assess reproductive performance. Female calves from the second lactation were necropsied after the observation period.

Statistics:

Cow 85690, diagnosed with paratuberculosis, and her offspring (twins) were excluded from all analyses. Cows removed early from the study (85075, 85117, and 85005 during the first lactation; 85771 and 85017 during the second lactation) were excluded from the production, reproduction, blood, and urine data analyses for the lactations in which they

were dropped. However, these cows were included in the health and mastitis database so that all health and mastitis incidents were included in the analyses.

Average daily yield of 3.5 % fat-corrected milk (FCM) and milk fat, protein, and lactose percents for 16 treatment cycles (224 days) were analyzed separately for each year of study and for each parity group (primiparous and multiparous). Analysis was also performed on daily averages of each variable calculated over four 56-day periods of treatment to allow for comparison of treatment effects during different time intervals of the entire treatment period. In addition, FCM production standardized to 224 days of treatment as described in Section 6.c was analyzed. Milk minerals (ash, calcium, phosphorus, zinc, and magnesium) were analyzed at each sample period, separately for each year of study and parity group.

Each production variable was subjected to separate normality and heterogeneity tests and model building. The basic model included treatment, block, covariate and, where appropriate, interactions of treatment by block, treatment by parity and block by parity. Cows were blocked by location within the barn. The covariate was the first-year, 2-week pretreatment average, expressed as deviation from mean pretreatment data for the appropriate parity.

Blood data were analyzed separately for each year of study and for each parity group by repeated measures analysis (exceptions listed below). Each variable was subjected to separate normality and heterogeneity tests. Pretreatment data were analyzed by a linear model including treatment and, where appropriate, block, parity and all two-way interactions. Data were pre-adjusted to a baseline covariate using the slope from a model that included the effects listed above for production variables and a repeated measures part including week of treatment, the interaction between treatment and week and, where appropriate, interactions between week and block and/or parity. Modified marginal means for pre-adjusted data were estimated and appropriate standard errors and mean squares for constructing confidence intervals and testing hypotheses were calculated. When the treatment by week interaction or any one of three contrasts comparing control versus sometribove dosage by time was significant ($P < 0.1$) then differences between control and treated cows were examined at each week. Otherwise, only treatment main effects were examined for differences of sometribove groups from control.

Exceptions to the repeated measures analysis were chi-square analyses used for band neutrophils, basophils, eosinophils and monocytes. The treatment week means were categorized and separate dose by category analyses were run for primiparous and multiparous cows for each week sampled. If an overall treatment effect by week was significant ($P < 0.1$) then differences between control and treated cows were examined for each week by running individual chi-square analyses.

Production Response:

FCM production and milk composition were evaluated in this study to determine whether high doses of sometribove over multiple lactations had a negative effect on milk production and also to verify that the study conditions represented a valid system to evaluate animal safety.

During the first lactation of treatment, sometribove treatment increased FCM production at all dosage levels and the response was maintained throughout the treatment period (Table 20). Production in the 4th quarter of treatment for primiparous cows was numerically, but not statistically increased, probably because of low animal numbers. However, FCM production over the entire treatment period was significantly increased in both parity groups.

During the second lactation of treatment, animal numbers were low, particularly in the second parity group. FCM yield was numerically higher in all treated groups compared to controls over all periods of treatment (Table 21).

Milk fat, protein, and lactose percents were not significantly affected by sometribove treatment during each lactation of treatment. Similarly, milk ash, calcium, phosphorus, magnesium, and zinc content were not significantly affected by sometribove treatment.

These results indicated that sometribove-treated cows were able to produce greater yields of milk with normal composition over multiple lactations at doses up to 6 times the intended commercial level.

Pathology:

The pathologic examination of cows, calves, and fetuses included a review of clinical observations, terminal body and organ weights, a macroscopic examination, microbiological culturing of tissues (if necessary), microscopic evaluation, data summarization, statistical analysis, and interpretation of data. For each completely necropsied cow, over 60 samples representing 30 different tissues and representing all major organ systems were collected (see Section 6.a, Table 12) and 17 tissues or organs were weighed routinely at necropsy.

Sometribove did not have any observed effect on nervous (brain, pineal gland, pituitary, spinal cord, sciatic nerve, and eyes), lymphoreticular (cervical, mediastinal, mesenteric or supramammary lymph nodes, spleen, and thymus) and gastrointestinal (esophagus, rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, colon, and exocrine pancreas) systems of cows or calves. Moreover, the incidence of macroscopic and microscopic lesions in parathyroid, trachea, pancreas, urinary bladder, gall bladder, aorta, skin, calves, and fetuses was not influenced by sometribove. This was also true for accessory sex organs of male calves (prostate, seminal vesicles, epididymides, and testes). However, in the necropsies performed following the first year of the study, the percentage of cows (parities pooled) with synovitis/articular erosions in the 0, 600, 1800, and 3000 mg dose groups was 0, 23, 36, and 17 % , respectively. Following the second year of study, the percentage of cows with synovitis/articular erosions was 28, 25, 14, and 22 % across the respective dose groups. (See discussion on the effects of sometribove on lameness in Section 6.f.)

Sometribove also did not affect absolute organ weights and organ-to-body and organ-to-brain weight ratios for brain, pituitary, parathyroids, liver, or lungs of cows. The data suggest that organ weights and organ weight ratios for thyroid and adrenal glands were minimally increased. There were no sometribove-related lesions in thyroid or adrenal glands.

Gross and microscopic findings were generally unremarkable in animals treated with sometribove. Small multifocal adhesions were scattered on the pleural surfaces of the thoracic cavity of 25 treated and 5 control cows. These adhesions were associated with microscopic diagnoses including chronic pleuritis, adhesions/pleural hyperplasia or thickening, chronic pericarditis, hyperplasia of pericardial membranes, epicardial fibrosis, and/or villus hyperplasia of visceral pleura. The pleural and pericardial lesions are common sequelae to severe respiratory disease and/or complications of traumatic reticuloperitonitis. No clinical cardiac abnormalities were detected antemortem.

The incidence and severity of interlobular and interstitial fibrosis in the mammary gland were numerically higher in the 3000 mg cows than controls and, for this reason, mammary tissue was examined microscopically from all cows in the study. The higher incidence of mastitis in the 3000 mg sometribove-treated group (see Section 6.j) was the likely cause of interlobular and interstitial fibrosis in mammary tissue of this group.

Gravid uteri of control and sometribove-treated cows were normal. At or near the completion of the first lactation of treatment, the incidence of microscopic lesions found in non-gravid uteri was higher among sometribove-treated animals.

Intramuscular injection sites were characterized by a localized inflammatory reaction and subsequent persistent scarring. Lesions produced by IM injections were considered to be unacceptable due to hidden injection site carcass damage. The Chronic Animal Toxicity Study employing the IM route of administration was acceptable to support the safety of the compound using a subcutaneous (SC) route of administration because exposure of IM treated animals to the drug was equal to or greater than with SC administration (see Section 6.c). In addition, histological data from injection site reactions following SC administration of sometribove were required to evaluate the acceptability of this injection route (see Section 6.g).

Sixteen fetuses were examined when cows were sacrificed as scheduled during the first or second year of treatment. All fetuses were developing normally.

Nineteen male (first year) and 16 female calves (second year) were sacrificed as scheduled at the completion of the 4-5 week observation period. Neither sometribove nor parity of cows at the start of the study appeared to influence the terminal body weights, absolute organ weights or organ weight ratios of calves necropsied after their dams had been treated for one or two lactations.

Other Results:

Nutritional variables and performance in the early subsequent lactational periods are summarized in Section 6.h. Effects on mastitis incidence are discussed in Section 6.j, reproduction is reviewed in Section 6.i, and cow health in Section 6.k. Blood variable results are provided in Section 6.m.2, circulating anti-somatotropin binding activity is discussed in Section 6.m.1, and urine variable data are reviewed in Section 6.m.4. Effects on rectal body temperature are provided in Section 6.m.3. Effects on offspring are discussed in Section 6.l.

As discussed in the above referenced sections, sometribove-treated cows in the Chronic Animal Toxicity Study, in general, had more health and mastitis incidents and more

reproductive problems than control cows. FDA concluded that effects on cow health, mastitis, and reproduction at doses nearer to the expected use level would need to be examined in the pivotal effectiveness and clinical studies to further evaluate the effects of sometribove on animal safety.

FDA also concluded that intramuscular injection of sometribove was unacceptable due to muscle scarring and that an IM/SC bridging study would determine whether the IM route of administration would be acceptable to support the safety of sometribove administered subcutaneously. Histological data from injection site reactions following SC administration were needed to determine the acceptability of this injection route

Table 20.

The effect of sometribove administered at 0, 600, 1800, and 3000 mg on 3.5 % fat-corrected milk (FCM) production of 1st, 2nd, and 3rd parity cows during each period and overall for the first year of the TAS study.

Parity	Period*	Sometribove Dosage (mg) Control	600	1800	3000	Prob**
		Mean FCM Production***---kg/d ± standard error (number of animals)				
1	Pre	24.5 ± 1.8 (6)	23.5 ± 1.6 (7)	25.2 ± 1.8 (6)	26.4 ± 1.8 (6)	.670
	1	23.0**a** ± 1.0 (6)	29.8**b** ± 0.9 (7)	30.8**b** ± 1.0 (6)	32.2**b** ± 1.0 (6)	<.001
	2	23.2**a** ± 1.2 (6)	30.0**b** ± 1.1 (7)	31.0**b** ± 1.2 (6)	30.4**b** ± 1.3 (6)	.001
	3	23.5**a** ± 2.1 (6)	29.8**b** ± 1.9 (7)	30.6**b** ± 2.1 (6)	26.3**ab** ± 2.1 (6)	.097
	4	20.7 ± 2.8 (5)	25.6 ± 2.3 (7)	24.4 ± 2.8 (5)	22.2 ± 2.9 (5)	.539
	1-4	22.6**a** ± 1.2 (6)	28.7**b** ± 1.1 (7)	29.6**b** ± 1.2 (6)	27.9**b** ± 1.2 (6)	.003
2,3	Pre	29.8 ± 1.0 (14)	28.1 ± 1.0 (13)	30.8 ± 1.1 (12)	31.4 ± 1.0 (14)	.128
	1	26.0**a** ± 0.9 (14)	34.4**b** ± 0.9 (13)	38.2**c** ± 0.9 (12)	37.1**c** ± 0.9 (14)	<.001
	2	21.4**a** ± 1.3 (14)	31.8**b** ± 1.5 (13)	35.2**bc** ± 1.5 (12)	36.3**c** ± 1.4 (14)	<.001
	3	16.4**a** ± 1.8 (14)	26.8**b** ± 2.0 (13)	29.1**b** ± 2.0 (12)	28.5**b** ± 1.9 (14)	<.001
	4	11.9**a** ± 2.4 (10)	16.5**ab** ± 2.2 (12)	21.7**b** ± 2.2 (11)	20.1**b** ± 2.2 (12)	.025
	1-4	19.5**a** ± 1.1 (14)	27.8**b** ± 1.2 (13)	31.4**c** ± 1.2 (12)	31.1**c** ± 1.2 (14)	<.001

a,b,c Least-squares means within a row with unlike letters are different (P< 0.10).

***** Pre = Pretreatment phase. The treatment phase is presented as 4, 8-week periods: 1 = weeks 1-8; 2= weeks 9-16; 3 = weeks 17-24; 4 = weeks 25-32; 1-4 = overall for weeks 1-32.

****** Prob = probability that F-value exceeds calculated F-statistic for treatment main effect.

******* Least-squares means are presented for parity 1 and parity 2,3. Pretreatment and treatment models for parity 1 included treatment and block. Treatment model also included pretreatment values. Pretreatment and treatment models for parity 2,3 included treatment, parity, block, and treatment-by-parity interaction. Treatment model also included pretreatment values.

Table 21.

The effect of sometribove administered at 0, 600, 1800, and 3000 mg on 3.5 % fat-corrected milk (FCM) production of 2nd, 3rd, and 4th parity cows during each period and overall for the second year of the TAS study.

Parity	Period*	Sometribove Dosage (mg)																Prob**
		Control				600				1800				3000				
		Mean FCM Production***---kg/d ± standard error (number of animals)																
2	1	34.1	±	0.6	(4)	45.3	±	2.9	(2)	37.2	±	13.7	(2)	39.0			(1)	NA
	2	26.2	±	1.1	(4)	41.1	±	0.9	(2)	26.6	±	13.1	(2)	32.8			(1)	NA
	3	19.9	±	2.3	(4)	35.2	±	1.1	(2)	19.2	±	12.4	(2)	27.5			(1)	NA
	4	13.5	±	4.5	(3)	28.5	±	1.9	(2)	16.5			(1)	18.7			(1)	NA
	1-4	24.4	±	1.4	(4)	37.5	±	0.2	(2)	24.6	±	10.0	(2)	29.5			(1)	NA
3,4	1	36.3	±	2.8	(10)	44.2	±	4.8	(4)	42.6	±	4.0	(5)	38.8	±	3.3	(8)	.427
	2	29.2	±	2.5	(10)	38.2	±	4.4	(4)	36.0	±	3.6	(5)	33.1	±	3.0	(8)	.257
	3	20.7	±	2.8	(10)	30.2	±	4.8	(4)	28.2	±	4.0	(5)	26.2	±	3.4	(8)	.258
	4	12.9**a**	±	2.2	(8)	19.7**ab**	±	3.5	(4)	19.5**ab**	±	3.2	(4)	24.6**b**	±	2.8	(7)	.031
	1-4	25.3	±	2.3	(10)	33.3	±	3.9	(4)	32.1	±	3.3	(5)	29.8	±	2.7	(8)	.209

a,b Least-squares means within a row with unlike letters are different (P <.10).

***** The treatment phase is presented as 4, 8-week periods: 1 = weeks 1-8; 2 = weeks 9-16; 3 = weeks 17-24; 4 = weeks 25-32; 1-4 = overall for weeks 1-32.

****** Prob = probability that F-value exceeds calculated F-statistic for treatment main effect.

NA = not statistically analyzed.

******* Means are presented for parity 2. Least-squares means are presented for parity 3,4. The model included treatment, parity, treatment-by-parity interaction, and pretreatment values from

the first year of study.

c. Intramuscular Versus Subcutaneous Route of Injection Study (IM/SC Bridging)

Study No. 100-DDC-COW-TCW-86-032

In order for animal safety data from IM studies to be supportive of studies where sometribove was injected SC, exposure of treated animals to the drug would need to be equal to or greater than with SC administration. The purpose of this study was to determine whether studies in which sometribove was administered IM would be supportive of studies where sometribove was injected SC, the intended route of administration. Two measurements were used as prime indicators of relative exposure for the two modes of administration ((IM and SC): 1) production of salable 3.5 % fat-corrected milk standardized to 252 days of treatment ((SSFCM), and 2) blood concentration of somatotropin.

The study was conducted at the Monsanto Animal Research Center, Dardenne, MO.

Investigator: T. C. White, Ph.D.
Monsanto Company
St. Louis, MO 63198

Materials and Methods:

Animals were housed in a tie-stall facility with artificial lighting and evaporative cooling. Sixty-four Holstein cows were randomly assigned within parity (1st or 2nd and 3rd lactation) to receive 500 mg sometribove, IM or SC, or excipient alone (control), IM or SC. The distribution of cows started on treatment is provided below:

Table 22.

Parity	0 mg-IM	0 mg-SC	500 mg-IM	500 mg-SC
Primiparous	3	4	7	7
Multiparous	8	7	14	14

Cows were purchased to arrive at the facility approximately two months prior to expected calving to allow acclimation. Treatment started 60 ± 3 days after calving, and all cows started on treatment within an 8-week span of time. Treatment or excipient was administered every 14 days. In cows that became pregnant, treatment continued until a minimum of 74 days prior to expected calving to allow a sufficient dry period, until average daily milk production in an injection cycle decreased below 5 kg/day, or through 18 injection cycles (36 weeks; 312 ± 3 days of lactation), whichever occurred first. In

open cows, treatment continued through 18 injection cycles or until average daily milk production during an injection cycle decreased below 5 kg/day, whichever occurred first.

Injection sites were shaved prior to administration. Intramuscular injections were administered using a 17-gauge, 1.5-inch hypodermic needle into the left semitendinosus, left semimembranosus or the right semitendinosus muscles. Injections were rotated according to week of study so that six weeks elapsed between repeated use of the same administration area. The SC injections were administered using a 16-gauge, 5/8 or 1-inch needle in the left postscapular, left prescapular, or right postscapular areas. Sites were rotated in a similar manner as for IM administration.

Cows were milked twice daily at approximately 0600 and 1800 hours using standard location procedures. Weight of milk yield was recorded for each cow at each milking from calving until the end of the treatment period. The appearance of mastitis/abnormal milk was recorded. Individual cows' milkings that were discarded (withheld from human consumption) because the animal was administered medication that had a milk withdrawal period were identified.

During injection cycles 1, 7, and 13 (i.e., treatment weeks 1, 2, 13, 14, 25, and 26), milk was sampled from each cow at every PM milking. During remaining injection cycles, milk was sampled from each cow at consecutive PM and AM milkings every 7 days (days 5 and 12 post-injection). Milk samples were analyzed for fat, protein, and lactose percent and somatic cell count. Individual quarter milk samples were collected from each cow at approximately 60-day intervals and cultured to determine the level of subclinical mastitis.

Cows were fed individual diets formulated to meet or exceed nutritional requirements recommended by NRC (1978). Daily feed intake was determined for each cow throughout the study. Animals were weighed weekly starting at parturition. In addition, body condition scores were determined during the pretreatment period and at approximately 30 day intervals throughout the study period.

All cows were bred according to established Monsanto research center procedures. Breeding commenced at approximately 40 days postpartum. Cows which had not conceived after 180 days postpartum were declared open and breeding discontinued.

Cows were observed daily (AM and PM) for signs of disease, injury or other disorders and all adverse and health-related experiences were documented. In addition to daily health observations, general physical examinations were performed at approximately 40 and 180 days postpartum and at completion of the study (approximately 300 days postpartum). At those times, whole blood for hematology and serum/plasma for clinical analyses were obtained. Rectal temperatures were measured daily during the study.

Injection sites were examined and scored on days 2, 9, and 16 postinjection. A description of the scoring system can be found in Section 6.g.

Blood samples were obtained for clinical analyses at the time of physical examinations. Intensive sampling was also conducted in cycles 1, 4, 7, 10, 13, and 16. These samples were analyzed for glucose, thyroxine, creatinine, ß-hydroxybutyrate, non-esterified fatty

acids, blood urea nitrogen, insulin, and somatotropin concentrations. Relative serum anti-somatotropin binding was determined on day 7 (postinjection) samples in cycles 1 and 4.

Data Handling:

Three cows were removed from the study prior to completing their treatment period:

Table 23.

Dose	Parity*	Cow ID	Days on Treatment	Reason
0 mg-IM	M	814	175	Bovine Leukosis
0 mg-SC	P	830	83	Misinjected (Wrong Do
	M	815	155	¾2/3 treatment period low production

* P=Primiparous; M=Multiparous

These cows were excluded from the evaluation of relative exposure, reproduction, and nutritional variables, but their data were included in the analysis of clinical health, mastitis, and injection site reactions. Although cow #814 completed >2/3 of the 252-day standardized treatment period, she was included only in the analysis of clinical health, mastitis, and injection site reactions because she was diagnosed with a chronic disease (bovine leukosis).

Data from the IM and SC-injected control cows were pooled within parity groups for analysis.

For the purposes of evaluating milk production, a cow's last injection cycle was considered complete if at least 11 treatment days of data were available beyond the last injection. If fewer than 11 consecutive days of treatment were available, milk production was considered to be 0 kg/day during this cycle.

An **average daily milk yield** for each week of study was calculated for each cow. If a milk weight during a week was not recorded, the entire day's yield for that cow was considered missing. All of her remaining milk weights for the week were averaged and then multiplied by 2.

An **average daily 3.5 % fat-corrected milk (FCM) yield** for each week was calculated for each cow. A weekly milk fat percent was calculated from the average of all milk samples analyzed that week. The cow's average milk fat percent and average daily milk yield for the week were used to calculate an average daily FCM yield per week as described in Section 5.a.

The **average daily standardized FCM (SFCM) yield** and **average daily salable standardized FCM (SSFCM) yield** over the 36-week standardized treatment period was calculated for each cow using the procedures discussed in Section 5.a.

For the evaluation of serum concentrations of somatotropin, the average circulating concentration, peak circulating concentration, and area-under-the-curve estimate was calculated for each cow during each of the injection cycles of intensive blood sampling (1, 4, 7, 10, 13, and 16).

Analysis of data was as described in Section 5.b. except that no covariate was used for analysis of the area-under-the-curve estimates for circulating somatotropin.

Results:

The results for milk production expressed as salable standardized 3.5 % fat-corrected milk (SSFCM) and standardized 3.5 % fat-corrected milk (SFCM) are summarized in Table 24. In both primiparous and multiparous cows, IM and SC administration of sometribove each produced significant increases (18.2 to 27.3 %) in SSFCM compared with control cows. There was no statistical difference in the SSFCM production between the SC or IM routes of administration.

The results for milk composition analyses are also summarized in Table 24. Percent milk fat was not significantly changed by sometribove treatment in primiparous or multiparous cows. Percent milk protein was slightly but significantly increased in both parity groups. Percent lactose was also increased significantly in multiparous cows but not in primiparous cows. Although statistically significant, the small increases in these variables were within normal variation for Holstein cows and not considered to be of biological importance in terms of milk composition.

Table 24.

The effect of sometribove administered intramuscularly (IM) or subcutaneously (SC) to cows on salable 3.5 % fat-corrected milk (SSFCM) and 3.5 % fat-corrected (SFCM) standardized to 252 days of treatment and on milk composition.*

Lactation Group/ Variable	Sometribove Dosage		
	Control	IM (500 mg)	SC (500 mg)
Primiparous Cows			
(N)	6	7	7
SSFCM, kg/day	24.2**a**	30.8**b**	28.
SFCM, kg/day	25.1**a**	32.2**b**	29.
Fat, %	3.17	3.29	3.
Protein, %	3.13**a**	3.22**b**	3.
Lactose, %	5.08	5.12	5.
Multiparous Cows			
(N)	13	14	14
SSFCM, kg/day	25.1**a**	31.1**b**	31.
SFCM, kg/day	27.2**a**	33.8**b**	34.
Fat, %	3.45	3.39	3.
Protein, %	3.18**a**	3.29**b**	3.
Lactose, %	4.88**a**	4.96**ab**	5.

a,b Means within a row with different letters are significantly different ($P < 0.05$).

***** Results reported as least squares means from covariate analysis from each group.

IM administration of sometribove produced larger increases than SC administration in average and peak circulating somatotropin concentrations and area-under-the-curve estimates (Table 25).

Table 25.

The effect of sometribove administered intramuscularly (IM) or subcutaneously (SC) to cows on circulating somatotropin concentrations.*

Variable/ Lactation Group	**Sometribove Dosage**		
	Control	**IM (500 mg)**	**SC (500 m**
Average Circulating Concentration, ng/ml			
Primiparous Cows	0.6**a**	5.3**b**	3.7**c**
Multiparous Cows	0.2**a**	6.1**b**	3.2**c**
Peak Circulating Concentration, ng/ml			
Primiparous Cows	2.6**a**	12.9**b**	7.9**c**
Multiparous Cows	1.5**a**	11.9**b**	7.8**c**
Area-Under-the-Curve Estimates, ng*day/ml			
Primiparous Cows	9.0**a**	78.5**b**	56.3**c**
Multiparous Cows	2.8**a**	90.0**b**	48.1**c**

a,b,c Means within a row with unlike letters are significantly different ($P<0.05$).

***** Results reported as least-squares means of repeated measures analysis of variance.

Data on nutritional variables are summarized in Section 6.h. Reproduction data are discussed in Section 6.i, mastitis data are reviewed in Section 6.j, and cow health data are discussed in Section 6.k. Circulating anti-somatotropin binding data are reviewed in Section 6.m.1, and hematology and blood chemistry results are presented in Section 6.m.2. Injection site reactions are discussed in Section 6.g, and effect on rectal temperatures is presented in Section 6.m.3.

In summary, the IM route of administration produced a greater exposure to circulating somatotropin than did the SC administration. Therefore, studies in which the drug was administered IM, including the 14-Day Drug Tolerance Study, the Multi-Lactation

Chronic Animal Toxicity Study, and IM clinical studies, were valid in providing animal safety data to support SC administration of sometribove. Effectiveness, as measured by SSFCM yield, was not significantly different between the two injection routes and superior to controls. However, due to the greater exposure to the drug when administered IM, only pivotal studies in which the drug was administered SC were used to provide effectiveness data.

d. Intramuscular Dose Titration Study (Dose-IM)

Study No. 100-FRE-COW-PJE-86-023

A controlled, single lactation study was conducted to evaluate the effects of sometribove injected intramuscularly (IM) over multiple doses. Based on results of the IM/SC Bridging Study, this study was used to provide data on health, nutritional variables, and reproduction of treated cows. Milk production and milk composition were also examined to verify that the study conditions represented a valid system to evaluate animal safety. The study was conducted at Monsanto Animal Research Center, French Village, MO.

Investigator: P.J. Eppard, Ph.D.
Monsanto Company
St. Louis, MO 63198

Eighty-five lactating Holstein cows in their 1st, 2nd, or 3rd lactation were used. Cows were housed in two tie-stall facilities with artificial lighting and evaporative cooling. All cows were brought to the test facility prior to their pretreatment parturition, and individual health and production records were maintained beginning at parturition. The study was conducted as a randomized complete block with a two-way classification. The two classification factors were sometribove dosage (0, 250, 500, or 750 mg/14 days) and parity (primiparous vs. multiparous). All doses were administered from 60 ± 3 days postpartum via IM injection, alternately into the distal and proximal semitendinosus and semimembranosus muscles on the left and right sides (i.e., 8 rotating sites) at 14-day intervals until one of the following: 1) 74 days prior to expected calving; 2) average milk production for the 14-day injection cycle fell below approximately 5 kg/cow/day; 3) moribundity or death; or 4) 400 days postpartum was reached in the case of open, lactating cows. Pregnant cows remained on study and were monitored three weeks following subsequent parturition. The distribution of cows started on treatment is provided in Table 26.

Table 26.

Parity	0 mg	250 mg	500 mg	750 mg	Totals
Primiparous	7	7	7	7	28
Multiparous	14	15	14	14	57

Cows were milked twice daily at approximately 12-hour intervals. Weight of milk yield was recorded for each cow at each milking throughout lactation. The appearance of mastitic/abnormal milk was recorded.

Individual milk samples were collected from each cow weekly on consecutive PM and AM milkings throughout lactation (days 4 and 11 post-injection) for analysis of fat, protein, and lactose percent and somatic cell count. Milk ash, zinc, magnesium, phosphorus, and calcium concentrations were measured in samples collected on consecutive PM and AM milkings during treatment weeks -6, -4, -2, -1, 1, 2, 5, 6, 11, 12, 17, 18, 23, 24, 29, 30, 35, and 36.

All cows were fed diets formulated to meet or exceed requirements recommended by NRC (1978). Cows were offered sufficient feed to allow for a refusal rate of approximately 5 %. Feed refusals were determined for each cow daily throughout the lactation. Rations were sampled daily, composited weekly, and analyzed for nutrient content. Body weights were measured weekly beginning at parturition. Body condition was scored on week 1 of treatment and at the end of each subsequent calendar month.

Estrus detection and breeding were conducted using accepted management practices. Management tools such as KAMAR® strips and cow-side progesterone kits were used to facilitate estrus detection. Breeding commenced approximately 40 days postpartum and animals that had not conceived by 200 days were declared open. Birth weight, height, and heart girth were recorded for all male and female calves born to cows on study. Female calves were raised until approximately 4 weeks of age, and their body weights were measured on a consistent day of each week during this period.

The general health status of all animals was evaluated daily throughout the study. Rectal temperatures were measured daily beginning two weeks prior to treatment initiation. Blood was sampled on days -14 and -7 of pretreatment and on days 0, 7, and 14 of treatment cycles 1, 5, 11, 16, and 22. Serum or plasma was analyzed for somatotropin, insulin, thyroxine, nonesterified fatty acids, beta-hydroxybutyrate, glucose, creatinine, triglycerides, calcium, phosphorus, magnesium, and urea nitrogen. Blood and urine samples were also collected at veterinary physical examinations at approximately 40 and 180 days postpartum, at the last week of treatment, and at two weeks postpartum in the subsequent lactation. These samples were analyzed for hematology parameters, calcium, magnesium, phosphorus, creatinine, and triglycerides.

Four multiparous cows were removed from the study prior to completing their treatment period:

Table 27.

Dose	Parity*	Cow ID	Days on Treatment	Reason
250 mg	M	657	11	Died; Injury
		663	149	Dried Off Early; Lam
750 mg	M	584	168	Chronic Laminitis
		602	26	Died; Aneurism of Cr Mesenteric Artery

*P=Primiparous; M=Multiparous

These cows were excluded from the analysis of production, reproduction, and nutritional variables, with the exception of cow 663, who was excluded from the production and nutritional data but was included in the reproductive data. Each of the four cows was included in the analysis of clinical health and mastitis.

Average daily milk and FCM yields and average milk composition were calculated for each cow using all completed injection cycles through 210 days treatment (15 cycles). Also, an **average daily standardized FCM (SFCM) yield** over the 252-day treatment period was calculated for each cow as described for the IM/SC Bridging study (see Section 6.c). The effective dosage range was analyzed, adjusting for effects of block and pretreatment (14-day) average daily FCM yield.

The results for milk production and milk composition are provided in Tables 28 and 29 for primiparous and multiparous cows, respectively.

Table 28.

The effect of sometribove administered IM at 0, 250, 500, and 750 mg biweekly on milk production and milk composition of primiparous cows.*

Variable	0 mg	250 mg	500 mg	750 mg
Number of Primiparous Cows	7	7	7	7
Milk Yield, kg/d	24.0**a**	26.3**a**	29.4**b**	31.0**b**
FCM, kg/d	25.7**a**	28.3**a**	31.1**b**	33.3**b**
SFCM, kg/d	24.1**a**	26.8**ab**	29.3**b**	32.4**c**
Fat, %	4.02	4.00	3.90	3.97
Protein, %	3.20	3.23	3.28	3.25
Lactose, %	5.20	5.15	5.19	5.18
Ash, %	0.70	0.70	0.70	0.70
Calcium, ppm	1118	1073	1026	1013
Phosphorus, ppm	904	943	919	912
Magnesium, ppm	112	114	115	114
Zinc, ppm	3.3**a**	3.9**b**	3.3**a**	3.4**a**

***** Least squares means; except for SFCM, all variables analyzed through 15 injection cycles (210 days of treatment).

a,b,c Means with unlike letters are significantly different (P<0.05).

Table 29.

The effect of sometribove administered IM at 0, 250, 500, and 750 mg biweekly on milk production and milk composition of multiparous cows.*

Variable	0 mg	250 mg	500 mg	750 mg
Number of Multiparous Cows	14	13	14	12
Milk Yield, kg/d	27.3**a**	29.3a**b**	31.7**b**	29.9a**b**
FCM, kg/d	27.8**a**	30.1**a**	33.2**b**	30.0**a**
SFCM, kg/cow	25.4**a**	27.5**a**	30.8**b**	27.5**a**
Fat, %	3.64	3.72	3.72	3.61
Protein, %	3.14	3.22	3.23	3.19
Lactose, %	4.98	5.00	5.01	4.94
Ash, %	0.70	0.70	0.70	0.71
Calcium, ppm	1024	1030	995	988
Phosphorus, ppm	824**a**	864**b**	873**b**	875**b**
Magnesium, ppm	110	112	114	115
Zinc, ppm	3.0	3.0	3.1	2.9

***** Least squares means; except for SFCM, all variables analyzed through 1 injection cycles (210 days of treatment).

a,b Means with unlike letters are significantly different (P <0.05).

Milk production was higher in treated cows, indicating that study conditions were valid for the evaluation of animal safety. A linear response pattern for production variables was present for primiparous but not multiparous cows. Mean proportions of major milk constituents during the treatment period were not significantly affected by sometribove administration regardless of dosage. Similarly, magnesium and calcium concentrations were not significantly different in treated and control cows. Zinc concentrations were higher ($P < 0.05$) in the 250 mg group (primiparous only) while phosphorus was elevated ($P < 0.05$) for all sometribove-treated groups in multiparous cows. However, the small increases in zinc and phosphorus concentrations for these groups were not consistent across all dosages and both parities. Also, the concentrations did not exceed normal ranges for these variables and were not considered to be of biological importance.

Data for nutritional variables and performance in the early subsequent lactation are summarized in Section 6.h. Reproduction data are reviewed in Section 6.i, mastitis data in Section 6.j, and cow health data in Section 6.k. Calf growth and health data are reviewed in Section 6.l. Blood clinical chemistry and hematology results are summarized in Section 6.m.2. Body temperature data are reviewed in Section 6.m.3. Urine data are discussed in Section 6.m.4.

e. Multi-location Intramuscular Single Dose Study (Single Dose-IM)

The effects of sometribove injected intramuscularly were evaluated using 92 primiparous Holstein cows and 272 multiparous Holstein cows in their second or third lactation at four U.S. locations. Based on results of the IM/SC Bridging Study, this study was used to provide additional data on health, nutritional variables, and reproduction of treated cows. Milk production and milk composition were also examined to verify that the study conditions represented a valid system to evaluate animal safety. Trials were conducted at the University of Arizona, Tucson; Cornell University, Ithaca, NY; Utah State University, Logan; and Monsanto's Dardenne Dairy Center, Dardenne, MO.

Investigators: D. E. Bauman, Ph.D.
Cornell University
Ithaca, NY 14853

J. T. Huber, Ph.D.
University of Arizona
Tucson, AZ 85721

R. C. Lamb, Ph.D.
Utah State University
Logan, UT 84322

W. A. Samuels, Ph.D.
Monsanto Company
St. Louis, MO 63198

All cows calved on-location prior to treatment initiation to allow acclimation. Animals were randomly assigned to one of two treatments, sometribove (500 mg) or placebo (excipient), administered by intramuscular injection every 14 days. Treatments began at 60 ± 3 days postpartum and continued until a minimum of 74 days prior to the expected calving date or approximately 14 days prior to dry off for low production in pregnant cows. Non-pregnant cows received treatments until 400 days postpartum or until approximately 14 days prior to dry off for low production. Site of treatment was rotated among four areas (semimembranosus and semitendinosus muscles) in the rear legs. The distribution of cows started on treatment at each study location is provided in Table 30.

Table 30.

Number of Cows Started on Treatment

Location and Study No.	Parity*	0 mg	500 mg	Totals
Arizona	P	9	9	1
100-ARI-COW-LK-85-039	M	32	31	6
Cornell	P	12	12	2
100-COR-COW-DLH-85-038	M	30	30	6
Dardenne	P	13	13	2
100-DDC-COW-WAS-85-021	M	50	50	10
Utah	P	12	12	2
100-UTA-COW-LK-86-003	M	24	25	4
POOLED	P	46	46	9
	M	136	136	27

* P=Primiparous; M=Multiparous

Animal management followed generally accepted practices for dairy cows. Milking occurred at approximately 12-hour intervals, and milk weights per cow were recorded twice daily beginning at parturition. Milk samples were collected from two consecutive milkings at weekly intervals throughout lactation and analyzed for fat, protein, and lactose percent and somatic cell count. These AM/PM samples were composited at Arizona and Utah and analyzed separately at Cornell and Dardenne. In addition, milk ash, calcium, and phosphorus content were determined on samples collected at one and two weeks prior to treatment initiation and at four-week intervals throughout the remainder of lactation.

Diets were formulated to meet or exceed nutritional requirements recommended by NRC (1978). Feedstuffs and total mixed diets were analyzed for composition on a regular basis. Diets were fed *ad libitum* to obtain a 5 % or greater refusal. Feed ingredients and specific feeding practices varied with location; however, feed consumption was measured daily throughout lactation. Water was available at all times. Animals were weighed weekly (biweekly at Cornell) and body condition scores were assigned at calving, at each physical examination, and throughout lactation at intervals specific to each location.

Reproductive management varied with location but estrus detection was primarily by visual observation of animals at least twice per day by dairy personnel. A scoring system of one to six was used when recording observed signs. At two of the locations (Dardenne and Utah), KAMAR® heat-mount detectors were also used as reproductive aids. All breeding was by artificial insemination during the prescribed breeding period. Animals were bred between days 40 or 50 until at least day 170 postpartum. Breeding beyond this point was at the herdsman's discretion. Cows diagnosed as having a cystic ovary were treated, and prostaglandins were used for estrus synchronization in animals that were problem breeders.

Clinical mastitis was detected by forestripping milk from each quarter onto the floor prior to each milking, and observation of abnormal milk was recorded. Standard operating procedures required the antibiotic infusion of infected quarters, but this practice was inconsistent across locations. Antibiotic infusions were infrequently used for mastitis cases at the Utah location. As discussed in Section 5.a, the lack of an approach to control clinical mastitis rendered the mastitis data from the location incompatible with that from the other three locations. Thus, the Utah mastitis data were excluded from analysis.

Physical examinations were performed by a veterinarian on all cows at days 40 and 180 ± 3 postpartum and during the last treatment cycle. In addition, all animals were observed daily for signs of disease, injury, or other disorders, and observations were recorded. Blood samples (for hematology and serum chemistry measurements) were collected at the day 40 physical examination. Serum chemistry analysis was also carried out on blood samples collected from the cows at day 180 ± 3 postpartum and during the last treatment cycle. However, no blood samples for hematology were collected during the treatment period of the study. Anti-somatotropin binding activity was determined from blood samples collected at 14 and 21 days after the initial treatment, at days 40 and 180 ± 3 of lactation, and during the last treatment cycle. Physical examination, body weight, and general health data were also collected on the calves from these cows through nine weeks of age.

All animals that calved following the lactation of treatment were observed for the first nine weeks into the subsequent lactation. Milk weights, feed consumption, and daily observation data were collected as previously described. No sometribove treatments were administered during this time.

Fourteen multiparous cows were removed from the study prior to completing their treatment period, had chronic health problems through much of the treatment period, or were dried off early (¾ 2/3 standard 252-day treatment period) due to low production:

Table 31.

Dose	Parity*	Study Location	Cow ID	Days on Treatment	Reas
0 mg	M	Arizona	4320	38	Traumatic Reticulopericarditis
		Cornell	3511	61	Paratuber
			3750	21	Misinject (Wrong Day)
			3785	196	Chronic I
		Dardenne	85271	154	< 2/3 tre period due to low produ (remained on study thr calving)
		Utah	5586	73	Lymphosar
500 mg	M	Arizona	3009	140	< 2/3 tre period due to low produ (remained on study unt calving)
		Cornell	3690	54	Paratuber
			3747	21	Misinject (Wrong Day)
		Dardenne	305	70	Systemic Mastitis
			321	86	Died of U Causes
			396	7	Misinject (Wrong Dose and Day)
			85230	121	Klebsiell Mastitis
		Utah	5710	14	Misinject (Wrong Dose)

* M=Multiparous

These cows were excluded from the evaluation of production, reproduction, blood, and nutritional data, but they were included in the analysis of clinical health and mastitis so that all health and mastitis incidents were included in the analysis.

Average daily milk production, average daily FCM production, and average milk composition values were determined for each cow, using data from completed treatment

cycles up to 252 days of treatment. Also, an **average daily standardized FCM (SFCM) yield** to 252 days of treatment was calculated for each cow as described previously (see Section 6.c).

Statistical analyses of milk production and composition data were conducted using the Statistical Analysis System (SAS). Within location variances for FCM milk were found to be homogeneous across the four locations so that weighted analyses were not necessary in pooling the data. The model was Y(ijk) = U + T(i) +S(j) + TS(ij) + [beta]PRE(ijk) + e(ijk); where U = overall mean; T(i) = treatment effect; S (j) = location effect; TS(ij) = treatment by location interaction; PRE(ijk) = covariate; [beta] = regression coefficient for PRE(ijk); and e(ijk) = residual. Whenever the treatment-by-location interaction was significant at $P < 0.25$, the treatment-by-location error term was used to test the significance of the treatment effect. Where location differences were expected, the average two-week pretreatment level for each cow was expressed as a deviation from the location mean for each variable and included in the model to reduce experimental variation.

Results:

Effects on milk production and composition are provided in Table 32.

Table 32.

	Primiparous		**Multiparous**	
Variable*	**0 mg**	**500 mg**	**0 mg**	**500 mg**
Number of Cows	46	46	130	128
Milk Yield, kg/d	26.2a	29.7b	27.0a	30.8b
FCM Yield, kg/d	25.3a	28.8b	26.1a	30.0b
SFCM Yield, kg/d	24.9a	28.3b	24.4a	28.9b
Fat, %	3.36	3.36	3.42	3.41
Protein, %	3.14	3.17	3.15	3.21
Lactose, %	5.03	5.03	4.88	4.90
Ash, %	0.74	0.74	0.74	0.75
Calcium, ppm	1115	1078	1098	1075
Phosphorus, ppm	911a	942b	876	922

a,b For each variable **within parity group**, means with different superscripts are significantly different (P< .05).

* Results are reported as least squares means.

Production variables were increased 13-18 % by sometribove during the treatment period, indicating that study conditions were valid to evaluate animal safety. Milk composition was not significantly affected with the exception of a small increase in milk phosphorus content for treated primiparous cows. A similar trend was noted for treated

multiparous cows, but the slight increases were within normal variation for Holstein cows and not considered to be of biological importance.

Nutritional variables, reproduction, mastitis, and health data from this study are summarized in Sections 6.h, 6.i, 6.j, and 6.k, respectively. Performance in the early subsequent lactation is reviewed in Section 6.h. Hematology and serum chemistry results are included in Section 6.m.2, and circulating anti-somatotropin binding data are discussed in Section 6.m.1. Health and growth of offspring are presented in Section 6.l.

f. Lameness

Effects of sometribove treatment on the musculoskeletal system were evaluated in the TAS study and the clinical studies discussed in previous sections, as well as a clinical field study.

From the previous sections, the following studies provided data on the effects of sometribove on the musculoskeletal system:

Multi-lactation Chronic Animal Toxicity Study (TAS)
Multi-location SC Dose Response Clinical Study (4 Dose-SC)
Multi-location IM Single Dose Study (Single Dose-IM)
IM Dose Titration Study (Dose-IM)
IM/SC Bridging Study (IM/SC)

Clinical signs, including lameness and injuries to the musculoskeletal system, from 848 dairy cows in the above studies were analyzed. Results and analyses of health data, including musculoskeletal effects, are discussed in Section 6.k on Cow Health.

As presented in Section 6.k (Cow Health), treated primiparous cows in the TAS Study had significant increases in the number of cows affected, days observed, cases observed, and duration of cases of lameness or abnormal gait during the first year of the study. In the second year of the TAS Study, lameness was not increased in the treated primiparous cows. Treated multiparous cows did not have a clear pattern of increased lameness for either the first or second lactation during treatment with sometribove.

In the pooled analysis, lameness tended to be increased in treated multiparous cows compared to controls, but not in treated primiparous cows.

Results of the pooled analysis indicated that, in primiparous cows, there was an increase in total cows affected and total days observed for hock abnormalities. An increase in cows affected with hock abnormalities, particularly swollen hocks, was also evident from analysis of the physical examination data from the pooled clinical trials.

In multiparous cows, an increase in total cows affected and total days observed for hock abnormalities, particularly swollen hocks, was observed in the pooled analysis. An increase in disorders of the foot region was also seen in these trials. An increase in cows affected with knee abnormalities, specifically knee calluses or knee fibrosis, was observed in the physical examination data from the pooled analysis for both parity groups (Section 6.k).

As discussed in Section 6.k on cow health and the following paragraph, the following clinical study was conducted in order to resolve questions on the effects of sometribove on the musculoskeletal system of treated dairy cows.

Study No. 100-USA-COW-RJC-92-007 - A Clinical Field Study

In addition to the unresolved questions on the effects of sometribove on lameness as discussed above, a clinical lameness study was precipitated by results of a postmortem study conducted to evaluate injection sites in sometribove-treated cows from commercial dairy farms (Study 100-DDC-COW-PJE-91-068). Five cows from two commercial dairy herds that had been chronically treated with sometribove (no controls) were sacrificed and complete postmortems were performed. Multiple articular (subchondral) erosions and other joint pathologies were observed in multiple joints of all of the animals at post mortem. However, of 25 subarticular eroded surfaces identified from proximal and distal articular cartilage of long bones from the five cows, only 5 (20 %) occurred in the posterior (rear) limbs while 20 (80 %) occurred in the anterior (front) limbs. If the effect were drug related, a more equal distribution of lesions among joints would be expected. Also, ante mortem swellings (enlarged tibio-tarsal [hock] and radio-carpal [knee joint]) observed in the five cows did not correspond to the intra-articular lesions, 7 of 10 posterior limb joints being enlarged (70 %) versus 4 of 10 anterior joints being enlarged (40 %). This was the opposite result from that which would be expected if joint swellings were a reliable indicator of intra-articular joint lesions. The results were suggestive that some other factor peculiar to the front limbs, such as higher weight bearing, was responsible for the articular lesions rather than any direct effect of sometribove.

Nonetheless, since three of the five cows were clinically lame upon presentation prior to sacrifice, and since the question of lameness as reflected in TAS and pooled analysis results was not fully resolved, the sponsor conducted a further study to evaluate prevalence of lameness in a larger population of chronically treated cows on commercial farms.

Investigators:

R. J. Collier, Ph.D.
W. J. Cole, DVM, Diplomate ACT.
Monsanto Company
St. Louis, MO 63198

Clinical lameness was evaluated in field trial dairy cows that had received sometribove for at least two consecutive lactations. These field trials were designed to evaluate sometribove use under practical farming conditions. Field trial were conducted in Michigan (3), New York (4), and Pennsylvania (1).

Herds for this study were selected based on cow availability, milk yield, management, and feeding practices. All cows were housed in loose housing/free stall arrangements and milked in herringbone-type milking parlors.

Cows selected for lameness evaluation were part of the ongoing field trials evaluating the effect of sometribove on longevity of cows in a specific herd. All cows (188, 94 sometribove-treated, 94 control) were multiparous. Sometribove-treated cows had

completed at least two lactations in which they received at least 11 sometribove injections (500 mg/14 days) per lactation. Control and sometribove-treated cows were matched by parity, age (up to 4 months difference), and stage of lactation. Matched animals also had resided on the farm for a similar time period.

All cows were examined in the standing position by two evaluators (veterinary experts in the study of lameness in dairy cattle) for at least three minutes prior to the evaluation of gait. A similar surface (grooved concrete alleyway) was used at each location for the cows to walk on. The evaluators were positioned in a similar location so as to afford an equal opportunity to observe the animals in motion. Cows were first encouraged to walk away from the evaluators, beginning a distance of ten feet from the evaluators and walking a distance of approximately 40 feet. The cows were allowed to proceed at a walk. Cows were then turned and walked toward the evaluators. Cows were walked a second time and additional times at the request of either evaluator. Evaluators were blinded to treatment and to each other's recorded observations (Table 33). Results were recorded independently by each evaluator.

Table 33. Scoring system for evaluating gait.

Category	Degree of Gait Abnormality	Description
0	None	No visible gait abnormality at a walk; reluctance to walk.
1	Mild	Mild variation from normal gait at wal includes intermittent mild gait asymme mild bi- or quadrilateral restriction movement.
2	Moderate	Moderate and consistent gait asymmetry symmetrical gait abnormality but able without continuous stimulation.
3	Severe	Marked gait asymmetry or severe symmet abnormality.

Following the evaluation of gait, cows were restrained for a physical examination of the limb. Both evaluators jointly conducted each examination and completed a physical examination form after agreeing on results. The following areas of the limbs were examined: shoulder, pelvis, humerus, femur, stifle, tibia, elbow, radius, hock, carpus, metacarpus, metatarsus, fetlock, pastern, coronet, interdigital, and hoof. Each area was evaluated for the following lesions: swelling/enlargement (superficial), swelling/enlargement (deep/bone), swelling/enlargement (synovial), laceration (superficial), laceration (deep), laceration (articular), infection, growth, atrophy, hoof

overgrowth, hoof abnormal overgrowth, hoof crack, and other (defined in comments). The grades given to each area and lesion type were: 0 (none), 1 (mild), 2 (moderate), or 3 (severe).

Kappa coefficients, used to assess evaluator agreement within each gait score as well as across all scores, were greater than 0.7 for all scores. Analyses were conducted on gait abnormality scores from each evaluator separately and on the maximum evaluator score assigned to each animal. The proportions of control and treated animals with gait abnormalities were compared using McNemar's test which is appropriate for dichotomous matched pair data. Because an animal judged by the evaluators to have a gait abnormality score of 1 was not considered lame, lameness proportions were evaluated by collapsing the gait scores into two categories, namely non-lame (0 or 1) and lame (2 or 3). Differences in lameness proportion were tested using McNemar's test. McNemar's test was likewise used to compare the proportions of animals with unequal weight bearing stance. Limb lesions were statistically evaluated within each limb section (shoulder, humerus, etc.) and lesion type. Within each animal, the maximum lesion grade assigned to similar limb sections was analyzed. Proportions of control and treated animals with lesions present were compared using McNemar's test.

There were no differences detected between treated and control cows based on the evaluation of stance (Table 34). Overall 29.0 % of treated and 28.0 % of control cows had uneven stance (P=1.0). The frequency of lameness was not different between treated and control animals (Tables 35 and 36). The frequency of lameness (score 2 and 3) was 39.4 % for controls and 46.8 % for treated cows across all farms (P=.323, Table 35).

Table 34. Evaluation of stance in pairs of sometribove-treated and control cows*.

	----------Control cows----------		
Treated cows	**Even**	**Uneven**	**Total**
Even	48 51.61	18 19.35	66 70.97
Uneven	19 20.43	8 8.60	27 29.03
Total	67 72.04	26 27.96	93** 100.00

* Reported as Frequency/Percent

** One pair dropped due to missing stance evaluation for one cow.

Table 35.

Comparison of gait scores of 0 or 1 versus 2 or 3 in pairs of sometribove-treated and control cows*

	----------Control cows----------		
Treated cows	**0,1**	**2,3**	**Total**
0,1	35 37.23	15 15.96	50 53.19
2,3	22 23.40	22 23.40	44 46.81
Total	57 60.64	37 39.36	94 100.00

* Reported as Frequency/Percent

Table 36.

Comparison of gait scores in pairs of sometribove-treated and control cows.*

	----------Control cows----------				
Treated cows	**0**	**1**	**2**	**3**	**Total**
0	12 12.77	10 10.64	6 6.38	0 0.00	28 29.79
1	9 9.57	4 4.26	9 9.57	0 0.00	22 23.40

In Table 37 are listed the variables from the physical examinations which were found to be statistically significant. Based on maximum lesion grade score, significantly more treated cows than controls had superficial lacerations of the hock (P=.088) and superficial swellings/enlargements of the fetlock (P=.07) (Table 37). Significantly more controls than treated cows had overall abnormalities of the femur (P=.04) and superficial lacerations of the femur (P=.077). In certain herds, specific lesions were noted with increased frequency compared to other herds.

Table 37.

Frequency of significant lesions by farm.

Farm	Hock laceration-superficial	Fetlock swelling-superficial	Femur combined	Femur laceration-superficial
A	0/0*	0/0	0/0	0/0
B	5/4	0/0	0/1	0/1
C	0/0	0/1	1/0	0/0
D	3/1	0/0	0/2	0/2
E	1/0	4/0	1/0	1/0
F	8/2	5/0	0/5	0/3
G	0/0	0/0	0/0	0/0
H	2/2	0/1	0/2	0/1

*Treated/control

In summary, based on results of the TAS, pooled analysis, and field lameness studies, increased enlargements of the hock and calluses of the knee (carpal region) of both parity groups and increased disorders of the foot region in multiparous cows occurred but were unrelated to the prevalence of lameness as determined by the field lameness study. The product labeling states that treated cows may have increased numbers of enlarged hocks and lesions (lacerations, enlargements, calluses) of the knee (carpal region). Treated multiparous cows may have increased disorders of the foot region. The results of these studies did not indicate that use of sometribove increased lameness.

g. Injection Site Reactions

Injection site reactions resulting from sometribove treatment were evaluated in several studies.

Multi-location SC Dose Response Clinical Study (4 Dose-SC)

The methods for the 4 Dose-SC study are described in Section 5.a. Injection site reaction data from the University of Florida location were excluded from analysis because the rotation schedule for injections was different from the other three trials (see Section 5.a).

Injection sites were scored using the following system: 0 = no visible swelling; 1 = a visible swelling of < 10 cm in longest surface dimension and/or < 1 cm in height; 2 = a visible swelling of 10 to 16 cm in longest surface dimension and/or 1 to 2 cm in height; 3 = a visible swelling > 16 cm in longest surface dimension and/or > 2 cm in height and/or other complications (e.g., draining lesion, lameness, hematoma, etc.).

The results of injection site scoring (parities pooled) are presented in Tables 38-41.

Table 38.

Frequency of injection site scores 0, 1, 2, or 3 each week post-sometribove (0 or 500 mg) injection; Arizona Dose-SC Trial.

INJECTION SITE SCORE

	Dosage of Sometribove (0 or 500 mg/14 days)							
	----0----		----1------		-----2------		---3-----	
Week Post-Injection	0 mg	500 mg	0 mg	500 mg	0 mg	500 mg	0 mg	500 mg
1	257 (91)*	74 (28)	25 (9)	152 (58)	1 (0)	37 (14)	0 (0)	1 (0)
2	279 (99)	90 (34)	4 (1)	168 (64)	0 (0)	6 (2)	0 (0)	0 (0)
3	285 (100)	196 (75)	0 (0)	66 (25)	0 (0)	1 (0)	0 (0)	0 (0)
4	284 (100)	236 (90)	0 (0)	25 (10)	0 (0)	0 (0)	0 (0)	0 (0)
5	284 (100)	251 (97)	0 (0)	9 (3)	0 (0)	0 (0)	0 (0)	0 (0)
6	283 (100)	255 (99)	0 (0)	2 (1)	0 (0)	0 (0)	0 (0)	0 (0)
7	283 (100)	257 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
8	282 (100)	254 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*Values within the parentheses are percentages of the total count.

Table 39.

Frequency of injection site scores 0, 1, 2, or 3 each week post-sometribove (0 or 500 mg) injection; Utah Dose-SC Trial.

INJECTION SITE SCORE

	Dosage of Sometribove (0 or 500 mg/14 days)							
	----0----		---1 ------		-----2------		---3-----	
Week Post-Injection	0 mg	500 mg	0 mg	500 mg	0 mg	500 mg	0 mg	500 mg
1	307	34	20	126	1	139	0	27

(Eds. note: The following table consists of 9 columns.)

Table 41.

Frequency of injection site scores 0, 1, 2, or 3 each week post-sometribove (0 or 500 mg) injection; pooled Arizona, Utah, and Cornell Dose-SC Trials.

INJECTION SITE SCORE

	Dosage of Sometribove (0 or 500 mg/14 days)							
	----0----		----1------		-----2------		----3-----	
Week Post-Injection	0 mg	500 mg	0 mg	500 mg	0 mg	500 mg	0 mg	500 mg
1	783 (93)*	206 (26)	60 (7)	368 (46)	3 (0)	191 (24)	0 (0)	29 (4)
2	810 (95)	241 (27)	41 (5)	488 (62)	0 (0)	79 (10)	0 (0)	7 (1)
3	855 (99)	497 (63)	11 (1)	273 (35)	0 (0)	18 (2)	0 (0)	0 (0)
4	856 (99)	689 (88)	9 (1)	93 (12)	0 (0)	3 (0)	0 (0)	0 (0)
5	865 (100)	750 (96)	0 (0)	33 (4)	0 (0)	0 (0)	0 (0)	0 (0)
6	861 (100)	761 (98)	1 (0)	18 (2)	0 (0)	1 (1)	0 (0)	0 (0)
7	862 (100)	772 (99)	0 (0)	7 (1)	0 (0)	0 (0)	0 (0)	0 (0)
8	859 (100)	768 (99)	0 (0)	5 (1)	0 (0)	0 (0)	0 (0)	0 (0)

*Values within the parentheses are percentages of the total counts.

Over 90 % of scores from placebo injections were 0. The few control animals with injection site reactions generally had scores of 1 or 2, and they resolved (score of 0) by week 3 or 4. At the Cornell and Arizona locations, the SC injection of sometribove

generally resulted in little or no injection site reaction (score 0-1). However, at Utah, approximately half the scores during the first week after sometribove injection were scored 2 or 3. At all three study locations, over 95 % of scores were completely resolved within 5 weeks of injection.

Comparison of milk production of the cows that experienced injection site scores of 2 and 3 with those scoring 0 and 1 revealed that there was no relationship between injection site score and milk yield.

IM/SC Bridging Study (IM/SC)

The methods for this study are described in Section 6.c. Scoring of injection sites was as described above for the 4 Dose-SC study.

Injection site scores pooled across parities are presented in Table 42.

Table 42.

Frequency of injection site scores 0, 1, 2, or 3 each week post sometribove (0 or 500 mg) injection; IM/SC Bridging Study.

Inj. Site Score	Dose	WEEK OF SCORE 1	2	3	4	5	6
0	0	294 (97)*	300 (99)	289 (100)	271 (100)	271 (100)	252 (100)
	500IM	238 (66)	273 (77)	312 (91)	320 (100)	320 (100)	299 (100)
	500SC	63 (18)	25 (7)	155 (46)	313 (100)	313 (100)	292 (100)
1	0	8 (3)	2 (1)	1 (0)	0 (0)	0 (0)	0 (0)
	500IM	87 (24)	65 (18)	21 (6)	0 (0)	0 (0)	0 (0)
	500SC	206 (59)	202 (58)	154 (46)	0 (0)	0 (0)	0 (0)
2	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	500IM	28 (8)	17 (5)	6 (2)	0 (0)	0 (0)	0 (0)
	500SC	72 (21)	116 (33)	23 (7)	1 (0)	0 (0)	0 (0)
3	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	500IM	5 (1)	1 (0)	2 (1)	0 (0)	0 (0)	0 (0)
	500SC	6 (2)	4 (1)	2 (1)	0 (0)	0 (0)	0 (0)

* Values within parentheses are percentages of the total count.

Responses to SC administration of sometribove were more readily seen and therefore tended to persist longer than IM injections. In both primiparous and multiparous cows, SC administration sites scored significantly higher than the IM sites due to closer proximity to the skin. Most of the swellings observed in IM treated animals decreased in size by day 9 post-administration, and over 90 % were resolved (site score = 0) by three weeks post-administration. Scores were higher for SC treated cows than for control and IM treated sites but were resolved by week 4 post-administration.

Comparison of milk production of the SC injected cows that experienced injection site scores of 2 and 3 with those scoring 0 and 1 revealed that there was no relationship between injection site score and milk yield.

Study No. 100-USA-COW-RJC-91-072 - A Clinical Field Study

Investigators:

R. J. Collier, Ph.D.
M. F. McGrath, Ph.D.
Monsanto Company
St. Louis, MO 63198

Injection site responses from cows treated with sometribove were evaluated at a single point in time from five commercial dairy farms that were part of a larger clinical field study. Two hundred thirty-two lactating dairy cows were chronically treated with 500 mg of sometribove every 2 weeks with SC injections in the postscapular and/or tailhead region. Of the 232 cows, 13 (6 %) were selected because of persistent injection sites, and 6 of these cows had multiple sites scoring at least 2. The mean number of injections these cows had received was 30. All of the injection sites for these cows were scored for size. The scores were awarded as follows: 0 = no swelling; 0.5 = slightly visible swelling (< 5 cm in longest surface dimension and/or < 0.5 cm in height); 1 = minimal swelling (5 to 10 cm in longest surface dimension and/or 0.5 cm to 1 cm in height); 2 = moderate swelling (10 to 16 cm in longest surface dimension and/or 1 to 2 cm in height); 3 = severe swelling (>16 cm in longest surface dimension and/or >2 cm in height and/or other complications associated with injection site reactions). Injection sites that scored 2 or 3 were collected for histological and microbiological examination by excisional biopsy.

The majority of injection sites scoring 2 or 3 were fewer than 8 days old, suggesting that many of these sites would resolve. Only 4 cows had injection site scores of 2 or 3 that were at least 30 days old. Of the 19 samples examined for microbiological contaminants, 14 were negative. Two sites contained *Actinomyces pyogenes*. The other three samples contained common air contaminants. Therefore, the microbiological evaluation of the injection sites that scored 2 or 3 indicated that there is no direct microbial involvement in the formation of or persistence of the injection site reactions observed.

Five cows that represented both typical (minimal) and large/persistent injection site responses were necropsied. As discussed below (see Study No. 100-DDC-COW-PJE-91-068), it was determined that injection site reactions did not affect surrounding structures (e.g., muscle, uterus, nerves).

Study No. 100-DDC-COW-PJE-91-068 - A Non-Clinical Laboratory Study

Investigator:

P. J. Eppard, Ph.D.
Monsanto Company
St. Louis, MO 63198

Five mature Holstein cows from commercial herds were utilized. The animals had previously received sometribove, 500 mg/14 days, as part of an ongoing field study. In that field study, sometribove had been administered SC in the postscapular and/or the ischiorectal fossa (tailhead).

Clinically, two animals had chronic reactions (persisting for 6 to 12 months) while the other 3 animals had more typical reaction sites. Fibrous masses were identified at the injection sites of all 5 animals. At gross necropsy, articular erosions and other joint pathology were noted in multiple joints of each cow. (See discussion in Section 6.f on lameness.)

Microscopically, granulomatous inflammation was found at nearly all sites characterized by multifocal areas containing macrophages, lymphocytes, polymorphonuclear leukocytes, and giant cells. Granular material was again seen within the macrophages. The overall reaction was supported by fibrous connective tissue while foci of residual sometribove were apparent. These foci were usually surrounded by multinuclear giant cells. Lymphocytes were clumped at the periphery of the inflammation.

Injection site lesions were similar in size and appearance for the two chronic reactor cows and the three typical cows (animals in which injection lesions regress over 14 to 28 days). The only notable difference was an increase in the presence of polymorphonuclear leukocytes in the sites from chronic reacting animals. The injection sites did not affect surrounding tissue or the general health of the animal.

Study No. 100-VER-COW-WAS-86-031 - A Clinical Field Study in Jersey Cows

Investigator:

A. P. Pell, Ph.D.
University of Vermont
Burlington, VT 05405

Forty-six Jersey cows received SC injections of either 500 mg of sometribove or excipient biweekly beginning at about 60 days of lactation and continuing until dry-off. The study was conducted at the University of Vermont. Injection sites were scored on days 3 and 10 after each injection using the same scoring system described for the 4 Dose-SC and IM/SC Bridging studies.

Jersey cows that received sometribove had more swellings at the site of injection than did the controls. On day 3 after injection, 12 % of injection site reactions in sometribove-treated cows (primiparous and multiparous combined) had a score of 0, 37 % had a score of 1, 46 % had a score of 2, and 6 % had a score of 3. On day 10 after injection, 7 % of

injection site reactions in sometribove-treated cows had a score of 0, 37 % had a score of 1, 48 % had a score of 2, and 8 % had a score of 3.

The injection site reactions were usually localized swellings, although some persisted for several months. In some cases, swellings broke open and drained. The study suggested that Jersey cows might be more sensitive to sometribove treatment than Holsteins with respect to injection site reactions. Additional studies, discussed below, were conducted to further evaluate injection site reactions in Jersey cows.

Study No. 100-AZF-COW-JAD-89-075 - A Clinical Field Study in Jersey Cows

Investigator:

J. A. Duque, M.S.
Monsanto Company
St. Louis, MO 63198

The effect of sometribove administration on milk production response was evaluated in lactating Jersey cows on a commercial dairy farm in Arizona, as described in Section 5.b. Eighty-eight cows between 59 and 179 days postpartum were randomly assigned by parity into treated (500 mg sometribove/14 days) or control (untreated) groups. Sometribove injections were given SC in the postscapular or tailhead regions. The experimental period was 14 weeks or 7 injection cycles. Injection sites were evaluated on days 3, 10, 17, and 24 after each injection using the same scoring system described in Study No. 100-USA-COW-RJC-91-072.

No adverse injection site reactions were noted. The highest score assigned was an injection site score of 1 given to cows receiving sometribove postscapularly. A total of 4.2 % of the injections for the animals receiving sometribove postscapularly demonstrated minimal injection site swelling that persisted through 24 days. Only one animal of those treated with sometribove in the tailhead region was noted as having a slight injection site swelling at day 3 postinjection.

Study No. 100-DDC-COW-PJE-92-003 - A Preclinical Study in Jersey Cows

Investigator:

P. J. Eppard, Ph.D.
Monsanto Company
St. Louis, MO 63198

Thirty-eight Jersey cows were assigned to a 2 x 2 factorial design with excipient or 500 mg sometribove treatment and normal or high dietary calcium levels. The study was conducted at Monsanto's Animal Research Center at Dardenne, MO. Cows were injected SC with sometribove or excipient for three, 2-week cycles beginning approximately 4 weeks prior to expected calving date (2 injections in the dry period, 1 injection in the peripartum period). All injections occurred in the tailhead region. If a cow calved 10 or more days earlier or later than expected, fewer or additional injections were administered to allow similar treatment effects during the immediate peripartum period. Injection sites were scored 3 and 10 days after injections so that the number of scores per animal varied

slightly, depending on actual calving date relative to expected date. The scoring system used was the same as that used in Study No. 100-USA-COW-RJC-91-072, above. The highest score assigned on day 3 out of 55 total sometribove injections was one score of 1. On day 10, no injection site swellings were observed.

Conclusions.

In cows injected SC with 500 mg sometribove at 14-day intervals, few injection sites scoring greater than 1 on the scoring scale of 0-3 should be expected. Some cattle may consistently experience reactions scoring 2 or 3, and an occasional site may break open and drain, as happened in the Utah SC-Dose and Vermont Jersey trials. Labeling of the product reflects that a mild transient swelling of 3-5 cm in diameter may occur at the injection site beginning about 3 days after injection and may persist up to 6 weeks following injection. Some cows may experience swellings up to 10 cm in diameter that remain permanent but are not associated with animal health problems. The typical swellings of injection sites are of cosmetic concern only; if permanent blemishes are objectionable to the user, administration of the product to the particular animal should be discontinued. Use of sometribove in cows in which injection site swellings repeatedly open and drain should be discontinued.

There was no significant deleterious effect of repeated injections into the ischiorectal fossa (tailhead) region of cows.

Study No. 100-USA-COW-SCB-89-049 - Carcass Evaluation Study

Investigators:

S. C. Bussen, B.S.
R. J. Collier, Ph.D.
Monsanto Company
St. Louis, MO 63198

Twenty-seven Holstein cows located in New York (7), Michigan (11), and Utah (9) and 4 Jersey cows located in Arizona were examined postmortem to evaluate injection sites and carcass condition. All of the cows had received SC injections of 500 mg sometribove in the postscapular, tailhead, or flank areas. Each cow received between 5 and 27 injections at 14-day intervals. The animals were slaughtered at commercial meat processing plants and the comments of each USDA inspector were documented.

Overall comments from the USDA inspectors regarding carcass condition indicated no observable differences in general carcass condition from other dairy animals processed through their respective facilities. However, there were more injection sites noticed in these cows due to the short time elapsed since the last sometribove injection in most cows (1-12 days).

Of the twenty-seven cows, 17 had lesions that were identified, excised, photographed, and examined histologically. The range in injections identified per cow was from 5 to 28. The 17 cows had received a total of 238 sometribove injections. The time from last injection to post mortem was one to 12 days except for three cows in which the last injections were administered 8-10 months before slaughter.

Examination of photographs comparing injection sites in carcasses injected in either the postscapular or tailhead (ischiorectal fossa) regions led to the conclusion that tailhead injections resulted in less carcass damage, especially hemorrhage and swelling, that might require trimming of underlying muscle. Also, tailhead injection sites were more likely to be removed during the normal trimming process. Most of the injection sites in either region remained on the carcass after the hide was removed. The most recent injections sites (1-2 days old) were characterized by slight swelling and hemorrhage, and were easily noticed after the hide was removed. Histological examination revealed that maximum edema, infiltration of fibrin, and neovascularization was evident at 5-7 days post-injection. During days 7-10 post-injection, infiltration of collagen proceeded while edema and fibrin subsided. From both gross and microscopic observation of the progression of injection response, it appeared that the sites generally reached maximum size and reactivity at days 5-7.

The number of cull dairy cattle coming into some slaughtering plants with **recent** injection sites as a result of sometribove injections could cause an increase in the number of carcasses held for testing for antibiotic residues. Also, because of the gross and microscopic timing of injection site resolution and because the management decision as to whether to reinject or cull a cow would occur at 14-day intervals, in order to minimize injection lesions on carcasses at slaughter, product labeling includes a statement recommending that no injections be given within 2 weeks of expected slaughter.

h. Nutrient Intake, Body Weight, and Body Condition Score

Adequate intake of nutrients by lactating dairy cows is necessary to support milk production, to maintain normal health and reproductive functions and, in primiparous cows, to support growth. Dairy cows are also dependent upon sufficient body energy reserves, as reflected by body condition, to sustain production particularly in subsequent lactations. Thus, the effect of sometribove on nutritionally related variables, including dry matter and nutrient intakes, nutrient balances, body weights, and body condition scores was measured. The need for additional nutrients to support higher milk production was evaluated, and it was determined whether sometribove-treated cows could consume sufficient nutrients during various phases of the lactation cycle to meet these requirements while maintaining appropriate body weight and body condition. In addition, production performance during the beginning of the subsequent lactation was evaluated to determine if there were carry-over effects due to sometribove treatment in the previous lactation. The following studies were used to evaluate these effects:

Multi-lactation Chronic Animal Toxicity Study (TAS)
Multi-location SC Dose Response Clinical Study (4 Dose-SC)
Multi-location IM Single Dose Study (Single Dose-IM)
IM Dose Titration Study (Dose-IM)
IM/SC Bridging Study (IM/SC)

The experimental design of each of these studies is described in Sections 6.b, 5.a, 6.e, 6.d, 6.c, respectively. Animals were fed total mixed diets. In all studies cows were fed

diets that met or exceeded the nutrient requirements as recommended by the National Research Council during the conduct of the study.

Nutrient Intakes and Nutrient Balances

In general, the results indicated that sometribove treatment increased nutrient intake when cows responded with an increase in milk production. Peak feed intake followed peak milk yield by several weeks. The increase in intake associated with sometribove treatment occurred earlier for primiparous cows than multiparous cows. Energy balance is subject to extensive variability which limits the ability to evaluate absolute values; nevertheless, the relative differences in energy balance between treatment groups could be evaluated. Nutrient balances when averaged over the treatment period were unaffected by sometribove, except for calcium and phosphorus balances.

Multi-location SC Dose Response Clinical Study.

Dry matter (DM) and net energy for lactation (NE) intakes were greater at all dosage levels of sometribove compared to the controls for both parities through 252 days of treatment (Table 43). Dry matter intake for primiparous cows was increased by 1.9 kg/day (9.5 %) at the 500 mg dose compared to the controls over the 252-day treatment period. Dry matter intake was increased by 1.5 kg/day (6.6 %) during the 252-day period for the multiparous cows administered 500 mg sometribove compared to control cows. Also, the increases in DM and NE intake were greater for cows treated with the 750 mg dose compared to the 500 mg dose across both parities, although only significantly for the primiparous cows.

When intake parameters were summarized in 56-day periods, the pattern of increase in treated cows was different by parity. Primiparous cows significantly increased net energy intake at each of the four 56-day periods. The multiparous cows treated with sometribove, however, did not increase their net energy intake until the second 56-day period (Table 44). Thereafter, intake of sometribove-treated multiparous cows was higher than the controls for the remaining 224 days of treatment. Thus, there was a longer delay in the response in net energy intake for the multiparous cows compared to the primiparous cows. Dry matter intake response of cows treated with sometribove was similar to net energy intake for both primiparous and multiparous cows. In comparing dry matter intake plots (Figures 7 and 9) with the milk production plots (Figures 8 and 10, see pages 64 and 65), dry matter intake peaked after the peak in milk yield for both parities.

Dry matter and NE intake of treated primiparous and multiparous cows peaked higher than the control cows, and the higher level of intake was maintained throughout the treatment period (Figures 7 and 9).

Energy and protein balances averaged over 252 days of treatment were not affected by sometribove administration (Table 43). However, energy balance of treated cows differed from controls when data were summarized in 56-day periods and also differed by parity (Table 44). The variability in energy balance increased with each 56-day period, because the pattern of repletion of body tissue and the persistency of lactation varied among animals. Significant effects due to sometribove treatment were as follows: 1) energy balance was higher in primiparous cows treated at the 750 mg dose level for days 113 to

168 of treatment compared to controls due to higher intake in the treated cows; and 2) energy balance was reduced in treated multiparous cows during the first 56 days of treatment compared to controls due to an increased milk energy output and a delay in energy intake.

Calcium and phosphorus balances averaged over 252 days of treatment were similar among all treated groups for the multiparous cows (Table 43). In the primiparous cows, calcium balance was higher in the 500 (93 grams/day) and 750 mg/14 days groups (99 grams/day) than the control (87 grams/day). Phosphorus balance was significantly higher in the 750 mg/14 days group (41 grams/day) as compared to the controls (32 grams/day; Table 43).

Table 43.

Effect of sometribove on body weight and condition, nutrient intake, and nutrient balance through 252 days of treatment. Multi-location SC Dose Response Clinical Study (4 Dose-SC).

	----------Sometribove Dosage (mg/14 days)--			--
Parameter**1**	0 mg	250 mg	500 mg	
Primiparous Cows (n)	26	27	27	
Dry Matter Intake (kg/day)	20.0**a** ± .36	21.6**b** ± .36	21.9**b** ± .37	23
.37
DMI as % of BW**2**	3.55 ± .087	3.70 ± .085	3.73 ± .087	3.
.088
Net Energy Intake (Mcal/d)	33.2**a** ± .65	36.2**b** ± .64	36.6**b** ± .65	39
.66
Energy Balance (Mcal/day)**2**	7.01 ± .515	8.00 ± .507	7.77 ± .523	8.
.526
Protein Balance**2** (kg/day)	0.96 ± .059	1.05 ± .058	1.05 ± .059	1.
.060
Calcium Balance (g/day)	87**a** ± 1.9	92**ab** ± 1.9	93**b** ± 1.9	99**c**
Phosphorus Balance (g/day)**2,3**	32**a** ± 1.7	36**ab** ± 1.6	36**ab** ± 1.6	41**b**
Body Weight (kg)	571**a** ± 4.3	584**b** ± 4.2	586**b** ± 4.3	59
4.3
Body Condition Score**2**	3.11 ± .038	3.06 ± .038	3.01 ± .039	2.
.039
Multiparous Cows (n)	35	34	32	

Dry Matter Intake (kg/day)	22.9**a** ± .37	24.3**b** ± .37	24.4**bc** ± .39 ± .36
DMI as % of BW**2**	3.46**a** ± .059	3.67**b** ± .060	3.71**b**c ± .063 ± .059
Net Energy Intake (Mcal/day)	38.2**a** ± .66	40.8**b** ± .67	40.9**bc** ± .70 ± .65
Energy Balance (Mcal/day)**2**	9.57 ± .447	9.92 ± .452	9.77 ± .477 ± .442
Protein Balance (kg/day)**2**	1.3 ± .05	1.3 ± .05	1.3 ± .06 .05
Calcium Balance (g/day)**2**	105 ± 2.8	107 ± 2.8	109 ± 3.0 2.8
Phosphorus Balance (g/day)**3**	40 ± 1.5	43 ± 1.5	43 ± 1.6 1.4
Body Weight (kg)**2**	667 ± 4.4	671 ± 4.4	663 ± 4.6 4.3
Body Condition Score**2,3**	3.20 ± .042	3.15 ± .042	3.03 ± .046 ± .042

a,b,c For each parameter, means with different letters are significantly different (P< .05).

1 Results are reported as least-squares means (± SE of least-squares means).

2 The treatment X site interaction was used as the error term because it was significant (P< .25).

3 Analysis weighted to adjust for unequal variance across sites.

Table 44.

Effect of sometribove on net energy intake and energy balance during four 56-day periods of treatment and over the 224-day combined per Multi-location SC Dose Response Clinical Study (4 Dose-SC).

	Sometribove Dosage (mg/14 days)			
Parameter**1**	0 mg	250 mg	500 mg	750 m
Primiparous Cows**2** (n)	26	27	27	25
Net Energy Intake (Mcal/day)				
Days 1 - 56	33.5**a** ± .52	34.7**ab** ± .51	35.0**b** ± .52	36.0**b** ± .5
Days 57 - 112	34.6**a** ± .69	37.2**b** ± .68	37.8**bc** ± .70	39.3**c** ± .7
Days 113 - 168	34.2**a** ± .86	36.7**b** ± .85	37.8**b** ± .87	41.3**c** ± .8
Days 169 - 224**3**	32.0**a** ± 1.15	37.0**b** ± 1.15	36.1**b** ± 1.17	40.5**c** ± 1.
Days 1 - 224	33.6**a** ± .63	36.3**b** ± .62	36.7**b** ± .63	39.3**c** ± .6
Energy Balance (Mcal/day)				
Days 1 - 56	6.68 ± .406	5.94 ± .400	5.37 ± .412	5.39 ± .41
Days 57 - 112**3**	7.91 ± .540	8.25 ± .531	8.24 ± .548	8.16 ± .55
Days 113 - 168	7.64**a** ± .629	8.21**a** ± .619	8.76**ab** ± .638	10.29**b** ±
Days 169 - 224**3**	6.43 ± .946	9.42 ± .945	8.46 ± .961	10.31 ± .9
Days 1 - 224**3**	7.17 ± .498	7.92 ± .490	7.71 ± .505	8.54 ± .50
Multiparous Cows**4** (n)	35	34	32	35

Net Energy Intake (Mcal/day)				
Days 1 - 56	43.1 ± .59	42.8 ± .59	42.9 ± .63	43.7 ± .58
Days 57 - 112	41.1**a** ± .67	43.2**b** ± .68	43.0a**b** ± .72	44.6**b** ± .6
Days 113 - 168	37.8**a** ± .88	41.7**b** ± .89	41.5**b** ± .94	43.4**b** ± .8
Days 169 - 224	34.1**a** ± 1.06	38.4**b** ± 1.02	38.6**b** ± 1.06	41.2**b** ± 1.
Days 1 - 224	38.9**a** ± .68	41.5**b** ± .68	41.5**b** ± .72	43.2**b** ± .6
Net Energy Balance (Mcal/day)				
Days 1 - 56	10.47**c** ± .449	8.79**b** ± .455	7.30**a** ± .480	7.28**a** ± .4
Days 57 - 112**3**	10.78 ± .548	10.27 ± .555	9.72 ± .585	9.07 ± .54
Days 113 - 168**3**	9.75 ± .629	10.76 ± .636	10.53 ± .671	10.48 ± .6
Days 169 - 224**3**	8.19 ± .841	10.66 ± .810	11.11 ± .844	11.30 ± .8
Days 1 - 224**3**	9.83 ± .443	10.08 ± .448	9.64 ± .473	9.46 ± .43

a,b,c For each parameter, means with different letters are significantly different (P< .05).

1 Results are reported as least-squares means (± SE of least-squares means).

2 Number of primiparous cows in days 169-224 analysis is 26, 26, 27, and 25, respectively.

3 The treatment X site interaction was used as the error term because it was significant (P< .25).

4 Number of multiparous cows in days 169-224 analysis is 31, 33, 31, and 34, respectively.

Figure 7 - Effect of varying doses of sometribove on dry matter intake of primiparous cows

Figure 8 - Effect of varying doses of sometribove on actual milk of primiparous cows

Figure 9 - Effect of varying doses of sometribove on dry matter intake of multiparous cows

Figure 10 - Effect of varying doses of sometribove on actual milk of multiparous cows

Multi-lactation Chronic Animal Toxicity Study.

In general, sometribove-treated primiparous cows tended to have a greater intake (DM and NE) than controls during the first year of the study. By contrast, sometribove had no effect on DM or NE intake in the second lactation of study. (However, numbers of primiparous cows during year two were very low.) Intakes of DM and NE were increased for sometribove-treated multiparous cows for years one and two of the study. The effect

of sometribove on intakes of crude protein, calcium, and phosphorus were similar to the effect of sometribove on DM intake for both groups over the two lactations of treatment.

Analysis of intake data using the cows that completed both lactations showed no effect of sometribove on DM or NE intake for primiparous cows over the 2-lactation period. Numerically, intakes of DM and NE were higher for the primiparous cows treated at the 1800 and 3000 mg levels compared to the control and 600 mg dosage levels for year one. The multiparous cows administered sometribove had significantly greater intakes of DM and NE for both years of treatment (Table 45).

Energy balance was higher in year two compared to year one for the primiparous cows but was not affected by treatment. There was a dose by year interaction for energy balance of multiparous cows. The treated multiparous cows had numerically lower energy balances than the control cows during the first year of treatment and numerically higher energy balances during the second year of treatment. This appears to have been due to a lower energy balance for the control multiparous cows during year two compared to year one (Table 45).

Table 45.

Average dry matter intake (DMI), net energy intake (NEI), and energy balance (EB) of cows administered sometribove for two lactations for the 36-week standardized treatment period (includes only cows that completed two lactations). Multi-lactation Chronic Animal Toxicity study (TAS).

		Sometribove Dosage				Dose	Ye
DxY**1**		--------(mg/14 days)--------					
Item	Year	0	600	1800	3000	P	P
Primiparous Cows (n)		4	2	2	1		
DMI (kg/day)	1	19.7	19.9	21.4	22.7	.61	.67
	2	21.9	22.6	21.9	23.8		
NEI (Mcal/day)	1	32.4	31.8	35.9	38.2	.54	.79
	2	36.4	48.4	36.4	40.3		
EB (Mcal/day)	1	6.82	9.68	7.40	5.88	.30	.07
	2	6.84	10.80	8.25	9.49		
Multiparous Cows (n)		10	6	5	8		

DMI (kg/day)	1	19.4	21.9	22.7	22.5	.0002	.21
	2	19.6	22.8	22.3	23.7		
NEI (Mcal/day)	1	30.5	36.1	38.1	37.7	.0001	.22
	2	32.4	38.8	37.3	39.9		
EB (Mcal/day)	1	8.00	7.40	7.87	6.51	.671	.60
	2	5.94	7.98	7.95	8.51		

1DxY = Dose x Year interaction

Multi-location IM Single Dose Study.

Mean dry matter intake for the 252-day treatment period was increased by 1.3 and 1.5 kg/day for the primiparous and multiparous sometribove-treated cows, respectively. Sometribove-treated primiparous and multiparous cows consumed more NE (2.4 and 2.8 Mcal/day, respectively) than the controls (Table 46).

Table 46.
Dry matter intake (DMI) and net energy intake (NEI) of cows administered sometribove averaged over 36 weeks of the treatment period. Multi-location Intramuscular Single Dose Study.

	Sometribove Dosage (mg/14 days)		
Item	**0**	**500**	**Probability**
Primiparous Cows (n)	46	46	
DMI (kg/day)	19.35 ± .332**1**	20.63 ± .330	.072
NEI (Mcal/day)	31.85 ± .645	34.21 ± .641	.081
Multiparous Cows (n)	130	128	
DMI (kg/day)	21.03 ± .190	22.54 ± .189	.009
NEI (Mcal/day)	34.56 ± .366	37.38 ± .363	.010

1 Each value represents the least-squares mean ± SE of the least-squares mean.

Following pretreatment, sometribove-treated primiparous cows decreased their average daily dry matter intake approximately .5 to 1 kg during the first 4 weeks of treatment. In contrast, the control primiparous cows increased DM intake about 2 kg over pretreatment

levels during this time period. Control primiparous cows peaked in dry matter intake at approximately week 5 to 9 of treatment, which would be week 13 to 17 of lactation. Treated primiparous cows reached a peak in dry matter intake at approximately week 12 to 15 of treatment (week 20 to 25 of lactation) and essentially maintained that level with a small decrease through week 36 of treatment. Intake of treated primiparous cows exceeded controls beginning at approximately 17 to 19 weeks of treatment.

The sometribove-treated multiparous cows increased their DM intake by approximately .5 kg from pretreatment levels over the first four weeks of treatment while the control cows increased DM intake approximately 3 kg over the same time period. The control cows peaked in DM intake at 4 to 6 weeks of the treatment period, whereas, the treated multiparous cows peaked in DM intake at 10 to 13 weeks of treatment and did not achieve the peak levels of intake of the control cows. Intake of the sometribove-treated cows was maintained at this level with only small decreases throughout the treatment period (36 weeks) while the control cows deviated from the treated cows and decreased at a greater rate starting at approximately 21 weeks of treatment.

Mean energy, protein, calcium, and phosphorus balances of the sometribove-treated cows were similar to the controls for primiparous and multiparous cows (Table 47).

Table 47.

Nutrient balances of cows administered sometribove averaged over 36 weeks of the treatment period. Multi-location intramuscular Single Dose Study.

Parameter	Sometribove Dosage (mg/14 days) 0	500
Primiparous Cows (n)	46	46
Energy Balance (Mcal/day)	5.0 ± .28	5.2 ± .28
Protein Balance (g/day)	700 ± 29	680 ± 29
Calcium Balance (g/day)	83 ± 1.7	83 ± 1.7
Phosphorus Balance (g/day)	34 ± .99	35 ± .99
Multiparous Cows (n)	130	128
Energy Balance (Mcal/day)	6.5 ± .16	6.5 ± .16
Protein Balance (g/day)	930 ± 17	885 ± 17
Calcium Balance (g/day)	96 ± 1.1	96 ± 1.1
Phosphorus Balance (g/day)	36**a** ± .67	38**b** ± .67

ab For each parameter, means with different letters are significantly different (P< .05).

1 Results are reported as least-squares means (± SE of least-squares means).

IM Dose Titration Study.

Increases in mean dry matter and net energy intakes across doses for primiparous cows (Table 48) corresponded with the increase in milk production (section 6.d). For the multiparous cows, only the 500 mg dose group had significantly increased milk production (section 6.d.), whereas dry matter and net energy intakes were not significantly affected by treatment at any dose (Table 48).

Table 48.

Dry matter intake (DMI) and net energy intake (NEI) of cows administered sometribove averaged over 36 weeks of the treatment period. Intramuscular Dose Titration Study (Dose-IM).

	Sometribove Dosage (mg/14 days)				
Item	0	250	500	750	P
Primiparous Cows (n)	7	7	7	7	
DMI (kg/day)	21.1 ± .50**1**	21.8 ± .50	24.6 ± .49	24.3 ± .50	.0001
NEI (Mcal/day)	34.6 ± .92	35.9 ± .92	41.2 ± .91	41.0 ± .91	.0001
Multiparous Cows (n)	14	14	14	13	
DMI (kg/day)	24.2 ± .57	24.0 ± .57	25.7 ± .57	25.2 ± .59	.128
NEI (Mcal/day)	39.6 ± 1.1	39.6 ± 1.1	42.2 ± 1.1	41.5 ± 1.1	.213

1 Each value represents the least-squares mean ± SE of the least squares-mean.

IM/SC Bridging Study.

When averaged over the treatment period, sometribove-treated primiparous and multiparous cows increased dry matter intake 2.8 and 2.7 kg/day and net energy intake 5.0 and 5.1 Mcal/day, respectively, over the controls (Table 49). Within two to four weeks of the initial sometribove treatment, the treated primiparous cows had increased their DM and NE intake compared to controls. The multiparous cows treated with sometribove deviated from the control cows beginning at approximately seven weeks of treatment. These results suggest that the intake response to sometribove treatment is delayed for the multiparous cows.

Table 49.

Dry matter intake (DMI) and net energy intake (NEI) of cows administered sometribove in the IM/SC Bridging Study.

	--Sometribove Dosage (mg/14 days)--		
Item	0	500**1**	Probabili
Primiparous Cows (n)	6	14	
DMI (kg/day)	18.5 ± .751**2**	21.3 ± .466	.008
NEI (Mcal/day)	31.1 ± 1.46	36.1 ± .909	.013
Multiparous Cows (n)	13	28	
DMI (kg/day)	21.0 ± 3.33	23.7 ± 2.20	.0007
NEI (Mcal/day)	35.4 ± 1.20	40.5 ± .819	.001

1 IM and SC groups combined.

2 Each value represents the least-squares mean ± SE of the least-squares mean.

Mean net energy balances over the treatment period were not significantly different from controls for the primiparous cows (controls, 5.3 Mcal/day; 500 mg IM, 5.3 Mcal/day; 500 mg SC, 5.8 Mcal/day) or the multiparous cows (controls, 6.3 Mcal/day; 500 mg IM, 7.3 Mcal/day; 500 mg SC, 7.1 Mcal/day).

Body Weight and Body Condition Score

Overall, body weight was not affected by sometribove treatment for multiparous cows over the course of the treatment period. In contrast, there was evidence to suggest that sometribove administration increased body weight of primiparous cows. Body condition scores were more reflective of the tissue reserve (energy status) throughout the course of the lactation than body weight. The change in body condition score over the treatment period was the variable chosen to evaluate the effect of sometribove on tissue energy repletion since it corrects for within animal variation. Use of sometribove may reduce the amount of body condition that is regained during lactation. This change in body condition from the pretreatment period through the end of treatment was significantly less in the sometribove-treated primiparous and multiparous cows compared to their controls.

Multi-location SC Dose Response Clinical Study.

Mean body weight over the treatment period was higher in sometribove-treated primiparous cows compared to controls (Tables 43 and 50). The increase in body weight

in the sometribove-treated groups was evident within the first 56 days and remained throughout the treatment period (Table 50). In contrast, body weight and change in body weight were unaffected in multiparous cows treated with sometribove (Tables 43, 50, and 51).

Table 50.

Effect of sometribove on body weight and condition score during four 56-day periods of treatment and the 224-day combined period. Multi-location SC Dose Response Clinical Study (4 Dose-SC).

	Sometribove Dosage (mg/14 days)			
Parameter**1**	0 mg	250 mg	500 mg	750 mg
Primiparous Cows**2** (n)	26	27	27	25
Body Weight (kg)				
Days 1 - 56	533**a** ± 3.2	542**b** ± 3.1	543**b** ± 3.1	548**b**±3.2
Days 57 - 112	553**a** ± 4.5	567**b** ± 4.4	569**b** ± 4.5	575**b**±4.6
Days 113 - 168	578**a** ± 4.9	590**ab** ± 4.8	592**b** ± 4.9	602**b**±4.9
Days 169 - 224	603**a** ± 5.4	614**ab** ± 5.3	619**bc** ± 5.4	631**c**±5.4
Days 1 - 224	567**a** ± 4.1	579**b** ± 4.0	581**b** ± 4.1	589**b**±4.2
Body Condition Score				
Days 1 - 56**3**	2.96 ± .036	2.94 ± .036	2.91 ± .036	2.89±.037
Days 57 - 112	3.04 ± .033	3.04 ± .032	2.99 ± .033	2.95±.033
Days 113 - 168**3**	3.13 ± .046	3.07 ± .046	3.04 ± .047	2.97±.047
Days 169 - 224**3**	3.24**b** ± .047	3.11**ab** ± .047	3.07**a** ± .047	3.01**a**±.0
Days 1 - 224**3**	3.09 ± .039	3.05 ± .038	3.00 ± .039	2.95±.040

Multiparous Cows**4** (n)	35	34	32	35
Body Weight (kg)				
Days 1 - 56**3**	632 ± 3.1	632 ± 3.1	632 ± 3.2	639 ± 3.0
Days 57 - 112**3**	652 ± 4.5	655 ± 4.5	646 ± 4.8	652 ± 4.4
Days 113 - 168**3**	672 ± 5.9	676 ± 5.9	663 ± 6.2	672 ± 5.8
Days 169 - 224**3**	699 ± 6.6	707 ± 6.3	692 ± 6.6	705 ± 6.3
Days 1 - 224**3**	663 ± 4.3	667 ± 4.3	658 ± 4.6	666 ± 4.3
Body Condition Score				
Days 1 - 56**4**	2.95 ± .026	2.94 ± .026	2.89 ± .028	2.95 ± .0
Days 57 - 112**4**	3.11**c** ± .036	3.08**bc** ± .036	2.97**a** ± .040	2.99**ab** ±
Days 113 - 168**3,5,6**	3.24 ± .054	3.16 ± .054	3.05 ± .059	3.05 ± .0
Days 169 - 224**3,5,6**	3.40 ± .063	3.31 ± .062	3.16 ± .068	3.18 ± .0
Days 1 - 224**3,5,6**	3.17 ± .042	3.12 ± .042	3.02 ± .046	3.04 ± .0

a,b,c For each parameter, means with different letters are significantly different (P< .05).

1 Results are reported as least-squares means (± SE of least-squares means).

2 Number of primiparous cows in days 169-224 analysis is 26, 26, 27, and 25, respectively.

3 Treatment X site interaction used as the error term because it was significant (P< .25).

4 Number of multiparous cows in days 169-224 analysis is 31, 33, 31, and 34, respectively.

5 Analysis weighted to adjust for unequal variances across sites.

6 Excludes one 0 mg and one 250 mg multiparous cows from Arizona missing pretreatment values.

Table 51.

Pretreatment body weight (PreBWT) and change in body weight (BWTDIF) during the standardized 252-day sometribove treatment period. Multi-location SC Dose Response Clinical Study (4 Dose-SC).

	Sometribove Dosage (mg/14 days)					
Item	0	250	500	750	SEM	P
Primiparous Cows (n)	26	27	27	25		
PreBWT (kg)	511.2**1**	531.0	527.4	528.9	10.3	.5
BWTDIF (kg)	100.0	112.5	111.3	129.0	8.7	.1
Multiparous Cows (n)	35	34	32	35		
PreBWT (kg)**2**	626.8	614.8	619.9	609.0	11.6	.7

Body condition when averaged over the entire treatment period was similar among control and treated primiparous cows (Table 43). Only when averaged over treatment days 169-224 was the body condition score of the 500 and 750 mg dose sometribove groups below that of the controls. For the multiparous cows, only during treatment days 57-112 were the 500 and 750 mg groups significantly below the controls (Table 50). In contrast, when the change in body condition score (18-cycle score minus pretreatment score **or** end of treatment score minus pretreatment score) was analyzed, the 500 and 750 mg/14 days sometribove groups did not increase body condition to the same extent as the 250 mg and control groups for both primiparous and multiparous cows (Table 52). The treated cows remained on the high energy rations for a longer period of time than controls.

Table 52.

Change in body condition score (BCS) between cycle 18 of sometribove treatment and pretreatment, and between the end of sometribov treatment and pretreatment. Multi-location SC Dose Response Clinical Stu (4 Dose-SC).

	Sometribove Dosage (mg/14 days)				
Item	0	250	500	750	SEM
	---------Change in BCS---------				
Primiparous Cows (n = 74)					
BCS (18 cycle-start)	.472**1**	.287	.168	.131	.070
BCS (end-start)	.457	.403	.143	.235	.078
Multiparous Cows (n = 97)					
BCS (18 cycle-start)	.607	.666	.270	.474	.095
BCS (end-start)	.670	.762	.494	.536	.076

1 Each value represents the least-squares mean.

Multi-lactation Chronic Animal Toxicity Study.

There was no significant effect of sometribove on the change in body weight during the treatment period in either lactation for primiparous cows. However, during year two with limited animal numbers, change in body weight was numerically but not significantly greater for treated primiparous cows compared to controls. Treated multiparous cows did not differ from controls in change in body weight during the treatment period in either lactation (Table 53).

Differences between the body weight at the end of the second lactation of treatment and body weight prior to the first lactation of treatment were examined to evaluate long term

effects. Only cows that completed the two lactations of treatment were included in the analysis. The results are shown in Table 54. The primiparous cows treated with sometribove had a significantly greater gain in body weight compared to their controls. No differences across treatment groups were observed in body weight change for the multiparous cows. This suggests that sometribove administered during the first two lactations for a primiparous cow may result in a greater body weight.

(Eds. note: The following table consists of 7 columns.)

Table 53.

Pretreatment body weight (preBWT) and change in body weight between pretreatment and end of treatment (BWTDIF) for cows administered sometribove for each year of the multi-lactation Chronic Animal Toxicity Study (TAS).

	Sometribove Dosage (mg/14 days)				
Parameter	0	600	1800	3000	SEM
YEAR 1					
Primiparous Cows (n)	6	7	6	7	
PreBWT (kg)	522.7**1**	517.6	509.4	538.7	17.9
BWTDIF (kg)	129.9	125.4	130.1	114.6	14.8
Multiparous Cows (n)	14	13	12	14	
PreBWT (kg)	692.4	596.1	685.4	645.2	13.2
BWTDIF (kg)	130.6	125.6	137.0	144.6	11.7
YEAR 2					
Primiparous Cows (n)	4	2	2	1	
PreBWT (kg)	588.6	665.9	657.4	639.3	38.3
BWTDIF (kg)	58.7	74.0	78.3	156.0	26.3
Multiparous Cows (n)	10	6	5	8	
PreBWT (kg)	654.0	671.4	657.0	613.9	22.9
BWTDIF (kg)	49.5	39.8	68.1	98.8	24.1

1 Values are least-square means.

Table 54.

Difference in body weight (BWTDIF) over two lactations of treatment with sometribove. Multi-lactation Chronic Animal Toxicity Study (TAS).

	Sometribove Dosage (mg/14 days)					
Parameter	0	600	1800	3000	SEM	P
Primiparous Cows (n)	4	2	2	1		
PreBWT (kg)	514.3**1**	538.2	502.5	553.2	31.2	.742
BWTDIF (kg)	135.6	196.5	228.0	219.2	13.5	.002
Multiparous Cows (n)	10	6	5	8		
PreBWT (kg)	577.5	591.9	555.0	550.4	18.2	.233
BWTDIF (kg)	120.4	119.3	170.0	160.0	24.0	.208

1Values are least-square means.

Multi-location IM Single Dose Study.

Primiparous cows treated with sometribove tended to be lighter at the start of treatment but did not differ from control cows in body weight change during the standardized treatment period. Treatment with sometribove had no effect on body weight change for the multiparous cows in this study (Table 55).

The sometribove-treated primiparous and multiparous cows had a significantly smaller increase in body condition during the standardized treatment period compared to the controls (Table 55). This trend was also observed for each location.

Table 55.

Pretreatment body weight (PreBWT) and body condition score (PreBCS), and change in body weight (BWTDIF) and body condition score (BCSDIF) between pretreatment and end of treatment (standardized to 252 days) for cowsadministered sometribove. Multi-location Intramuscular Single Dose Study (Single Dose-IM).

	Sometribove Dosage (mg/14 days)		
Parameter	0	500	Probability
Primiparous Cows (n)	46	46	
PreBWT (kg)	539.5 ± 6.97**a**	524.1 ± 6.97	.123
BWTDIF (kg)	96.6 ± 5.64	104.4 ± 5.64	.329
PreBCS**b**	2.68 ± .045	2.75 ± .046	.295
BCSDIF**b**	.621 ± .076	.389 ± .077	.032
Multiparous Cows (n)	130	128	
PreBWT (kg)**c**	586.2 ± 6.71	587.0 ± 6.69	.940
BWTDIF (kg)**c**	100.8 ± 5.85	107.2 ± 5.83	.487
PreBCS	2.66 ± .037	2.59 ± .037	.190
BCSDIF	.753 ± .037	.580 ± .045	.006

a Results are reported as least-squares means (± SE of least-squares means).

b For primiparous cows, n = 45 and 44 for the 0 and 500 mg groups, respectively.

c Location by dose interaction used for the error term.

IM Dose Titration Study.

Body weight change was not affected by sometribove treatment in either the primiparous or multiparous cows. Primiparous cows treated with 250 and 750 mg sometribove lost body condition during treatment compared to an increase in cows in the 500 mg dose level and controls (Table 56). Multiparous cows did not differ in body condition score over the treatment period across dose groups.

IM/SC Bridging Study.

Increase in body weight tended to be greater for the treated primiparous cows compared to the controls over the treatment period. Sometribove treatment had no effect on body weight gain in multiparous cows (Table 57). Body condition score of treated primiparous cows was reduced during the treatment period. By contrast, body condition score for the controls was increasing during this time period. Also, there was a significant decrease in the replenishment of body condition during the treatment period for treated multiparous cows compared to their controls.

Table 56.

Analysis of pretreatment body weight (PreBWT) and body condition score (PreBCS), and change in body weight (BWTDIF) and body condition score (BCSDIF) throughout the 252-day sometribove treatment period. Intramuscular Dose Titration Study (Dose-IM).

	Sometribove Dosage (mg/14 days)					
Item	0	250	500	750	SEM	P
Primiparous Cows (n)	7	7	7	7		
PreBWT (kg)	528.5**1**	490.5	514.2	492.2	15.8	.2
BWTDIF (kg)	96.9	123.2	129.5	97.1	12.7	.1
PreBCS	3.07	3.00	2.79	3.00	.102	.2
BCSDIF	.250	-.107	.393	-.286	.122	.0
Multiparous Cows (n)	14	14	14	13		
PreBWT (kg)	579.0	579.1	604.6	610.7	13.5	.1
BWTDIF (kg)	120.7	123.3	121.2	132.7	14.4	.9
PreBCS	2.71	2.61	2.82	2.93	.111	.2
BCSDIF	.750	.554	.429	.468	.177	.5

1 Each value represents the least-squares mean.

Table 57.

Pretreatment body weight (PreBWT) and body condition score (PreBCS) and the change in body weight (BWTDIF) and body condition score (BCSDIF) between pretreatment and end of treatment for cows administered sometribove. IM/SC Bridging Study.

	Sometribove Dosage (mg/14 days)			
Parameter	0	500	SEM	Probabil
Primiparous Cows (n)	6	14		
PreBWT (kg)	472**1**	524	11.6	.03
BWTDIF (kg)	74.4	98.8	11.2	.09
PreBCS	3.3	3.4	.14	.58
BCSDIF	.355	-.004	.13	.04
Multiparous Cows (n)	13	28		
PreBWT (kg)	592	580	11.8	.40
BWTDIF (kg)	57.8	71.2	7.4	.15
PreBCS	3.35	3.21	.07	.10
BCSDIF	.589	.219	.06	.001

1 Results are reported as least-squares means.

Performance In Subsequent Lactation

The Multi-lactation Chronic Animal Toxicity Study (two dry periods and early lactation following first lactation of treatment), Multi-location IM Single Dose Study (8 weeks of the subsequent lactation), and the IM Dose Titration Study (3 weeks of the subsequent lactation) were evaluated to assess the effects of sometribove on performance in the subsequent lactation when no sometribove was administered.

Increased dry matter intake without a corresponding increase in milk production was observed primarily in multiparous cows administered sometribove in the previous lactation. The effect was variable among study locations. Based on the results of these studies, cows may have increased dry matter intakes in the dry period and early in the subsequent lactation without a corresponding increase in milk production early in the subsequent lactation, as compared to the first few weeks of the previous lactation.

Multi-lactation Chronic Animal Toxicity Study.

Milk yields during the early portion of the second lactation tended to be increased over the previous lactation for the control, 600 mg, and 1800 mg groups, in contrast to a numerical decrease for the 3000 mg group in both primiparous and older cows. After one lactation of sometribove treatment, multiparous cows consumed more feed during the early part of the second lactation than controls. No difference in dry matter intake or net energy intake was observed in the treated primiparous cows (Table 58).

Multi-location IM Single Dose Study.

Data collected for the 8 weeks of the lactation following sometribove treatment were analyzed for primiparous and multiparous cows (Table 59). Dry matter intake, body measurements, and milk production did not differ for treated and control primiparous cows over the first eight weeks of the lactation. The multiparous cows treated with sometribove had significantly greater DM and NE intakes and were significantly lower in body condition score than control cows during the early part of the subsequent lactation.

Table 58.

Dry matter intake (DMI) and net energy intake (NEI) during the 7 weeks prior to sometribove treatment in the second lactation. Multi-lactation Chronic Animal Toxicity Study (TAS).

	Sometribove Dosage (mg/14 days)					
Parameter	0	600	1800	3000	SEM	Probability
Primiparous Cows (n)	4	2	2	1		
DMI (kg/day)	23.5**1**	20.2	24.6	23.1	3.04	.628
NEI (Mcal/day)	40.4	35.2	42.3	39.7	5.24	.668
Multiparous Cows (n)	10	6	5	8		

DMI (kg/day)	21.0	23.6	25.1	21.8	1.02	.016
NEI (Mcal/day)	36.1	40.8	43.2	37.5	1.68	.017

1 Values are least-square means.

Table 59.

Dry matter intake (DMI), net energy intake (NEI), body weight (BWT), body condition score (BCS), and milk and FCM production for the first 8 weeks of the lactation subsequent to the lactation of sometribove treatment. Multi-location Intramuscular Single Dose Study (Single Dose-IM).

	Sometribove Dosage (mg/14 days)		
Parameter	0	500	Probability
Primiparous Cows (n)	42	33	
DMI (kg/day)	20.38 ± .592**1**	21.07 ± .663	.489
NEI (Mcal/day)	34.45 ± 1.03	35.50 ± 1.15	.310
BWT (kg)	600.0 ± 7.3	602.2 ± 8.2	.854
Milk (kg/day)	37.21 ± 1.33	35.67 ± 1.50	.496
FCM (kg/day)	38.01 ± 1.11	35.37 ± 1.26	.209
BCS (n)	40	31	
BCS	2.89 ± .085	2.68 ± .101	.208
Multiparous Cows (n)	112	95	
DMI (kg/day)	19.28 ± .354	21.56 ± .375	.0001
NEI (Mcal/day)	32.48 ± .620	36.35 ± .656	.0001
Milk (kg/day)	36.11 ± .708	37.57 ± .745	.152
FCM (n)	107	92	
FCM (kg/day)	38.44 ± .627	38.35 ± .662	.913
BCS & BWT (n)	111	94	
BWT (kg)	641.0 ± 4.9	653.9 ± 5.1	.165
BCS	3.00 ± .078	2.82 ± .081	.061

1 Results are reported as least-squares means (± SE of least-squares means).

IM Dose Titration Study.

Treatment with sometribove during the previous lactation had no significant effect on body weight, body condition score, or DM or NE intake during the first three weeks of the subsequent lactation in primiparous cows, although FCM production was reduced for the 750 mg primiparous cows (Table 60). In contrast, treated multiparous cows did not differ in milk production and FCM in the subsequent lactation compared to controls. Although body weights did not differ in the first three weeks of the next lactation, body condition score was reduced for the multiparous cows treated with sometribove. This reduction was greatest for cows that had been administered the 750 mg dose. These results are based on only three weeks of data in early lactation when variability is high.

Table 60.

Body weight (BWT), body condition score (BCS), dry matter intake (DMI), net energy intake (NEI), milk production, and 3.5 % fat-corrected milk production (FCM) for the first 3 weeks of the lactation following the lactationof sometribove treatment. Intramuscular Dose Titration Study (Dose-IM).

	Sometribove Dosage (mg/14 days)				
Item**1**	0	250	500	750	P
Primiparous (n)	6	5	6	5	
BWT (kg)	550.8 ± 16.9	571.9 ± 19.4	580.0 ± 17.1	570.2 ± 18.9	.6
BCS	2.71 ± .133	2.49 ± .158	2.57 ± .150	2.26 ± .153	.2
DMI (kg/d)	16.9 ± 1.24	18.5 ± 1.49	21.5 ± 1.23	20.0 ± 1.46	.1
NEI (Mcal/d)	27.8 ± 2.13	30.5 ± 2.44	35.1 ± 2.10	32.2 ± 2.45	.1
Milk (kg/d)	27.2 ± 1.91	25.2 ± 2.48	29.5 ± 1.91	22.3 ± 2.88	.1
FCM (kg/d)	29.2 ± 1.88	29.2 ± 2.77	29.7 ± 2.01	20.3 ± 3.07	.0
Multiparous (n)	11	11	12	9	
BWT (kg)	650.1 ± 13.3	654.2 ± 13.4	656.9 ± 12.7	648.4 ± 14.8	.9
BCS	3.04 ± .102	2.81 ± .101	2.84 ± .096	2.53 ± .113	.0
DMI (kg/d)	17.3 ± 1.07	17.9 ± 1.05	20.6 ± 1.01	19.1 ± 1.19	.1
NEI (Mcal/d)	27.9 ± 1.78	29.2 ± 1.74	33.4 ± 1.67	31.3 ± 1.97	.1
Milk (kg/d)	28.8 ± 1.94	28.3 ± 1.86	28.5 ± 1.77	31.0 ± 2.10	.7
FCM (kg/d)	33.4 ± 2.21	29.7 ± 2.16	29.7 ± 2.06	30.5 ± 2.43	.5

1 Results are reported as least-squares means (± SE of least-squares means).

Conclusions

Feed intake increases over several weeks after initiating the use of sometribove in lactating dairy cows. This increase occurs earlier for primiparous cows than for multiparous cows. Use of sometribove may reduce the amount of body condition that is normally regained during lactation. This effect is more pronounced for multiparous cows. Also, voluntary feed intake may be increased and body condition decreased during both the dry period and subsequent early lactation. These effects are contained in product labeling. Also, the labeling recommends that cows be fed diets formulated to meet or exceed the nutritional requirements recommended by the National Research Council. The labeling also states that milk yield, stage of lactation, and body condition should be considered when making dietary changes. The feeding program should be managed to optimize energy intake and to have cows in appropriate body condition particularly during late lactation and the dry period.

i. Reproduction

Reproductive performance was evaluated in the following studies individually and from a pooled analysis:

Multi-lactation Chronic Animal Toxicity Study (TAS)
Multi-location SC Dose Response Clinical Study (4 Dose-SC)
Multi-location IM Single Dose Study (Single Dose-IM)
IM Dose Titration Study (Dose-IM)
IM/SC Bridging Study (IM/SC)

The experimental design and methods for each of these studies are described in Sections 6.b, 5.a, 6.e, 6.d, and 6.c, respectively. Location-specific practices were followed for vaccination programs, sire selection, management of reproductive problems, and calving management. All cows received a veterinary examination to evaluate reproductive health prior to initiation of the breeding program. All breeding was by artificial insemination. Estrus detection was by visual appraisal of standing estrus according to the following system: 1 = standing heat; 2 = bulling, sniffing, and attempting to mount other cows; 3 = bawling and restlessness; 4 = clear vaginal discharge, vulva lips swollen and/or flabby; 5 = metestrus bleeding; 6 = other, when 1-5 did not apply, explained in an observation column. Some locations may also have used heat detection aids such as heat mount detectors or tailhead chalking. Use of prostaglandins and gonadotropins to induce fertile estrus was not restricted in the IM studies and normal location practices were allowed. In the 4 Dose-SC study, use of any medication to alter the normal cycle was not allowed until after 120 days in milk. No restriction, however, was placed on the use of these products to treat a diagnosed ailment such as cystic ovarian disease or pyometra. Data were analyzed using all available information and again, separately, excluding those cows that received any medication intended to alter the estrous cycle. Since the trends regarding the effects of sometribove treatment on reproduction were the same in either case, only the overall analysis is discussed.

All locations participated in a routine herd health program. Pregnancy status was determined by rectal palpation by the herd veterinarian or other qualified person at regular intervals. This information was used to determine the conception date and to identify any loss of a previously diagnosed pregnancy. At parturition, a calving ease score was assigned, with increasing calving difficulty indicated by increasing score.

Calving ease scores were as follows: 1 = an unassisted delivery in which progressive fetal expulsion continued throughout and labor did not exceed 3 or 5 hours for multiparous or primiparous cows, respectively; 2 = a multiparous or primiparous cow delivered a calf after prolonged or difficult unassisted labor of >3 or >5 hours, respectively; 3 = manual assistance (traction only) was required; 4 = delivery was assisted due to improper calf positioning for parturition; 5 = calving occurred unobserved. Scores were grouped into high (scores 2, 3, and 4) versus low (score 1) calving difficulty for analysis. All calves were weighed at birth.

Data from each of the five studies were analyzed separately by parity. Data were then pooled where appropriate, i.e., where similar sometribove doses and similar reproductive management techniques were used, and analyzed by parity to allow the most complete evaluation of the effects of sometribove on reproduction. In all IM studies (including the IM/SC Bridging Study), attempts to breed cows began at approximately 40 days after calving. Because of this, approximately 40 % of all cows on these studies received at least one insemination prior to the first sometribove administration. In the 4 Dose-SC study, all inseminations occurred after the first administration of sometribove. For this reason, pooling across the IM and SC studies was not possible. Thus, the data from clinical studies using the IM route of administration were pooled for the control and 500 mg doses (including only the IM injected cows from the IM/SC study) and analyzed as the "Pooled IM" studies, and the 4 Dose-SC study was analyzed separately. Finally, the TAS Study was evaluated separately from the other studies because of the higher doses used.

Reproductive variables were analyzed over several separate study periods. In the Pooled IM studies, where breeding was initiated prior to sometribove treatment, reproductive performance was evaluated in the pretreatment period to determine, among other things, whether breedings occurring within a few weeks of initiating sometribove treatment resulted in greater failure in conception rates. Reproductive performance in the Pooled IM studies was also evaluated from the initiation of sometribove treatment (i.e., approximately 60 days postpartum) until the breeding cut-off (i.e., days postpartum in which attempts to breed a cow ceased), which was set at 170 days in these studies. Finally, reproductive performance over the entire breeding period (pretreatment through 170 days postpartum) was evaluated. In the 4 Dose-SC study, breeding initiated after the start of sometribove treatment and continued until at least 305 days postpartum. Evaluation of reproductive performance in this study covered the periods from 60 to 180 days postpartum, and from 60-305 days postpartum. (For example, for the 60-180 day evaluation, if a cow conceived at 250 days postpartum, she was nevertheless classified as "open" in the analysis.) Finally, in both the Pooled IM and 4 Dose-SC analyses, reproductive performance across dose groups was evaluated during the first 28 days of sometribove treatment to determine whether negative effects, if any, were predominantly associated with the earliest period of treatment when treated cows usually had a reduction in energy balance compared to controls. It should be noted that individual cows were only included in the periods of analysis for variables in which they were "eligible." For example, in the Pooled IM studies, a cow conceiving to a full-term pregnancy prior to the initiation of sometribove treatment was excluded from the day 60 to 170 analyses for selected variables because she was already pregnant. However, if she lost the fetus prior

to 170 days postpartum, she would have been included in the day 60 to 170 analyses for these variables because she was once again eligible for breeding.

A glossary of terms is included at the end of this Section (Table 65) and reflects most of the variables analyzed. Results of the analyses during the pretreatment period did not indicate that initiation of sometribove treatment shortly after breeding adversely affected conception or other reproduction indices. Effects during the first 28 days of treatment were not substantially different from those observed during the remainder of the breeding period.

The results of reproductive performance in the TAS study from days 60-140 postpartum in Year 1 and days 60-275 postpartum in Year 2, in the Pooled IM studies from day 60 to 170 postpartum, and the 4 Dose-SC study from days 60-180 postpartum in general were found to reflect the effects of sometribove treatment during the entire breeding period. Based on these data, both primiparous and multiparous cows experienced a reduction in pregnancy rates (see Table 61) and variables related to pregnancy rate (e.g., conception rate and successful calving rate). Primiparous cows had an increase in days open (see Table 62). Administration of sometribove was also associated with an increased rate of twinning and an increase in the incidence of cystic ovaries (Tables 63 and 64). Other parameters of reproductive performance, such as days to first estrus and insemination, interestrous interval, services per conception and incidence of fetal loss were not consistently affected by the administration of sometribove.

Conclusions

The product labeling states that treatment of cows with sometribove may result in reduced pregnancy rates and an increase in days open for primiparous cows. Also, the incidence of cystic ovaries and multiple births may increase. The labeling recommends the implementation of a comprehensive and ongoing herd reproductive health program preceding use of sometribove.

Table 61. Pregnancy rates (full term).

TAS Study**1**

Dose	0 mg	600 mg	1800 mg	3000 mg
Year 1; days 60-140 of lactation				
Primiparous	100 % (3/3)**2**	43 % (3/7)	40 % (2/5)	20 % (1/5)
Multiparous	100 % (10/10)	45 % (5/11)	43 % (3/7)	50 % (6/12)
Year 2; days 60-275 of lactation				
Primiparous	100 % (4/4)	100 % (2/2)	50 % (1/2)	0 % (0/1)
Multiparous	100 % (10/10)	17 % (1/6)	100 % (4/4)	75 % (6/8)

Pooled IM Analyses; days 60-170 of lactation**3**

Dose	0 mg	500 mg	P
Primiparous	90 % (37/41)	63 % (30/48)	.002
Multiparous	77 % (89/115)	70 % (90/129)	.115

4 Dose-SC Study; days 60-180 of lactation**1**

Dose	0 mg	250 mg	500 mg	750 mg	
Primiparous	77 % (20/26)	63 % (17/27)	70 % (19/27)	80 % (20/25)	.6
Multiparous	86 % (30/35)	62 % (21/34)	66 % (21/32)	66 % (23/35)	.1

1 Linear trend in proportions across dosage levels (Cochran-Armitage).

2 Number of cows pregnant full term divided by number of cows in group.

3 Cochran-Mantel-Haenszel Chi-square test for difference between control and treated.

Table 62.

Days Open A (average number of days from calving to final conception for cows pregnant to term).1

TAS Study

Dose	0 mg	600 mg	1800 mg	3000 mg	A**2**	Probabili
Year 1; days 60-140 of lactation						
P**4**	77±13.2(3)5	101±13.2(3)	91±16.2(2)	125±22.9(1)	.372	.1
M**4**	95± 8.5(10)	96±12.0(5)	86±15.5(3)	91±11.0(6)	.945	.6
Year 2; days 60-275 of lactation						
P	93±23.9(4)	196±33.9(2)	64±47.9(1)	± (0)	.116	
M	107**ab**±10.4(10)	223**c**±32.9(1)	84**a**±16.5(4)	135**b**±13.4(6)	.006	.18

Pooled IM Analyses; days 60-170 of lactation

Dose	0 mg	500 mg	A
P	89**a**±5.0(37)	105**b**±5.0(30)	.024
M	101±4.2(89)	103±3.5(90)	.646

Table 65. Glossary of Reproduction Terms

Number of Cows	Total number of cows available for analysis.
Estrous Period	Period of time the cow is receptive to breeding activity. Generally defined by immobility during mounting attempts of other cows. Activity is score (see below) and all scores within a 4 day period ar considered to be a single estrous period. The firs date of the 4 day period is used as the date of estrus which is used to calculate interestrous interval. The last date of the 4 day period is use to assign estrous period to a particular study peri
Total Estrous Periods	Total number of estrous periods during a given time This excludes estrous periods after conception in full term pregnancies and after conception and up t fetal loss in cows that later lost a pregnancy.
Average Number of Estrous Periods	The average number of estrous periods during a give time. Same exclusions as for Total Estrous Periods
Interestrous Interval	Average number of days between all estrous periods. Same exclusions as for Total Estrous Periods.
First Interestrous Interval	Average number of days between the first estrous period and the second estrous period. Same exclusions as for Total Estrous Periods.
Second Interestrous Interval	Average number of days between the second estrous period and the third estrous period. Same exclusio as for Total Estrous Periods.
Expected Estrous Periods	Calculated by using the open days divided by 21.
Days to First Estrus	Average number of days to first observation of estrus. Definitions for estrous scores follow: 1 = Standing estrus. 2 = Bulling, sniffing and attempting to mount othe 3 = Bawling and restlessness. 4 = Clear vaginal discharge, vulva lips swollen and/or flabby. 5 = Metestrus bleeding. 6 = Other; when 1-5 does not apply, explain in observation column.
Total Inseminations	The total number of inseminations for all cows with a group.
Average Number of Inseminations	The average number of inseminations per cow within group.
First Insemination Interval	The average number of days between the first and second insemination.
Second Insemination Interval	The average number of days between the second and third inseminations.

j. Mastitis

The effect of sometribove treatment on clinical mastitis, subclinical mastitis and somatic cell count (SCC) was evaluated from the following studies in separate and pooled analyses:

Multi-lactation Chronic Animal Toxicity Study (TAS)
Multi-location SC Dose Response Clinical Study (4 Dose-SC)
Multi-location IM Single Dose Study (Single Dose-IM)
IM Dose Titration Study (Dose-IM)
IM/SC Bridging Study (IM/SC)

The experimental design and sample collection schedule for each of these studies has been described (Sections 6.b, 5.a, 6.e, 6.d, 6.c, respectively).

Since the individual effectiveness trials lacked sufficient numbers of animals to examine any association between use of sometribove and effect on mastitis and milk somatic cell count, data from studies were pooled in order to augment animal numbers and increase the reliability of conclusions. As discussed in Sections 5.a and 6.e, data collected at one location (Utah) from the 4 Dose-SC study and the same location in the Single Dose-IM study were excluded from the clinical and subclinical mastitis analysis. Clinical mastitis was rarely treated during these trials, possibly affecting the incidence of mastitis and confounding any effects due to sometribove.

For clinical mastitis, data from the 4 Dose-SC, Single Dose-IM, Dose-IM and IM/SC studies (i.e., 8 trials) were pooled for analysis. The TAS study was excluded from the pooled analysis because of the higher doses used. A separate analysis of this study was carried out. For subclinical mastitis, data from the 4 Dose-SC study were pooled with the IM/SC study and the Dose-IM study. Finally, for somatic cell counts, several analyses were carried out on data from individual studies and on pooled data.

Detection of clinical mastitis.

In all studies, milkers were responsible for the detection of abnormal milk by the examination of foremilk from each quarter at each milking. There was some variation from location to location in routine practices and therapy related to mastitis. However, within a given location, control and sometribove-treated cows were assessed and administered therapy for mastitis by identical procedures.

Detection of subclinical mastitis.

Milk samples were obtained for determination of subclinical mastitis by microbiological culture once before initiation of treatment with sometribove and at approximately 8 week intervals during the treatment period.

Determination of SCC.

Milk samples for determination of SCC were taken once per week throughout the study for all of the studies. All determinations were done by independent laboratories.

Definitions.

Case Rate was the number of clinical mastitis cases per quarter per cow-day when a new case could be observed. Twenty-one (21) days must have elapsed between observations within a quarter or a new pathogen isolated for separate cases to be identified.

Days Affected Rate was the number of days affected per cow-day observed. Cases in more than one quarter on a given day were consolidated in this variable. Essentially, a yes/no response was created for each animal each day.

Results:

Clinical Mastitis

In the pooled analysis (excluding the TAS study), there was a total of 202911 quarter days when new cases could have been observed for 193 primiparous cows and 408468 quarter days when new cases could have been observed in 426 multiparous cows. Because of overlap, days observed were slightly smaller than one quarter of these values; primiparous cows were observed on 51069 days and multiparous cows on 103125 days.

Cochran-Mantel Haenszel (CMH) measures of general association were used to test for association, controlling various effects. Analyses were done (1) separately by parity, (2) controlling for parity, and (3) ignoring (collapsing) parity. There was an association between sometribove usage and the number of cows affected with clinical mastitis. Comparing the proposed use level (500 mg) to control, the relative risk of a treated animal showing signs of clinical mastitis during the treatment period was about 1.79 times that of a control animal (Table 66).

Table 66. Pooled analysis (0 and 500 mg sometribove only)

	Relative risk (500 mg/0 mg)		
Parity handling	Estimate	95 % Confidence Lower Bound	95 % Confidence Upper Bound
Primiparous	1.969	0.944	4.098
Multiparous	1.745	1.214	2.506
Controlled for Parity	1.789	1.292	2.475

Poisson-based regression was used to estimate the probability of observing a new case on a quarter-day when a new case could be observed. Case Rate per cow-day could then be obtained by assuming independence of the quarters. The probability was then the sum of the probabilities in each of the four quarters in the udder, or four times the probability in any one quarter. Poisson-based regression was also used to estimate the probability that a cow would be affected with clinical mastitis on any given day. The regression coefficients were used to calculate predicted Case Rates and Days Affected Rates for a standard treatment period of 252 days. Case Rate was increased for primiparous cows, with an expected 0.21 cases per control animal and 0.37 cases in treated (500 mg) primiparous cows. Case Rate was also increased for multiparous cows, with an expected 0.36 cases of clinical mastitis in control animals and 0.54 cases in treated (500 mg) cows.

The total Days Affected Rate for primiparous cows was not increased by treatment with sometribove. For multiparous cows, Days Affected Rate was increased from 1.49 in controls to 2.15 in treated (500 mg) animals. The results from this analysis are presented in Table 67. The P value reported in Table 67 refers to the probability associated with the regression, not to the comparison between the 0 and 500 mg dose group.

Table 67.

Comparison of expected mastitis Cases and total Days Affected for control and sometribove-treated cows -pooled analysis assuming a 252 day standardized treatment period.

	------- Study Groups -------		
Parity Group	Control	Sometribove**1**	P
Primiparous Cows			
Cases	0.21	0.37	0.0147
Days Affected	1.15	1.21	0.6779
Multiparous Cows			
Cases	0.36	0.54	0.0021
Days Affected	1.49	2.15	0.0001

1 Expected results for the 500 mg/14 day dose group.

A separate analysis was conducted for the TAS study because the range of doses of sometribove was considerably different from the range in the remainder of the studies. Over two years of treatment, this study covered 32961 quarter days when new cases could be observed in 27 primiparous cows (8466 days with overlap). In 56 multiparous cows, there were 76823 quarter days when new cases could be observed, which covered 19820 days, considering overlap. Although there was numerical evidence for a dose related increase in Case Rate and Days Affected Rate in this study, the high background level of clinical mastitis resulted in no significant differences detected in the pooled analysis at the use level of the drug (500 mg) although the linear trend was significant (Table 68).

Table 68.

Comparison of expected mastitis Cases and total Days Affected for control and 500 mg sometribove-treated cows - TAS study assuming a 252 da standardized treatment period.

	----- Study Groups -----		
Parity Group	Control	Sometribove	P
Primiparous Cows			
Cases	0.95	1.07	0.0784
Days Affected	3.50	4.33	< 0.0001
Multiparous Cows			
Cases	0.97	1.13	< 0.0001
Days Affected	4.43	5.62	< 0.0001

The average duration of clinical cases of mastitis was evaluated between control and sometribove (all doses) treated cows in the 8 clinical trials and TAS study, with parities pooled (Table 69).

(Eds. note: The following table consists of 3 columns.)

Table 69.

Average duration (in days) of cases of clinical mastitis in control and sometribove-treated cows.

	Sometribove Dose (mg/14 days)	
Trial	**0 mg**	**All Sometribove Doses**
Arizona IM	4.5	4.0
Arizona SC	4.0	5.9
Cornell IM	4.1	3.6
Cornell SC	2.0	4.1
Florida SC	2.0	3.3
Dardenne IM	5.2	4.3
Dose-IM	5.0	4.7
IM/SC	3.0	5.5
Weighted Average of Clinical Trials	4.5	4.6
TAS Study (Year 1)	5.4	5.5

The average case duration in controls versus treated cows in these clinical trials was 4.5 versus 4.6 days; in the TAS study the average durations were 5.4 versus 5.5 days, respectively. Thus, the increased total days affected with clinical mastitis in sometribove-treated cows reflected the greater number of clinical mastitis cases and not increased average duration per case.

Subclinical Mastitis

Analysis of subclinical mastitis on a per cow basis indicated that treatment with sometribove significantly increased the number of animals with this condition. The relative risks for contracting subclinical mastitis for the 250, 500, and 750 mg groups were 1.56, 1.55, and 1.51, respectively, compared to controls (Table 70).

On a quarter basis, the results showed a general association between sometribove level and testing positive for subclinical mastitis (P=0.001) for all dose levels. The relative risks for testing positive for subclinical mastitis for the 250, 500, and 750 mg groups were 1.60, 1.81, and 1.52, respectively, compared to controls (Table 70). A separate analysis (results not shown) indicated that for the four microbiological categories evaluated (i.e., pathogen, coagulase negative Staphylococcus (CNS), environmental, and other), at the use level of the drug the "pathogen" and the "CNS" categories were statistically significant (P=.001) for general association.

Table 70.

The effect of sometribove treatment at various dosages on the relative risk for occurrence of subclinical mastitis compared to controls.

	Sometribove Dose		
	250 mg	**500 mg**	**750 mg**
Per cow basis			
Relative risk	1.56	1.55	1.51
Probability	<.001	<.001	.003
Per quarter basis			
Relative risk	1.60	1.81	1.52
Probability	.001	.001	.001

Somatic Cell Counts (SCC)

Several analyses were conducted on somatic cell count (SCC) data from the TAS, 4 Dose-SC (all four locations), Single Dose-IM (all four locations), Dose-IM, and IM/SC studies.

First, data from each study location were analyzed individually by parity. Sometribove treatment resulted in significantly higher SCC ($P < 0.10$) for the following location-parity groups: Dardenne IM (multiparous only), Cornell IM (multiparous only), Arizona IM (multiparous only), Dose-IM (multiparous only), Utah SQ (multiparous only), and TAS year one (primiparous only).

Data were then pooled for the Single Dose-IM (excluding the Utah location) study. Analyses showed that multiparous cows treated with 500 mg sometribove had significantly higher SCC than control multiparous cows. Also, when parities were pooled, SCC were statistically higher in treated cows (Table 71).

Results were also pooled from the 4 Dose-SC (excluding the Utah location), Dose-IM, and IM/SC studies. This analysis revealed no significant increase in SCC due to sometribove treatment (Table 72).

Table 71. Least-squares means for log (base 10) transformed SCC data po across Dardenne, Arizona, and Cornell IM clinical trials.

	Sometribove Dose (mg)		
Parity Group (n)	**0**	**500**	**P**
Primiparous (68)	4.88	4.94	.2875
Multiparous (223)	5.03	5.15	.0001
Pooled parities (291)	4.99	5.10	.0001

Table 72.

Least-squares means for log (base 10) transformed SCC data pooled across Arizona SC, Cornell SC, Florida SC, Dose-IM, and IM/SC trials.

	-----Sometribove Dose (mg)-----				
Parity Group (n)	**Control**	**250**	**500**	**750**	**P**
Primiparous (126)	4.86	4.94	4.86	4.88	.6021
Multiparous (205)	4.97	5.05	5.03	5.05	.1997
Pooled parities (331)	4.93	5.01	4.97	4.99	.1391

To summarize, SCC were elevated in some herds. Removal of clinically mastitic cows from the analysis did not eliminate the effect. Since somatic cell counts are an indicator of the subclinical infection status of a herd, the elevations observed in some sometribove trials may have reflected the increased subclinical infection rate at these study locations.

Conclusions:

Administration of sometribove (500 mg/14 days): 1) increases the risk of clinical mastitis in both primiparous and multiparous cows; 2) increases the number of cases of clinical mastitis in both primiparous and multiparous cows; 3) increases the risk of subclinical mastitis in both parity groups; and 4) increases milk somatic cell counts in some herds. The product labeling advises of these effects to inform the user of risks associated on a per animal basis. Also, the labeling states that herd mastitis management practices should be thoroughly evaluated prior to initiating the use of sometribove.

FDA also concluded that the increase in clinical mastitis in sometribove-treated cows was not a public health concern with respect to antibiotic residues in milk being increased above tolerance due to therapeutic treatment of mastitis. Although the incidence of

clinical mastitis was increased in treated cows, there was no indication that these cases of mastitis were more difficult to treat, as reflected by the similar average duration of cases between treated and control cows. When examined on a per unit milk basis, the increase in the incidence of clinical mastitis due to sometribove (approximately 0.1 case per cow per year) is about 4 to 9 times **less** than the effects due to other sources of variation, such as season, parity, stage of lactation, and herd-to-herd variation. For example, on a per unit milk basis, the increase in mastitis incidence from winter to summer is at least nine times greater than the increase due to sometribove treatment. Also, the increase in mastitis between early lactation (when sometribove would not be administered) compared to late lactation is at least seven times greater than the effect of sometribove. (See references by Smith et al. [1985, J. Dairy Science 68:1531]; Morse et al. [1988, J. Dairy Science 71:848]; and Hogan et al. [1989, J. Dairy Science 72:1547]).

Another important factor is that therapeutic drugs, such as antibiotics for the treatment of clinical mastitis, are to be used in food-producing animals only under approved conditions and with appropriate withdrawal periods (as established by FDA) to ensure that food products are safe for human consumption. State and Federal regulatory bodies currently monitor milk supplies for drug residues, and any milk that contains illegal residues is discarded. In addition, the dairy industry currently tests every tanker truck of milk for penicillin-like, beta-lactam drugs prior to processing. The beta-lactams are the most commonly used drugs for the treatment of mastitis so that even if an increase in use of these drugs--and an increase in illegal residues--occurred as a result of increased mastitis, any residues would result in the rejection of the milk before it could enter the market.

Allergic reactions are the most common side effect of the beta-lactam antibiotics, the predominant therapeutic treatment for clinical mastitis, and these agents are the most common cause of drug allergies. Three to 10 % of the human population is allergic to penicillin. Penicillin allergies develop following long-term (weeks) exposure to high doses (therapeutic). Once an individual is allergic to penicillin, smaller doses can cause an allergic reaction. Typically these reactions consist of a skin rash and are not considered to be significant health risks. In the last 25 years, there have been less than 10 cases of allergic reactions worldwide following the consumption of penicillin residues in milk (Dewdney et al., 1991, Food Chem. Toxicol. 21:477-483). All of these adverse reactions involved penicillin residues in the milk at levels well above FDA's current tolerance of 5 parts per billion (ppb). Currently, the commonly used screening test for beta-lactam residues in milk is the *Bacillus stearothemophilus* disc assay, which detects penicillin residues at >=3-5 ppb. This provides an indication of the magnitude of the public health concern for violative antibiotic residues in food.

In view of the much larger variation in the number of clinical mastitis cases due to other factors, the monitoring of milk for illegal drug residues, and the minuscule public health concern for beta-lactam antibiotic residues in milk, FDA concluded that the use of sometribove was not important in considering the overall incidence of mastitis per unit of milk produced and therefore not a public health concern.

FDA's Veterinary Medicine Advisory Committee and expert consultants were convened for an open public hearing to discuss the issue of increased mastitis in sometribove-

treated cows and a potential increase in the risk of antibiotic residues in milk on March 31, 1993. After hearing presentations from interested parties, and after considering the data presented to the Committee, the Committee concluded that, while sometribove treatment might cause a statistically significant increase in mastitis, the increased risk to human health posed by mastitis and resultant use of antibiotics was insignificant.

k. Cow Health

An analysis of health observations from cows treated with sometribove was conducted using data from the following studies:

Multi-lactation Chronic Animal Toxicity Study (TAS)
Multi-location SC Dose Response Clinical Study (4 Dose-SC)
Multi-location IM Single Dose Study (Single Dose-IM)
IM Dose Titration Study (Dose-IM)
IM/SC Bridging Study (IM/SC)

The experimental design of each of these studies is described in Sections 6.b, 5.a, 6.e, 6.d, 6.c, respectively. Data were gathered from daily observations, log entries, calving reports, medication files, physical examinations, veterinary requests, animal release records, necropsy reports, and feed sheet records. Data were grouped by system, subsystem within system, and category within subsystem and then were analyzed at each of these levels. A listing of the system and subsystem groupings used is contained in Table 73. The categories within each subsystem are listed in the results tables described later.

Table 73. Grouping of health observations used in analysis

System	Subsystem
Circulatory/ Lymphatics	Heart
	Lymph Nodes
	Hypovolemic Shock
	Veins
	Bovine Leukosis
	Septicemia/Toxemia
	Anemia
Digestive	Rumen Motility
	Other rumen abnormalities
	Feces/stool
	Abomasum
	Cecum gas/dilatation
	Intestine
	Indigestion
	Parasites (internal)/Coccidiosis
	Reticulum
Genito-Urinary	Uterus
	Vulva/Vagina/Clitoris
	Cervix
	Ovary/Oviduct
	Kidney/Bladder
Musculoskeletal	Neck/shoulder/rib/back
	Hip/Thigh/Hook/Pelvis/Gluteal/Pinbone
	Leg
	Hock
	Knee/Carpus
	Stifle
	Foot/Hoof/Dew Claw/Fetlock/Pastern
	Gait
	Elbow
Metabolic Disorders	Ketosis
	Milk Fever/Hypocalcemia
	Acidosis
	Hypoglycemia
Respiratory	Lung/Thorax
	Upper Respiratory
Udder Disorders	Udder
	Teats
Eye and Conjunctiva	Eye
	Conjunctiva
Integumentary	Swelling/edema/enlarged
	Laceration/abrasion/lesion
	Abscess/infection
	Lump
	Warts (except teats)
	Dermatitis
	Scar Tissue(except repro tract)
Miscellaneous	Feed Intake

Health observations were summarized and analyzed in five different periods:

Pretreatment period - from calving to the day before start of treatment;

Standardized treatment period - from start of treatment up through 18 cycles (16 cycles for TAS) which usually was equivalent to the period used in production analysis;

Entire treatment period - from start of treatment through day of last milking if cow was pregnant, 14 days after last injection date while still milking if cow was not pregnant, or a cow's removal date from study if it preceded either the last milk or treatment dates;

Dry period - from day after last milking until day before next calving or a cow's removal date from study if it preceded the next calving;

Lactation subsequent to treatment - from calving through 56 days, a cow's last milk if it preceded 56 days or a cow's removal date from study if it preceded either the last milk or 56 days.

Data were analyzed both within each of the individual studies listed above and as a pooled analysis of all of the studies excluding the TAS study. Separate analyses were performed for primiparous and multiparous cows. Table 74 lists the total number of cows and cow days when health incidents could be observed.

Table 74. Health Observations

TAS; Primiparous	Period	Control	600**1**	1800**1**	3000**1**
N (# cows)	1st (full)	6	7	7	7
N (# cows)	2nd (pre)	4	2	2	1
N (# cows)	2nd (full)	4	2	2	1
N (# cows)	2nd (next)	4	2	1	
total cow days	1st (full)	1331	1789	1522	1584
total cow days	2nd (pre)	244	118	117	57
total cow days	2nd (full)	920	653	421	247
total cow days	2nd (next)	53	34	19	
TAS;Multiparous					
N (# cows)	1st (full)	14	13	13	14
N (# cows)	2nd (pre)	10	6	5	8
N (# cows)	2nd (full)	10	6	5	8
N (# cows)	2nd (next)	10	1	5	7
total cow days	1st (full)	3192	3169	2839	3373
total cow days	2nd (pre)	603	366	303	486
total cow days	2nd (full)	2325	1312	1227	2134
total cow days	2nd (next)	165	20	86	114
Pooled Analysis; Primiparous		Control	250**1**	500**1**	750**1**
N (# cows)	(full)	87	34	95	34
N (# cows)	(next)	49	5	40	5
total cow days	(full)	22217	9895	25094	9913
total cow days	(next)	2410	88	1963	95
Pooled Analysis; Multiparous					
N (# cows)	(full)	201	52	212	52
N (# cows)	(next)	126	11	109	9
total cow days	(full)	48138	13146	53336	13415
total cow days	(next)	6145	187	5235	167

1 Sometribove dose (mg/14 days)

For all conditions, the number of cows affected and the total days observed were analyzed. The TAS study analysis included two additional variables. The first was the total number of cases. For a given condition, a new case was defined when consecutive incidents were separated by at least seven days. Cases which extended between study periods (i.e., between the pretreatment and the treatment periods) were counted in the

period in which they began. The second variable, total duration of cases, was defined as the sum of the case lengths within a given condition.

Poisson regression was performed on total days observed, total cases observed, and total duration of cases, each with an appropriately calculated denominator, reflecting days at risk. When an analysis involved more than two doses, a residual chi square P-value was calculated. Analysis of the number of cows affected varied depending on the number of doses and the number of trials included in the analysis. The Cochran-Armitage trend test was used in the individual TAS and Dose-IM studies. Additionally, a residual chi square P-value was reported for these studies. The Cochran-Mantel-Haenszel nonzero correlation test was used for combined data across several locations. This included the individual Single Dose-IM and 4 Dose-SC study analyses as well as the pooled analyses.

Analysis of data from physical examinations was performed separately. Number of cows affected was the only variable analyzed because examinations were not conducted on a daily basis. Data were analyzed for each period of study that examinations were scheduled. Statistical methods identical to those previously discussed were used for the number of cows affected.

Results

There were no adverse effects of sometribove administration on the circulatory/lymphatic system or the integumentary system, nor did sometribove cause increased metabolic disorders or eye disorders/conjunctivitis.

Sometribove-treated cows had a greater incidence of disorders of the digestive system than controls. Within this system, various subsystems and categories were affected, for example, bloat and diarrhea (Tables 75 and 76).

Table 75. Bloat

TAS; Primiparous	Period	Control	600**1**	1800**1**	3000**1**	A**2**
# cows affected	1st (full)	0	0	2	3	.015
days observed	1st (full)	0	0	13	28	.000
total cases	1st (full)	0	0	9	13	.000
# cows affected	2nd (full)	0	0	0	0	1.000
TAS; Multiparous						
# cows affected	1st (full)	2	1	3	1	.865
days observed	1st (full)	3	2	7	4	.397
total cases	1st (full)	2	1	6	2	.537
# cows affected	2nd (full)	0	0	0	0	1.000
Pooled Analysis; Primiparous		Control	250**1**	500**1**	750**1**	A**2**
# cows affected	(full)	0	0	4	2	.038
days observed	(full)	0	0	9	3	.007
Pooled Analysis; Multiparous						
# cows affected	(full)	5	1	8	2	.370
days observed	(full)	11	2	12	2	.752

1 Sometribove dose (mg/14 days)

2 Probability A: Significance probability for test statistic from Cochran-Armitage test (# cows affected) or Poisson regression (day observed, total cases). N = Non-convergence.

3 Probability B: Significance probability for residual lack of fit from Cochran-Armitage test (# cows affected) or Poisson regressior observed, total cases). N = Non-convergence.

Table 76. Diarrhea1

TAS; Primiparous	Period	Control	600	1800	3000	A
# cows affected	1st (full)	3	4	4	5	.450
days observed	1st (full)	5	9	7	15	.050
total cases	1st (full)	4	8	6	13	.062
# cows affected	2nd (full)	2	2	1	0	.382
days observed	2nd (full)	2	3	1	0	.552
total cases	2nd (full)	2	2	1	0	.587
TAS; Multiparous						
# cows affected	1st (full)	5	8	7	7	.674
days observed	1st (full)	10	16	34	23	.013
total cases	1st (full)	6	10	18	20	.004
# cows affected	2nd (full)	5	2	4	3	.935
days observed	2nd (full)	6	5	6	5	.899
total cases	2nd (full)	6	3	6	4	.866
Pooled Analysis; Primiparous		Control	250	500	750	A
# cows affected	(full)	19	3	24	3	.685
days observed	(full)	33	4	44	5	.422
Pooled Analysis; Multiparous						
# cows affected	(full)	59	2	60	3	.288
days observed	(full)	92	2	134	4	.622

1 See Table 75 footnotes.

In the musculoskeletal system, sometribove-treated cows had more disorders of the hock (Table 77), which were primarily manifested as swellings or enlargements. This effects was also suggested in the physical examination data (not shown). Sometribove-treated multiparous cows tended to have more disorders of the hoof and foot region (e.g., bruises, infections, swellings) in the daily observation database (Table 78), but not in the physical examination database (no observations). Similarly, there was a trend for increased lameness in treated cows in the daily observation database (Table 79), but not in the physical examination data. The physical examination data indicated that treated cows had more lesions such as lacerations, enlargements, and calluses of the knee (carpal region) (Table 80). Because of the inconsistencies in these data, the lameness clinical field study, described in Section 6.f, was conducted to more thoroughly evaluate the effects of sometribove on the musculoskeletal system.

Table 77. Hock disorders1.

TAS; Primiparous	Period	Control	600	1800	3000	A
# cows affected	1st (full)	0	0	1	1	.198
days observed	1st (full)	0	0	2	2	.110
total cases	1st (full)	0	0	2	1	.242
# cows affected	2nd (full)	0	0	0	0	1.000
TAS; Multiparous						
# cows affected	1st (full)	1	2	1	4	.172
days observed	1st (full)	3	3	1	4	.875
total cases	1st (full)	1	3	1	4	.349
# cows affected	2nd (full)	1	2	3	1	.787
days observed	2nd (full)	1	2	4	1	.853
total cases	2nd (full)	1	2	4	1	.853
Pooled Analysis; Primiparous		Control	250	500	750	A
# cows affected	(full)	2	0	5	2	.091
days observed	(full)	7	0	32	2	.031
Pooled Analysis; Multiparous						
# cows affected	(full)	7	3	19	4	.018
days observed	(full)	19	9	89	11	.000

1 See Table 75 footnotes.

Table 78. Foot disorders1.

TAS; Primiparous	Period	Control	600	1800	3000	A
# cows affected	1st (full)	1	0	1	2	.297
days observed	1st (full)	1	0	1	3	.147
total cases	1st (full)	1	0	1	3	.147
# cows affected	2nd (full)	0	0	0	0	1.000
TAS; Multiparous						
# cows affected	1st (full)	2	3	1	4	.539
days observed	1st (full)	6	7	3	11	.342
total cases	1st (full)	6	4	1	6	.842
# cows affected	2nd (full)	2	1	1	4	.147
days observed	2nd (full)	3	4	3	13	.011
total cases	2nd (full)	2	2	1	4	.443
Pooled Analysis; Primiparous		Control	250	500	750	A
# cows affected	(full)	8	1	5	2	.322
days observed	(full)	15	3	23	5	.740
Pooled Analysis; Multiparous						
# cows affected	(full)	9	8	19	7	.126
days observed	(full)	25	17	46	35	.000

1 See Table 75 footnotes.

Table 79. Lameness1.

TAS; Primiparous	Period	Control	600	1800	3000	A
# cows affected	1st (full)	0	1	2	3	.051
days observed	1st (full)	0	2	3	5	.040
total cases	1st (full)	0	2	2	5	.042
# cows affected	2nd (full)	2	1	0	0	.167
days observed	2nd (full)	3	2	0	0	.229
total cases	2nd (full)	2	2	0	0	.317
TAS; Multiparous						
# cows affected	1st (full)	4	4	4	5	.696
days observed	1st (full)	9	9	5	18	.097
total cases	1st (full)	6	7	4	10	.450
# cows affected	2nd (full)	1	1	3	3	.098
days observed	2nd (full)	5	1	8	10	.042
total cases	2nd (full)	2	1	7	8	.019
Pooled Analysis; Primiparous		Control	250	500	750	A
# cows affected	(full)	7	3	8	3	.749
days observed	(full)	14	5	19	6	.769
Pooled Analysis; Multiparous						
# cows affected	(full)	19	9	33	7	.127
days observed	(full)	52	21	62	24	.177

1 See Table 75 footnotes.

Table 80. Knee calluses (physical examination data).

Pooled Analysis; Primiparous	Control	250	500	750	P**1**
# cows affected (180 d)	2	1	6	2	.181
# cows affected (end lac)	1	1	5	0	.430
Pooled Analysis; Multiparous					
# cows affected (180 d)	19	6	33	7	.052
# cows affected (end lac)	6	3	21	4	.001

1 Significance probability for test statistic from Cochran-Mantel-Haenszel nonzero correlation test.

For the genito-urinary system, sometribove-treated cows experienced increased disorders of the uterus (e.g., fluid in the uterus, adhesions; Table 81).

Table 81. Uterine disorders1.

TAS; Primiparous	Period	Control	600	1800	3000	A
# cows affected	1st (full)	0	2	0	2	.448
days observed	1st (full)	0	2	0	2	.463
total cases	1st (full)	0	2	0	2	.463
# cows affected	2nd (full)	0	0	0	0	1.000
TAS; Multiparous						
# cows affected	1st (full)	1	2	4	4	.112
days observed	1st (full)	1	2	8	5	.068
total cases	1st (full)	1	2	4	5	.093
# cows affected	2nd (full)	1	2	0	0	.201
days observed	2nd (full)	1	3	0	0	.178
total cases	2nd (full)	1	2	0	0	.260
Pooled Analysis; Primiparous		Control	250	500	750	A
# cows affected	(full)	5	1	10	4	.159
days observed	(full)	5	1	15	6	.025
Pooled Analysis; Multiparous						
# cows affected	(full)	12	6	29	3	.059
days observed	(full)	16	15	44	10	.007

1 See Table 75 footnotes.

For the respiratory system, there appeared to be an increase in the incidence of congestion and abnormal breathing in multiparous cows. However, the majority of these incidents occurred at the Dardenne Single Dose-IM trial. Because this disorder primarily occurred at one trial and was significant for only multiparous cows, FDA concluded that it was not a real effect of the drug.

Treated multiparous cows had an increased duration of udder dermatitis, but this was attributed to a small number of cows with long cases at the Cornell Single Dose-IM and SQ-Dose trials. Multiparous cows treated with sometribove had a greater incidence of udder swellings or edema than controls. However, these incidents were primarily associated with mastitis, dermatitis, and similar disorders.

Under the miscellaneous category, there was an increase in incidence of elevated body temperature in sometribove-treated cows. This observation is more fully discussed in Section 6.m.3. There was an increase in the number of observations of "off-feed" (feed refusal) and total days medicated for sometribove-treated cows (Tables 82 and 83).

Table 82. Off-feed (not eating)1.

TAS; Primiparous	Period	Control	600	1800	3000	A
# cows affected	1st (full)	1	1	3	6	.003
days observed	1st (full)	1	4	9	19	.000
# cows affected	2nd (full)	2	0	0	0	.174
days observed	2nd (full)	4	0	0	0	N
TAS; Multiparous						
# cows affected	1st (full)	6	3	3	5	.832
days observed	1st (full)	18	5	6	9	.132
# cows affected	2nd (full)	0	2	1	2	.283
days observed	2nd (full)	0	5	1	4	.400
Pooled Analysis; Primiparous		Control	250	500	750	A
# cows affected	(full)	5	10	20	5	.113
days observed	(full)	9	15	37	9	.015
Pooled Analysis; Multiparous						
# cows affected	(full)	26	16	31	11	.740
days observed	(full)	43	31	66	17	.187

1 See Table 75 footnotes.

Table 83. Total days medicated1.

TAS; Primiparous	Period	Control	600	1800	3000	A
# cows affected	1st (full)	6	7	7	7	1.000
days observed	1st (full)	27	39	55	114	.000
# cows affected	2nd (full)	3	2	1	1	.958
days observed	2nd (full)	49	21	17	34	.000
TAS; Multiparous						
# cows affected	1st (full)	14	13	13	14	1.000
days observed	1st (full)	109	79	114	330	.000
# cows affected	2nd (full)	9	6	3	8	.926
days observed	2nd (full)	100	149	26	319	.000
Pooled Analysis; Primiparous		Control	250	500	750	A
# cows affected	(full)	42	16	58	21	.005
days observed	(full)	199	74	295	78	.223
Pooled Analysis; Multiparous						
# cows affected	(full)	132	36	154	34	.036
days observed	(full)	669	238	1212	221	.000

1 See Table 75 footnotes.

During the dry period and in the first sixty days of the next lactation there were no effects on cows previously administered sometribove with the exception that the incidence of metabolic disorders, primarily milk fever, was reduced in multiparous cows (Table 84). A trend toward increased numbers of cows with retained placenta was evident in the pooled analysis (Table 85). The data from the five individual trials contributing to this data set (i.e., the Single Dose-IM Study trials and the IM Dose-Determination Study) were analyzed using a stratified analysis based on the Mantel-Haenszel Test. Analyses were conducted comparing controls and the 500 mg sometribove-treated cows with parities separated and with parities pooled, using a one-tailed test. The probability of the observed increase being due to chance, if there was no difference between the treatments, was .0209 for the analysis where the parities were kept separate and .0256 where parities were pooled. Thus, FDA concluded that the increase in the incidence of retained placenta in the subsequent post-calving period was a repeatable effect.

Table 84. Metabolic disorders1

TAS; Primiparous	Period	Control	600	1800	3000	A
# cows affected	2nd (pre)	0	0	0	0	1.000
# cows affected	2nd (next)	1	0	0		.453
TAS; Multiparous						
# cows affected	2nd (pre)	5	0	0	1	.089
# cows affected	2nd (next)	3	0	0	0	.057
Pooled Analysis; Primiparous		Control	250	500	750	A
# cows affected	(next)	2	0	1	0	.735
Pooled Analysis; Multiparous						
# cows affected	(next)	37	1	10	0	.000

1 See Table 75 footnotes.

Table 85. Retained placenta1.

TAS; Primiparous	Period	Control	600	1800	3000	A
# cows affected	2nd (pre)	0	1	0	0	.782
# cows affected	2nd (next)	0	0	0		1.000
TAS; Multiparous						
# cows affected	2nd (pre)	4	1	1	2	.567
# cows affected	2nd (next)	0	0	1	0	.711
Pooled Analysis; Primiparous		Control	250	500	750	A
# cows affected	(next)	6	1	9	1	.155
Pooled Analysis; Multiparous						
# cows affected	(next)	20	3	25	1	.158

1 See Table 75 footnotes.

Conclusions

Sometribove use is associated with increased frequency of use of medication in cows. Use of sometribove may result in an increase in digestive disorders such as indigestion, bloat, and diarrhea. Treated cows may have more disorders of the uterus, and there may be an increase in the number of cows experiencing periods of "off-feed" (reduced feed intake) during treatment with sometribove. Also, the incidence of retained placenta may be higher following subsequent calving. These effects are provided on the product labeling.

l. Calf Birth Traits, Growth, and Health

Analysis of health data from calves born to cows treated with sometribove was conducted. Cows received sometribove for approximately the first 7 months of the 9-month gestation. The following studies were included in the analysis:

Multi-lactation Chronic Animal Toxicity Study (TAS)
Multi-location SC Dose Response Clinical Study (4 Dose-SC)
Multi-location IM Single Dose Study (Single Dose-IM)
IM Dose Titration Study (Dose-IM)

The experimental design of each of these studies is described in Sections 6.b, 5.a, 6.e, and 6.d, respectively. A pooled analysis was conducted using studies that contained the control and 500 mg dosage groups. The calves from the TAS study were evaluated separately because of the higher doses administered to study cows.

The methods of gathering and analyzing health observations for calves were similar to those described for cows (Section 6.k). Birth traits measured were calving difficulty, gestation length, sex ratio, birth weight, height (TAS only), and girth (TAS only). Health data were analyzed separately by categories which are listed in Table 86. In addition, certain health observations were considered to be birth abnormalities (italicized in Table 86). Physical examinations were generally performed on calves within 72 hours of birth. Calves were observed for variable periods of time after birth depending upon the study. In the TAS study, all calves were observed for five weeks. In the 4 Dose-SC study, male calves were observed for one week and female calves for four weeks. In the Single Dose-IM study, all calves were observed for nine weeks. In the Dose-IM study, female calves (only) were observed for four weeks.

For purposes of the birth trait analyses all available data for calves were utilized. These included birth data from calves that were born alive or stillborn and calves from breedings that occurred beyond the breeding cut-off dates for each study. Data for the growth analyses included only the calves that completed the observation period as specified for each study. However, in the health and birth abnormality analyses, all calves observed for any part of the observation period were included.

Calf Performance in the Multi-lactation Chronic Toxicity Study (TAS)

There was no effect of sometribove on the birth traits with the exception of a trend for average calf birth weight, height, and girth to be reduced for the calves for both years from the multiparous cows treated at the two highest levels of sometribove compared to calves from control multiparous cows.

Average daily gains and feed intake were unaffected by the cow's previous treatment (Table 87). Gains were as expected for calves in this age group and breed. Similarly, hematology and blood chemistry variables were unaffected by sometribove treatment of the cow.

Table 86. Categories of birth abnormalities used for analysis

Category	Birth Abnormality	Category	Birth Abnormality
Nervous System	Disorientation	Heart/ Circulatory	Anemia
			*Heart Murmur**
			Redness gums
			Elevated heart rate
Skeletal	Underdeveloped tail		Septicemia/Toxemia
	*Toed in or Toed out**		
	Sickle hock	Urogenital	Female twin to male
	Bench knee		*Undescended testicle**
	*Curvature**		Underdeveloped uterus
	Conformation		Urogenital
	Swollen limb		Underdeveloped vulva
	Pain		Underdeveloped ovary
	Abnormal Sound/Abnormal		Enlarged
	*Enlarged Head**		
	Fracture/Cracked	Sensory	Hematoma eye
	Arthritis/	(eye,ear nose)	*Opacity**
	joint inflammation		Watery eye
	Hematoma		Congestion eye
Muscle	*Contracted tendons**		Redness/bloody nose
	*Hernia - inguinal**		Bloody eye
	Hyperextension		
	Lameness/Limping/Weak	Skin/hair	Extra teats
			Enlarged umbilical
Alimentary/	*Hernia - umbilical**		Abnormal umbilical
Digestive	*Underdeveloped teeth**		
	*Over or under bite**		

	Atresia	Endocrine glands	
	Pain abdominal		
	Hard/firm abdominal		
	Ulcer/erosion	Abnormal placenta	
	Abnormal suckling		
	Abnormal sounds		
	Swollen/enlarged/edema	Abnormal	Depressed
	Atony	appearance	Underdeveloped
Respiratory	Elevated rate	Stillbirths	
	Difficult breathing		
	Panting		

** Birth Abnormalities*

Table 87.

The effect of sometribove administered to the cow during the TAS study at dosages of 0, 600, 1800, and 3000 mg on 4-week average daily gain and dry matter intake of calves.

Year 1

	Sometribove Dosage (mg)				
Parity**1**	0	600	1800	3000	Prob**2**
	----Mean Average Daily Gain**3** (kg/d) ± standard error (n)----				

1	0.39±0.03	(4)	0.58±0.16	(2)	0.21	(1)	(0)	NA
2,3	0.34±0.05	(9)	0.33±0.05	(6)	0.31±0.06	(5)	0.35±0.05 (8)	.965

-----Mean Dry Matter Intake**3** (kg/d) ± standard error (n)-----

1	0.84±0.01	(4)	1.25±0.06	(2)	0.73	(1)	(0)	NA
2,3	0.75±0.05	(9)	0.89±0.06	(6)	0.72±0.07	(5)	0.78±0.05 (8)	.223

Year 2

---------- Sometribove Dosage (mg) ---------

Parity**1**	0	600	1800	3000	Prob**2**

----Mean Average Daily Gain**3** (kg/d) ± standard error (n)----

2	0.31±0.07	(4)	0.33±0.01	(2)	0.13	(1)	(0)	NA
3,4	0.19±0.06	(10)	0.17±0.19	(1)	0.25±0.07	(7)	0.25±0.07 (7)	.845

-----Mean Dry Matter Intake**3** (kg/d) ± standard error (n)-----

2	0.82±0.02	(4)	1.05±0.08	(2)	0.64	(1)	(0)	NA
3,4	0.68±0.06	(10)	0.93±0.20	(1)	0.77±0.07	(7)	0.69±0.07 (7)	.555

1 Parity refers to the cow's parity.

2 Prob = probability that F-value exceeds calculated F-statistic for treatment main effect. NA = not statistically analyzed.

3 Average daily gain is the regression coefficient of body weight on days of age through 4 weeks of age. Dry matter intake is average daily

intake from birth to 4 weeks, excluding days when colostrum was fed. In Year 1, means are presented for parity 1, and in Year 2, means are presented for parity 2. Least-squares means are presented for remaining parity groups. The model included treatment, parity, treatment-by-parity interaction, and sex for Year 1, and treatment, parity, and sex for Year 2. Analyses of dry matter intake excluded days colostrum was fed.

Total days affected with high body temperature and pneumonia could not be evaluated due to inaccuracies in the recording of the duration of these clinical signs and, therefore, the overall category of days with clinical signs also could not be evaluated. Numbers of calves affected by these variables, however, were accurately captured and, thus, the overall category of numbers of calves with clinical signs was evaluated. In general, days that calves were medicated were accurately recorded. Therefore, total days medicated was a more appropriate indicator of sometribove's effect on overall health of offspring than days of clinical signs.

In the TAS study, total days medicated for female and male calves over the 29-day period for offspring of treated primiparous cows was increased in year one of the study. There was an increase in total days medicated for female calves of sometribove-treated multiparous cows for the 29-day observation period in year one of treatment and for males and females in year two of the TAS study. However, since the number of calves in the TAS study was small, this effect was better evaluated in the pooled analyses of the clinical trials, which had greater numbers of animals.

In the TAS study there were no significant results noted from the physical examinations for any of the parameters analyzed for either parity group.

Heifer calves from the first lactation were monitored from birth through reproductive maturity and breeding (approximately 16 months of age or until confirmed pregnant at day 60 of gestation or later). Administration of sometribove to pregnant cows did not affect health and performance of resulting female offspring, except that wither height was lower for heifers from 14 to 16 months of age for heifers born to cows dosed with 600 and 1800 mg sometribove. Growth rates through 16 months of age averaged approximately .84 kg/day and were unaffected by sometribove. Size and growth of all animals fell within the range of growth standards reported for Holstein heifers. Conception rates were control: 8/8, 600 mg: 2/3, 1800 mg: 3/3, and 3000 mg: 3/3.

Bull calves born to treated cows were not monitored through reproductive maturity in this study; therefore, no conclusions were made regarding the effect of the cow's dose of sometribove on the reproductive performance of bull calves. Thus, the product labeling states that safety to replacement bulls from dairy cows injected with sometribove has not been established.

Calf Performance in the Pooled Analyses

Effect of sometribove on birth traits in the pooled analysis (4 Dose-SC, Single Dose-IM, and Dose-IM Studies) for the 0 and 500 mg dose groups are presented in Table 88. Calving difficulty and sex ratio of calves were unaffected by administration of sometribove to their dams during gestation. Gestation length was reduced in those cows receiving sometribove versus the controls. This reduction was approximately 2.6 days for primiparous cows and 3.1 days for multiparous cows. Birth weight of calves from cows administered sometribove was reduced by approximately 1.5 kilograms in both parity groups. This effect was independent of rate of twinning.

Growth rate and average daily gain were measured for the offspring. The growth data could not be pooled across studies since length of observation and sex of calves monitored varied by study location. The evaluation of calf growth utilized all available data for each study. Cow's treatment with sometribove did not affect growth of calves born subsequent to the lactation of treatment. These data were highly variable since body weights taken within the first weeks of life can vary markedly.

Table 88.

Effect of sometribove on birth traits in the pooled analysis.

Variable	-----Dose (mg)----- 0	500	Probability
Calving Difficulty1			
Primiparous			1.00
High	16 (23.2)**2**	15 (22.7)	
Low	53 (76.8)	51 (77.3)	
Multiparous			.347
High	44 (27.3)	30 (22.2)	
Low	117 (72.7)	105 (77.8)	
Sex Ratio of Calves			
Primiparous			.741
Female	32 (40.5)	27 (37.5)	
Male	47 (59.5)	45 (62.5)	
Multiparous			.442
Female	78 (45.1)	77 (49.4)	
Male	95 (54.9)	79 (50.6)	
Gestation length (days)3,4			
Primiparous	280.4 ± .773	277.8 ± .902	.028
n	71	55	
Multiparous	280.5 ± .805	277.4 ± .850	.020
n	155	124	
Birth weight (kg)3,4			
Multiple births included			
Primiparous	37.9 ± .780	36.4 ± .731	.083
n	76	69	
Multiparous	40.5 ± .529	39.0 ± .523	.007
n	172	149	
Multiple births excluded			
Primiparous	43.9 ± .579	41.9 ± .660	.023
n	69	55	
Multiparous	46.2 ± .585	43.2 ± .644	.003
n	154	120	

1 High calving difficulty includes calving ease scores of 2, 3, and 4. Low calving difficulty includes calving ease score of 1.

2 Numbers in parenthesis are percentages.

3 Location by treatment dose was used as the error term.

4 Results are reported as least squares means ± standard error of least squares means.

There was a decrease in total days medicated for female calves of sometribove-treated primiparous cows for the 29-day observation period. There was an increase in total days medicated for male calves from treated primiparous cows over the 9-day observation period, but a decrease in total days medicated for male calves from treated multiparous cows compared to values from calves of control cows. These differences in response indicated that there was no clear effect of sometribove treatment of the cow on days of medication for the calves.

A decrease in diarrhea was noted for calves from primiparous cows over the 29 day observation period. There was an increase in abnormal lung sounds for female calves from primiparous cows treated with sometribove; however, this was the result of only two observations in the 750 mg group and no observations in the other dose groups. Also, there were no similar trends in female calves of multiparous cows or in male calves of either parity group. There were additional statistically significant effects (both increases and decreases) due to sometribove treatment of cow on some additional clinical signs. However, these effects were not consistent across male and female calves and/or parity group of the cow, and FDA therefore did not consider these to be of biological importance.

Calf Abnormalities

Birth abnormalities which were observed from the pooled data are listed in Table 86. When all calves of control vs. 500 mg primiparous cows from the IM and SC trials were pooled there were increased overall birth abnormalities in female (12 vs. 6 for controls) and male (15 vs. 9) calves of 500 mg sometribove dosed primiparous cows, due primarily to increased hernias (2 treated vs. 0 for controls for females, and 5 treated vs. 2 controls for males) within the "Alimentary" category.

When selected important birth abnormalities considered to represent those which may affect animal health and survival (italicized in Table 86) were analyzed comparing calves of controls and 500 mg sometribove-treated cows, there was no increase in overall incidence of selected abnormalities. When all of the calves examined from 500 mg primiparous cows were considered (N=32 for female controls, N=42 for male controls, N=26 for female 500 mg calves, N=41 for male 500 mg calves), there was a slight increase in umbilical hernias for female (P=0.099) and for male (P=0.079) calves of treated primiparous cows. There was a numerical reduction for hernias in male calves of sometribove-treated multiparous cows (5 controls vs. 3 treatment group calves). When sex and parity were stratified and all control vs. 500 mg calves were compared, there was weak significance (P=0.093) for increased hernias (13 control vs. 21 treatment group calves). However, most of the hernias reported were of minimal size (the minimum being 2 cm). None of the hernias was serious enough to require surgery and, due to their small size, they were expected to resolve spontaneously. FDA concluded that a slight predisposition for calves of treated primiparous cows to have small hernias would be of no biological consequence.

There was an increase in contracted tendons for calves of treated primiparous cows (one male and one female calf compared to no control calves affected). There was no increase in calves of multiparous cows nor for combined parity and sex. Due to the small numbers

of calves involved and the inconsistency of effect, this result was considered to be due to chance.

Conclusions

FDA concluded that cows administered sometribove may have small decreases in gestation length and birth weight of calves, and they may have increased twinning rates. This information is provided on product labeling. Sometribove treatment of the cow during gestation had no adverse effects on the health of resulting offspring.

m. Miscellaneous Health Variables

1) Circulating Anti-Somatotropin Binding

The effect of sometribove on development of circulating anti-somatotropin antibodies in treated cows was estimated by measuring anti-somatotropin binding activity in blood samples collected from cows in the following studies and evaluating the correlation between binding activity and certain health and reproduction effects:

Multi-lactation Chronic Animal Toxicity Study (TAS)
Multi-location IM Single Dose Study (Single Dose-IM)
IM/SC Bridging Study (IM/SC)

The experimental design of these studies is described in Sections 6.b, 6.e, and 6.c, respectively. In the TAS study, blood was sampled for anti-somatotropin binding activity during treatment weeks -2, -1, 3, 7, and then every 8 weeks (year 1) or every 4 weeks (year 2) thereafter. In the Single Dose-IM studies, blood was sampled during weeks -3, 2, 3, 8-9, and during the last two weeks of treatment. In the IM/SC study, blood was sampled during treatment weeks -2, -1, 1, 7, and then every 6 weeks.

Binding was measured by incubation of serum with radiolabeled bovine somatotropin followed by precipitation. Binding activity was determined relative to each animal's pretreatment (pre-sometribove exposure) binding activity (negative control) and relative to rabbit polyclonal anti-somatotropin antibody (positive control).

Anti-somatotropin binding was considered positive if binding was greater than 25 % of the positive control. The control cows did not develop anti-somatotropin binding activity relative to their pretreatment levels. Anti-somatotropin binding was usually detected within the first 7 weeks of treatment in sometribove-treated cows in cases where binding was positive. In general, the level of binding gradually declined during the remainder of a lactation of treatment. Five cows in the 1800 or 3000 mg groups which achieved positive binding levels had sustained circulating anti-somatotropin binding greater than 25 % throughout the first lactation of treatment. During the second lactation of the study, two cows in the 3000 mg group had anti-somatotropin binding activity greater than 75 % after week three of treatment. There were no apparent effects upon production response, clinical pathology variables, fetuses, or postmortem findings in these animals. Binding was detected in 13 of 59 cows receiving sometribove during the first lactation of the TAS study and 2 of 24 cows in the second year. The binding was associated with the immunoglobulin, IgG. Binding also was detected in 3 of 42 sometribove-treated cows on the IM/SC study. Binding was detected in 38 of 179 cows receiving sometribove during the Single Dose-IM study.

Correlation coefficients were calculated between level of binding and the incidence of diarrhea, fetal loss, high feed refusal, high temperature, lameness, mastitis, and total clinical health signs. Comparisons of the incidence of pleural and peritoneal adhesions in post mortems of TAS cows were also made.

Excluding the pleural and peritoneal adhesion data, there were 208 comparisons encompassing six studies, two parities and seven health variables. Twenty-five of these 208 comparisons differed significantly ($P< 0.10$) from zero. Approximately 21 could be expected by chance alone; 10 were related negatively to the incidence of the variable compared to 15 which had a positive correlation with the incidence of the variable. Also, of the 25 significant comparisons, six were significant comparisons with occurrences of the variable for control cows.

Trends for variables which correlated significantly with increased binding activity in more than one comparison were not consistent. For example, mastitis was positively correlated in the Arizona IM multiparous cows and Dardenne IM primiparous cows, but negatively correlated in the TAS year one 600 mg dosed primiparous cows and TAS year two 600 mg dosed multiparous cows. Similarly, body temperature was negatively correlated in the TAS year two 600 mg dosed multiparous cows but positively correlated in the IM/SC 500 mg dosed primiparous cows. In the largest data sets, i.e., pooled Single Dose-IM study, there were no significant correlations for primiparous cows and a single negative correlation for multiparous cows (with the incidence of diarrhea). For the incidence of pleural and peritoneal adhesions from the TAS study, there also appeared to be no relationship with increased binding activity.

There was no relationship of anti-somatotropin binding activity with the health and reproduction variables examined.

2) Blood Variables

Hematology and clinical chemistry variables and selected hormones and metabolites were measured in blood samples collected from cows in the following studies:

14-Day Drug Tolerance Study
Multi-lactation Chronic Animal Toxicity Study (TAS)
Multi-location IM Single Dose Study (Single Dose-IM)
IM Dose Titration Study (Dose-IM)
IM/SC Bridging Study (IM/SC)

The experimental design and sampling schedule of each of these studies is described in Sections 6.a, 6.b, 6.e, 6.d, 6.c, respectively. Tables 89 and 90 list hematology and clinical chemistry variables, respectively, that were analyzed. In addition, selected hormones and metabolites (Table 91) also were analyzed for each study. Assays for prolactin, thyroid stimulating hormone, triiodothyronine, follicle stimulating hormone, luteinizing hormone, ACTH, cortisol, progesterone and estradiol were performed only for the TAS study. For the Dose-IM study, only calcium, urea nitrogen, triglycerides, magnesium, creatinine and phosphorus were measured. Total protein was not determined for the IM/SC Bridging study.

Table 89. Hematology Analyses1

Red blood cells	Mean corp. hemoglobin	Lymphocytes
White blood cells	Mean corp. hemoglobin conc	Eosinophils
Hematocrit	Red cell distribut. width	Basophils
Hemoglobin	Segmented neutrophils	Platelets
Mean corp. volume	Mean platelet volume	Monocytes
Band neutrophils		

1 All determinations were made by using an electronic hematology analyzer except individual white blood cell populations (lymphocytes, eosinophils, basophils, monocytes, segmented neutrophils, and band neutrophils), which were determined by manual differential count.

Table 90. Clinical Chemistry Analyses1

Calcium	Chloride	Phosphorus
Sodium	Magnesium	Bilirubin
Albumin	Potassium	Lactate Dehydrogenase (LDH)
Urea nitrogen	Creatinine	Alanine aminotransferase (ALT)
Triglycerides	Total protein	Aspartate aminotransferase (AST)
Globulin		

1 All chemistries were determined by using a clinical chemistry analyzer validated for bovine samples.

Table 91. Hormone and Metabolite Assays1

Somatotropin	Insulin
Thyroxine	Glucose
Nonesterified fatty acids	[[beta]]-hydroxybutyrate
Prolactin	Adrenocorticotropic Hormone
Triiodothyronine	Cortisol
Follicle Stimulating Hormone	Progesterone
Luteinizing Hormone Estradiol	

1 Somatotropin, thyroxine, and insulin were determined by using radioimmunoassay while nonesterified fatty acids, glucose, and [[beta]]-hydroxybutyrate were determined by using enzymatic spectrophotometry.

Hematology

Results for all variables remained within physiologically normal ranges. For the 14 Day Acute Toxicology study, there was a slight reduction in red blood cell (RBC) count detected among the sometribove-treated cows although values were within the normal

physiological range. No significant changes in total leukocytes, differential white cell counts or platelets were noted.

Concentrations of eosinophils, lymphocytes, monocytes, and band neutrophils were not altered by sometribove treatment in either lactation of the TAS study. Sometribove treatment lowered most erythrocyte-associated variables such as RBC count, hematocrit (Table 92), and hemoglobin (Table 93) during each lactation compared to controls. Although statistically significant differences were observed, mean values remained within normal ranges for lactating cows. Sometribove did not affect the incidence of immature erythrocytes and there was no evidence of pathologic hemolysis. No sometribove-related lesions were noted in bone marrow or spleen of necropsied cows (see Section 6.b).

Sometribove tended to increase platelet count during each lactation of the TAS study. Mean platelet volume was unaffected by sometribove. Total leukocyte and segmented neutrophil counts tended to be increased by sometribove during both lactations.

Hematology variables were determined at the time of physical examinations in the Single Dose-IM study. No differences were noted at the end of the first treatment period for any measured variable. At the examination on day 40 of the subsequent lactation, erythrocyte mean corpuscular volume was lower in primiparous cows that received sometribove in their first lactation. For multiparous cows at day 40 of the subsequent lactation, hematocrit, hemoglobin, and mean corpuscular hemoglobin were lower for the group treated with sometribove in the first lactation. Mean platelet volume was higher in this group for the same sampling period.

For the Dose-IM study, hematocrit, hemoglobin, mean corpuscular hemoglobin, and red cell distribution width were lower in both primiparous and multiparous cows treated with sometribove. This effect was noted for all cows during the treatment period and for primiparous cows at day 40 of the subsequent lactation. Segmented neutrophils were the only leukocyte population affected by sometribove treatment. Neutrophils were increased for the 750 mg primiparous cows and the 500 mg multiparous cows.

The hematology results for the IM/SC study indicated that RBC count, hemoglobin, hematocrit, mean cell volume, and mean cell hemoglobin were slightly lower for both parity groups in sometribove-treated cows.

Because there is expected to be a consistent lowering of hematocrit and hemoglobin values during treatment with sometribove, and because these values are commonly determined in field diagnostics, the product labeling states that its use has been associated with reductions in hematocrit and hemoglobin values during treatment.

Clinical Chemistry and Hormones

For the 14 Day Acute Toxicology study, serum electrolyte concentrations were within normal ranges. Blood urea nitrogen and creatinine levels were slightly reduced in sometribove-treated cows. Circulating concentrations of insulin, glucose and nonesterified fatty acids were elevated initially but declined throughout the rest of the study. Total bilirubin and sodium were increased while magnesium was decreased in treated cows.

Sometribove treatment significantly increased blood somatotropin concentrations during each year of the TAS study. Average somatotropin was increased ten to thirty times over control levels. The concentrations of several hormones and metabolites changed in response to sustained elevations in somatotropin. Insulin concentration was increased during each year of study, particularly for cows receiving 3000 mg. Glucose and nonesterified fatty acid concentrations also rose in response to sometribove treatment. Data supported results of previous studies showing a decreased sensitivity to insulin, allowing production of more glucose for eventual synthesis of milk lactose. Nonesterified fatty acids were primarily elevated during the early treatment period when milk response preceded the increase in feed intake and cows were in negative or reduced energy balance (see Section 6.h).

Serum thyroxine concentrations were increased during each lactation of sometribove treatment. However, little or no effect was noted for thyroid stimulating hormone or triiodothyronine, two other components of the thyroid axis which would be expected to change if basal metabolic rate was increased. The thyroid glands of sometribove-treated cows were histologically normal (Section 6.b). Thus, the change in thyroxine concentrations did not reflect a pathologic effect. Blood estradiol concentrations tended to be lower for sometribove groups than controls during the first year of treatment.

Several blood minerals were affected by sometribove treatment but remained within normal ranges. Blood calcium concentrations were increased, phosphorus concentrations were variably affected, and magnesium concentrations were decreased.

Sometribove treatment resulted in increased total protein concentrations, primarily due to increased globulin fraction. Especially during the early portion of each lactation, blood urea nitrogen and creatinine were decreased by sometribove relative to controls. These concentration changes were consistent with a protein sparing action of sometribove, e.g., incorporation of amino acids into milk protein rather than degradation for energy.

The lactate dehydrogenase and alanine aminotransferase activities in blood were transiently elevated and aspartate aminotransferase activities tended to be lower during sometribove treatment.

Blood analyses also were performed on samples collected in the IM/SC, Dose-IM, and Single Dose-IM studies. In general, values were within normal ranges for lactating dairy cattle and did not indicate disease or gross alteration of metabolism. Mean concentrations of somatotropin were higher in treated cows and differences were greatest at mid-cycle. The most commonly observed differences for sometribove-treated cows were reduced urea nitrogen and elevated nonesterified fatty acids, glucose, and insulin. Calcium levels were elevated in treated multiparous cows sampled at 180 days of lactation (following approximately 60 days of treatment).

In summary, blood clinical chemistry, endocrine, and metabolite results indicated sometribove treatment was safe at the use level (500 mg) and when chronically administered to dairy cows at dosages up to 6 times the intended use level for two lactations. With the exception of hematocrit and hemoglobin, the variables are not customarily determined for dairy cattle under field conditions and differences were within normal physiologic ranges and not associated with any clinical effect. The product

labeling includes a statement indicating that use of sometribove has been associated with reductions in hemoglobin and hematocrit values during treatment.

Table 92.

The effect of sometribove administered at 0, 600, 1800, and 3000 mg on hematocrit during the first year of the TAS study.

Week of Study 1	Control	600	1800	3000	P2
	Mean Hematocrit3 % ± standard error (number of animals)				
Primiparous					
Pre	26 ± 1 (6)	27 ± 1 (7)	27 ± 1 (6)	26 ± 1 (7)	.415
3	29 ± 1 (6)	28 ± 1 (7)	27 ± 1 (6)	25 ± 1 (7)	NT
7	32 ± 1 (6)	30 ± 1 (7)	29 ± 1 (6)	27 ± 1 (7)	NT
15	36 ± 1 (6)	32 ± 1 (7)	30 ± 1 (6)	30 ± 1 (7)	NT
23	33 ± 1 (6)	28 ± 1 (7)	28 ± 1 (6)	28 ± 1 (6)	NT
31	35 ± 1 (3)	30 ± 1 (7)	31 ± 1 (5)	30 ± 1 (5)	NT
Overall	33 ± 1 (6)	29* ± 1 (7)	29* ± 1 (6)	28* ± 1 (7)	< .001
Probability values (F-statistic) from repeated measures analysis:			Treatment-by-Time		.336
			Control vs 600 mg-by-Time		.106
			Control vs 1800mg-by-Time		.110
			Control vs 3000mg-by-Time		.689
Multiparous					
Pre	27 ± 1 (14)	27 ± 1 (13)	27 ± 1 (12)	27 ± 1 (14)	.958
3	28 ± 1 (14)	27 ± 1 (13)	26* ± 1 (12)	26* ± 1 (14)	NT
7	32 ± 1 (14)	30* ± 1 (13)	28* ± 1 (12)	28* ± 1 (14)	NT
15	34 ± 1 (14)	32* ± 1 (13)	32* ± 1 (12)	30* ± 1 (14)	NT
23	32 ± 1 (14)	31* ± 1 (13)	30* ± 1 (12)	30* ± 1 (14)	NT
31	34 ± 1 (8)	32* ± 1 (11)	31* ± 1 (10)	30* ± 1 (11)	NT
Overall	32 ±< 1 (14)	30 ±< 1 (13)	30 ±< 1 (12)	29 ± < 1 (14)	< .001
Probability values (F-statistic) from repeated measures analysis:			Treatment-by-Time		.575
			Control vs 600 mg-by-Time		.777
			Control vs 1800mg-by-Time		.478
			Control vs 3000mg-by-Time		.093

* Different from control (P< .10).

1 Pre = pretreatment period. Overall = Treatment period average.

2 NT = treatment effect not tested at individual treatment weeks.

3 Least-squares means are presented for pretreatment period. Estimated modified marginal means are presented for treatment weeks. C means are whole plot means from repeated measures analysis. Excluded primiparous cow 85075 (1800 mg) and multiparous cows 85690 (600 mg) and (1800 mg).

Table 93.

The effect of sometribove administered at 0, 600, 1800, and 3000 mg on hemoglobin during the first year of the TAS study.

Week of Study 1	Control	600	1800	3000	P2
	------Mean Hemoglobin3---g/dl ± standard error (number of animals)---				
Primiparous					
Pre	11.9 ± .4 (6)	12.0 ± .4 (7)	12.0 ± .4 (6)	11.0 ± .4 (7)	
3	12.7 ± .3 (6)	11.8 ± .3 (7)	11.6 ± .3 (6)	11.0 ± .3 (7)	
7	12.1 ± .3 (6)	10.8 ± .3 (7)	11.0 ± .3 (6)	10.3 ± .3 (7)	
15	11.9 ± .3 (6)	10.7 ± .3 (7)	10.7 ± .3 (6)	10.3 ± .3 (7)	
23	11.5 ± .3 (6)	10.1 ± .3 (7)	10.1 ± .3 (6)	10.0 ± .3 (6)	
31	12.3 ± .4 (3)	10.6 ± .3 (7)	10.5 ± .3 (5)	10.2 ± .3 (5)	
Overall	12.1 ± .2 (6)	10.8*± .2 (7)	10.8*± .2 (6)	10.3*± .2 (7)	
Probability values (F-statistic) from repeated measures analysis:		Treatment-by-Time		.764	
		Control vs 600 mg-by-Time		.370	
		Control vs 1800mg-by-Time		.528	
		Control vs 3000mg-by-Time		.673	
Multiparous					
Pre	11.9 ± .2(14)	11.8 ± .2(13)	11.8 ± .3(12)	11.7 ± .2(14)	
3	12.4 ± .2(14)	11.9 ± .2(13)	11.5 ± .2(12)	11.3 ± .2(14)	
7	12.0 ± .2(14)	11.4 ± .2(13)	11.2 ± .2(12)	10.3 ± .2(14)	
15	11.7 ± .2(14)	11.1 ± .2(13)	10.9 ± .2(12)	10.4 ± .2(14)	
23	11.5 ± .2(14)	10.8 ± .2(13)	10.8 ± .2(12)	10.2 ± .2(14)	
31	12.0 ± .3 (8)	11.5 ± .2(11)	10.8 ± .2(10)	10.3 ± .2(11)	
Overall	11.9 ± .1(14)	11.3*± .1(13)	11.0*± .1(12)	10.5*± .1(14)	<
Probability values (F-statistic) from repeated measures analysis:		Treatment-by-Time		.768	
		Control vs 600 mg-by-Time		.985	
		Control vs 1800mg-by-Time		.921	
		Control vs 3000mg-by-Time		.328	

* Different from control (P< .10).

1 Pre = pretreatment period. Overall = Treatment period average.

2 NT = treatment effect not tested at individual treatment weeks.

3 Least-squares means are presented for pretreatment period. Estimated modified marginal means are presented for treatment weeks. C means are whole plot means from repeated measures analysis. Excluded primiparous cow 85075 (1800 mg) and multiparous cows 85690 (600 mg) and (1800 mg).

3) Body Temperature

During the conduct of three pivotal studies, rectal body temperature of all cows on study was determined daily:

Multi-lactation Chronic Animal Toxicity Study (TAS)
IM Dose Titration Study (Dose-IM)
IM/SC Bridging Study (IM/SC)

Analysis of these data revealed a higher mean body temperature for cows receiving sometribove.

A Poisson-based logistic regression of observations of elevated rectal temperature as a proportion of total observations was done when more than one dose level was involved in a study (TAS and Dose-IM). All instances of elevated temperatures associated with a clinical health incident were eliminated from the analysis. Parities were pooled in the analysis.

Elevated temperature was defined as greater than 103 ° F. The results in Tables 94 and 95 (below) show a strong relationship between dose and proportion of observations of temperatures more than 103 ° F.

(Eds. note: The following table consists of 5 columns.)

Table 94.

Analysis of maximum likelihood estimates for logistic regression of elevated rectal temperature occurrence on sometribove dose in the firs lactation of TAS study.

Variable	Parameter Estimate	Standard Error	Wald Chi-Square	Prob > Chi-Squar
INTERCEPT	-4.0839	0.0777	2760.7957	0.0
DOSE	0.000499	0.000034	213.8282	0.0001

Table 95.

Analysis of maximum likelihood estimates for logistic regression of elevated rectal temperature occurrence on sometribove dose in the Dose study.

Variable	Parameter Estimate	Standard Error	Wald Chi-Square	Prob > Chi-Squar
INTERCEPT	-4.4776	0.0897	2491.3876	0.0
DOSE	0.00251	0.000152	274.3701	0.0001

The dose dependency was much steeper in the Dose-IM study, reflecting a smaller range of doses covered than in the TAS study (0 to 750 mg as opposed to 0 to 3000 mg).

Since sometribove increased the probability of observing elevated rectal temperature linearly over the 0 to 3000 mg dose range in the TAS study, analyses were carried out to determine if sometribove increased the probability of observing elevated rectal temperature at the usage dose level. It was important to distinguish between the "1X" dose level in the TAS study (600 mg injected IM) and the commercial dose level (500 mg injected SC). The probability of observing elevated rectal temperature at each dose level was predicted using the logistic regression equation above. The standard error of prediction was obtained from the quadratic form involving the level of interest and the covariance matrix of the estimates. A conservative standard error was used, predicting population means rather than individual animal means. Therefore, the predicted value was the expected proportion of all daily rectal temperature observations that might exceed 103 ° F. The predicted proportions were 0.0166 for control animals, 0.0212 for the usage level (500 mg SC) and 0.0222 for the 1X level in the TAS study (600 mg IM).

To test whether these levels differed from control, non-simultaneous 95 % confidence intervals were built around each predicted value. For the controls, the interval was (0.0142, 0.0192); for the commercial dose level, the interval was (0.0187, 0.0239); and

for the 1X level, the interval was (0.0198, 0.0250). Simultaneous intervals would be larger. The incidence of elevated rectal temperature in the 500 mg SC dose did not differ significantly from that in the control. Use of other cutoff points to define elevated rectal temperature (102.5 ° and 103.5 ° F) changed only the intercept of the equation, not the dose dependency (slope). In this instance, then, overdosing sometribove caused a greater proportion of observations of rectal temperature being above 103 ° F, while predicted use of the 500 mg dose showed no significant increase in the proportion of elevated observations.

The same confidence interval approach was used to compare 0 mg to 500 mg for the Dose-IM study. In this case, the 95 % confidence interval for 0 mg, around a predicted mean of 0.0112, was (0.0094, 0.0134). The 95 % confidence interval for 500 mg, around a predicted mean of 0.0383, was (0.0357, 0.0411). In this study, the projected use level significantly increased the probability of observing a rectal temperature greater than 103 ° F. This increase translated into 2.7 days of rectal temperature above 103.0 ° F per 100 cow days during sometribove treatment.

Because only two doses were represented in the IM/SC study (0 and 500 mg), regression was not a valid method of analysis of the results of this study. Instead, three way contingency tables (2 x 2 x 2) were constructed and various categorical techniques applied. Relative risk estimates, with 95 % confidence bounds, for observations of elevated temperature were calculated for IM dosing, SC dosing and across both routes. For IM doses, animals receiving 500 mg were 2.47 times more likely to be observed with elevated rectal temperatures than the controls. The confidence interval for relative risk was (2.04, 2.98). In the SC dosed animals, those receiving 500 mg were 2.52 times more likely to be observed with elevated rectal temperatures than the controls. In this instance, the 95 % confidence interval on relative risk was (2.03, 3.12). The common estimate across dosing methods was 2.49, with 95 % confidence bounds (2.16, 2.87).

Mean temperatures increased only slightly. For instance, means of the four dose levels in the first lactation of the TAS study ranged from 101.4 ° F for controls to 101.9 ° F for the 3X group. The standard deviation ranged from 0.52 in the controls to 0.76 in the 5X group. Due to lack of independence in the daily measurements, however, these values should not be compared to one another.

Tables 96-99 present the frequency distributions for percentage of body temperatures within specific temperature ranges across parities and entire trials.

Table 96.

Frequencies of body temperatures across parities for year one of the TAS study.

	Body Temperature Ranges				
Sometribove Treatment (mg)	< 102°	102°-103°	103°-104°	104°-105°	>105
control	89.1	10.3	.4	.2	.1
600	78.2	18.7	2.4	.6	.1
1800	57.5	35.2	5.3	1.5	.5
3000	60.7	32.2	5.5	1.3	.4

Table 97.

Frequencies of body temperatures across parities for year two of the TAS study.

	Body Temperature Ranges				
Sometribove Treatment (mg)	< 102°	102°-103°	103°-104°	104°-105°	>105
control	91.9	7.5	.6	.0	.0
600	79.4	17.8	2.3	.4	.2
1800	68.5	28.3	2.7	.5	.1
3000	66.2	28.3	4.1	1.1	.3

Table 98.

Frequencies of body temperatures across parities for the IM/SC study.

	Body Temperature Ranges				
Sometribove Treatment (mg)	< 102°	102°-103°	103°-104°	104°-105°	>105
control IM	72.6	21.7	5.0	.5	.2
control SC	73.9	21.1	4.3	.7	0
500 mg IM	59.9	26.8	8.9	3.7	.7
500 mg SC	59.6	29.1	7.3	3.0	1.0

Table 99. Frequencies of body temperatures across parities for the Dose-IM study.

	Body Temperature Ranges				
Sometribove Treatment (mg)	< 102°	102°-103°	103°-104°	104°-105°	>105

Sometribove may increase body temperature. This effect was not restricted to the warmer months of the year. However, the temperature-increasing effect of sometribove is enhanced during periods of high environmental temperature. Product labeling indicates that cows injected with sometribove may experience periods of increased body temperature unrelated to illness. To minimize this effect, the appropriate measures should be taken during periods of high environmental temperature to reduce heat stress. Also, care should be taken to differentiate increased body temperature due to use of sometribove from an increased body temperature that may occur due to illness.

4) Urinalysis

Urinalysis was performed utilizing commercially available urinalysis test strips with color sensitive areas which provided the following results: pH, bilirubin, blood, glucose, ketones, nitrate, protein, specific gravity, and urobilinogen for the following studies. Urine testing was performed during the following studies:

Multi-lactation Chronic Animal Toxicity Study (TAS)
Multi-lactation IM Single Dose Study (Single Dose-IM **Dardenne location only**)
IM Dose Titration Study (Dose-IM)
IM/SC Bridging Study

Values for pH were presented as either 7, 7.5, 8 or 8.5; bilirubin was reported as negative or small +; protein was reported as negative, trace, +, ++, +++ or ++++; specific gravity was reported as 1, 1.005, 1.01, 1.015, 1.02, 1.025, or 1.03; ketone values were reported as negative, trace, small 15, moderate 40, or large 80 or 160; the presence of blood in urine was reported as negative, trace, small +, moderate ++, or large +++; results for nitrates, urobilinogen and glucose were consistently negative.

During the TAS study, urine samples were collected at days 40 (pretreatment) and 180 of each lactation, at lactation end, and after calving at the beginning of the lactation following the second lactation of treatment. During the Dose-IM and the Dardenne IM trial urine samples were collected at day 40 (pretreatment), at day 180 of lactation, at lactation end and at the beginning of the subsequent lactation. During the IM/SC Bridging Study samples were collected at 40 and 180 days of lactation, and at lactation end.

For the Single Dose-IM Study, Dardenne location, ketone values for multiparous cows for end of lactation urine samples, 32/49 control samples were negative compared to 42/44 500 mg sometribove-treated cows. Ten control cows had trace values, 4 had "small 15" values and 3 had "moderate 40" values. Only 2 sometribove-treated multiparous cows had trace values for ketone and there were no other positive values for sometribove-treated cows. The significance for this distribution of results was P=0.001. In the IM/SC Bridging Study for ketone results for primiparous cows at lactation end, there were 3/6 control cows negative for urine ketones compared to 7/7 negative for IM dosed 500 mg sometribove-treated cows (P=0.070). However for SC sometribove-treated primiparous cows, 5/7 were negative, not significantly different from controls for this variable.

For the Dose-IM, for ketone values of multiparous cows, for samples taken during the early subsequent lactation following a full lactation of sometribove (or excipient) treatment, 10/11 control cows had positive ketone values. Four of the 11 control cows

had ketone values of "large." However, for the 250, 500, and 750 mg dosed sometribove-treated cows, 6/11, 9/12, and 6/9, respectively, were negative for ketones. The statistical value for this distribution indicated a high degree of significance at P=0.004. These data suggested that treatment of multiparous cows with sometribove resulted in reduced detectable urine ketones in the early subsequent lactation.

These results suggest a likely decrease in detectable ketone bodies (not a deleterious effect) in urine at the end of a lactation of sometribove treatment and at the beginning of a subsequent lactation following a lactation of sometribove treatment for multiparous cows.

There were no other significant differences in urine results reported from the trials.

7. Human Safety

a. Drugs for Use in Food Animals

The FDA Guideline for Toxicological Testing recommends appropriate toxicology tests for veterinary drugs in general and provides for the development of alternate testing procedures for certain classes of compounds such as, for example, biologically active proteins. As discussed in detail by Juskevich and Guyer ("Bovine growth hormone: human food safety evaluation," Science 249:875-884, 1990), the tests recommended are dependent on the potential exposure of people to residues and the possible biological effects of the protein in man. A determination of the potential oral activity of the protein in laboratory animals is the first step in the testing process. This determination involves the oral administration of exaggerated dosages daily for an appropriate time period. If the compound is determined to be orally active, more testing may be required. In the case of sometribove, there is convincing medical evidence that non-human somatotropins, like bovine somatotropin, are not biologically active in man (Recent Prog Horm Res. 15:71-114, 1959).

FDA has also required that the potential oral activity of the protein insulin-like growth factor-I (IGF-I) be assessed since this is the hormone that changes most dramatically in the blood following bovine somatotropin treatment and there was a general lack of available information on this hormone. IGF-I is a 70 amino acid protein whose amino acid sequence is identical in cows and humans (unlike bovine and human somatotropin). IGF-I is produced in various body tissues and its production is at least partially regulated by somatotropin. There is considerable evidence that IGF-I mediates some of the biologic effects of somatotropin. When bovine somatotropin is administered to dairy cows, the production of IGF-I in the cow is increased (J. Dairy Sci. 71:288, 1988).

To assess the safety implications of potential changes in IGF-I levels in milk, FDA required several studies to be conducted, including a study to assess the potential oral activity of IGF-I. When consumed orally, proteins are normally digested and destroyed in the digestive tract. Other studies were required to assess the effects of sometribove administration on the levels of IGF-I in cows' milk. To do this, it was first necessary to establish the normal range of IGF-I concentrations found in cows' milk.

b. Four Week Gavage Study in Rats with Sometribove Zinc

The study was carried out at Hazleton Laboratories America, Inc., Vienna, Virginia. Sometribove (zinc salt) was administered by daily oral gavage to groups of 20 male and 20 female Sprague Dawley rats per treatment level for 28 consecutive days. The control group was administered the dosing vehicle and the three treatment groups were given 60, 600, or 6000 micrograms of sometribove zinc per kilogram body weight each day. Animals were observed daily and a number of parameters were examined including body weight and food consumption, hematology, blood chemistry, urinalysis, postmortem examination, organ weights, and histopathology.

No adverse effects were observed in test animals. Body weights, food consumption, clinical parameters, organ weights, gross and microscopic pathology were unaffected at

all dosage levels. Therefore, the highest dosage tested, 6000 micrograms/kg body weight, was considered a "no-observed effect level" in the study.

c. Two Week Gavage Study in Rats with Insulin-Like Growth Factor-I (IGF-I)

The study was conducted at Hazleton Laboratories America, Inc. Rockville, Maryland. Insulin-like growth factor-I (IGF-I) was administered by daily oral gavage to groups of 20 male and 20 female rats for 16 consecutive days. The negative control group was administered the dosing vehicle by gavage and the other 3 oral gavage treatment groups were given 20, 200, or 2000 micrograms of IGF-I per kilogram body weight each day. The positive control groups were administered either IGF-I (50 or 200 micrograms/day) or 4000 micrograms/day alanyl porcine somatotropin (APS) via implanted osmotic pumps for 14 consecutive days. APS was used as the positive control because the release characteristics of this somatotropin from osmotic pumps and the subsequent increase in blood IGF-I were well-defined. Due to the large number of animals to be implanted with osmotic pumps, study initiation was staggered over two consecutive days so that one-half of the animals in each group were started each day. Animals were observed daily and a number of parameters were examined including body weight, food consumption, hematology, blood clinical chemistry, blood IGF-I levels, urinalysis, post-mortem examination, organ weights, histologic examination of the gastrointestinal tract, tibia length, and tibial epiphyseal width.

No adverse effects were observed in the study. Body weights and food consumption were not affected by gavage administration of IGF-I in male and female rats in the low and mid dose groups. Half of the high dose males started on one day exhibited a small but statistically significant increase in body weight gain. This finding was considered incidental and not related to treatment as it did not occur in the other group of high dose male rats started the following day nor in the high dose female rats. There were no dose related changes in organ weights, blood IGF-I levels or other measured clinical parameters in male or female gavage dosed rats, unlike rats given IGF-I systemically via osmotic pumps. The gastrointestinal tract of gavage treated rats was normal based on microscopic examination. Rats administered IGF-I systemically (pump), particularly at the higher dose, exhibited increased body weight gain, changes in clinical parameters and various organ weights, elevated blood IGF-I and increased tibial epiphyseal width. Rats given APS systemically exhibited the largest increases in body weight gain, greater changes in clinical parameters and organ weights, elevated blood IGF-I and increased tibial length and width. The highest oral gavage dosage of 2000 micrograms/kg body weight was considered a "no-observed effect level" since no significant toxicity was observed in rats at this level of exposure.

d. Survey of Insulin-like Growth Factor-I (IGF-I) Concentrations in Individual Milk Samples from Five Missouri Dairy Herds

The analytical phase of the study was conducted at the Monsanto Agricultural Company research facility at Chesterfield, Missouri. Lactating Holstein dairy cows (172 primiparous, 237 multiparous) from five separate farms in Missouri were used in the study. These animals had never been administered exogenous bovine somatotropin products. Individual milk samples were collected from each cow for measurement of endogenous levels of IGF-I using a validated assay.

The overall mean concentration of milk IGF-I for the 5 farms was 2.54 ng/ml. Mean concentration at each farm ranged from 0.29 to 4.21 ng/ml while individual cow values ranged from 0 to 30 ng/ml. Milk IGF-I concentrations were slightly higher in multiparous than primiparous cows and much higher during early lactation (6.2 ng/ml) than later lactation (1.9 ng/ml). Stage of lactation and farm location had the greatest effect on endogenous milk IGF-I concentrations.

e. Survey of Insulin-Like Growth Factor-I (IGF-I) Concentrations in Commercial Bulk Tank Milk Samples

The analytical phase of this study was conducted at the Monsanto Agricultural Research facility at Chesterfield, Missouri. In this study, milk samples from 100 bulk tanks were collected at a local commercial milk processing plant in Missouri. Bulk tank samples were obtained from commercial dairy farms that had not used exogenous bovine somatotropin products. Endogenous levels of IGF-I were measured in individual bulk tank milk samples using a validated assay. The mean concentration of IGF-I for all bulk tank samples was 4.3 ng/ml with concentrations for individual bulk tank samples ranging from 1.3 to 8.1 ng/ml.

f. Comparison of Milk Concentrations of Insulin-Like Growth Factor-I (IGF-I) Following Subcutaneous Injections of Sometribove

This study was conducted at the Monsanto Animal Research Center, O'Fallon, Missouri. Nine lactating Holstein dairy cows were administered 500 mg of sometribove by subcutaneous injection every two weeks. Treated cows were injected on study days 0, 14 and 28. Nine control cows were sham injected at the same study intervals. Milk levels of IGF-I were measured pre-test and in the middle (day 7) of each injection cycle using a validated assay.

Mean pre-treatment milk IGF-I concentrations were 5.05 ng/ml for controls and 3.94 ng/ml for treated cows. During the study, milk IGF-I concentrations ranged from 3.16 to 3.35 ng/ml for control cows and from 3.49 to 5.31 ng/ml for treated cows. The difference in milk IGF-I between control and treated cows was statistically significant at the 5 % probability level. However, the levels of IGF-I in milk from both control and treated cows are 100 to 1000-times lower than endogenous blood levels in humans. Thus, even if ingested IGF-I was not destroyed in the digestive tract and was absorbed intact, the dilution of a few nanograms of undigested IGF-I into the large endogenous plasma pool in humans would be physiologically insignificant. Therefore, milk IGF-I levels are safe for all consumers, including young infants.

g. Conclusions on Human Safety

The lack of oral activity of sometribove and IGF-I and the nontoxic nature of the residues of these compounds, even at exaggerated doses, demonstrates that sometribove and IGF-I present no human safety concern when consumed orally. Therefore, there is no need to establish a safe concentration of total residue or a residue method in meat or milk. Also, there is no need to establish a tolerance or withdrawal time for human consumption of meat or milk from dairy cows administered sometribove.

8. Agency Conclusions

This new animal drug application satisfies the requirements of Section 512 of the Federal Food, Drug, and Cosmetic Act (the Act) and demonstrates that Posilac® (sterile sometribove zinc suspension), when used under its proposed conditions of use, is safe and effective for its labeled indication.

Since neither meat nor milk from animals treated with sometribove presents any increased risks if consumed by people, it is not necessary to establish a safe concentration of total residue or to have a regulatory assay method or confirmatory assay method for milk or tissue residues. It is therefore unnecessary to establish a tolerance or withdrawal time for human consumption of meat or milk from dairy cows administered this drug product.

Sterile sometribove zinc suspension is not eligible for generic copying under section 512(b)(2) of the Act because it is a recombinant DNA-derived (i.e., biotechnology-derived) drug; section 106 of the Generic Animal Drug and Patent Term Restoration Act (GADPTRA) of 1988 provides that FDA may not approve an abbreviated application submitted under Section 512(b)(2) for a new animal drug which is primarily manufactured using, inter alia, recombinant DNA.

The Center for Veterinary Medicine has concluded that, for this drug product, adequate directions for use by the layman have been provided. Label directions are accompanied by pictorial diagrams and detailed instructions in plain language. Historically, the industry is familiar with subcutaneous injections. The syringe is a pre-loaded single-dose syringe, and this adds to the simplicity of use. The use of the product should be preceded by a comprehensive and ongoing herd management, mastitis control, and reproductive program, as recommended in product labeling. The drug is not a controlled substance. Thus, labeling is adequate for the intended use.

9. Labeling

a. Product made in Austria

1) Package Insert
2) Carton containing 25 single-dose syringes
3) 500-mg single-dose syringe
4) Shipping carton

b. Product made in Austria, filled and packaged in The Netherlands

1) Package Insert
2) Carton containing 25 single-dose syringes
3) 500-mg single-dose syringe
4) Shipping carton

Copies of these labels may be obtained by writing to the:

Freedom of Information Office
Center for Veterinary Medicine, FDA
7500 Standish Place
Rockville, MD 20855

FREEDOM OF INFORMATION SUMMARY
NADA 140-872
FIGURES

FIGURE 1
EFFECT OF VARYING DOSES OF SOMETRIBOVE ON PERCENT MILK FAT OF PRIMIPAROUS COWS

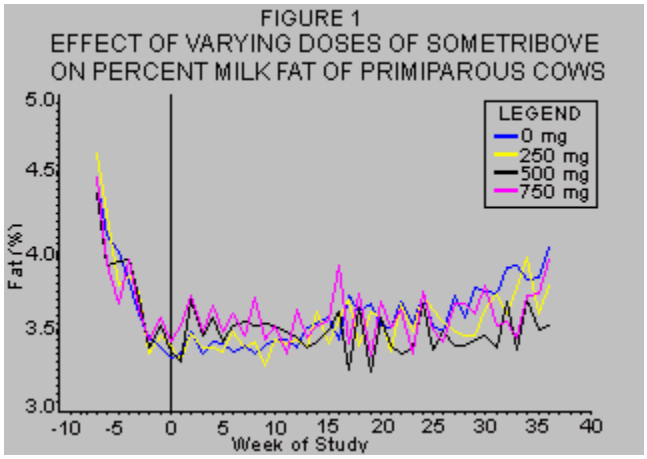


FIGURE 2
EFFECT OF VARYING DOSES OF SOMETRIBOVE ON PERCENT MILK FAT OF MULTIPAROUS COWS

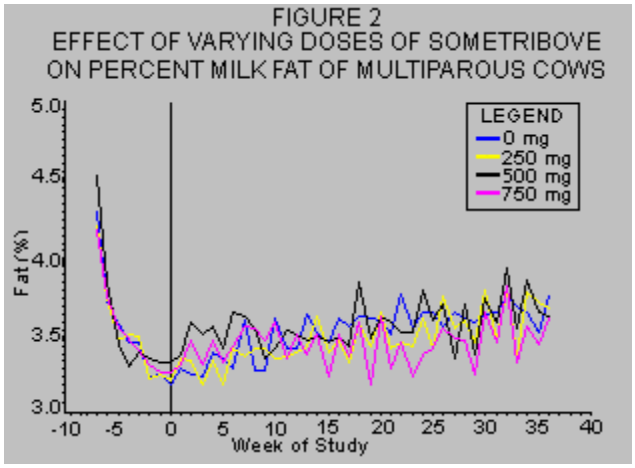


FIGURE 3
EFFECT OF VARYING DOSES OF SOMETRIBOVE ON PERCENT MILK PROTEIN OF PRIMIPAROUS COWS

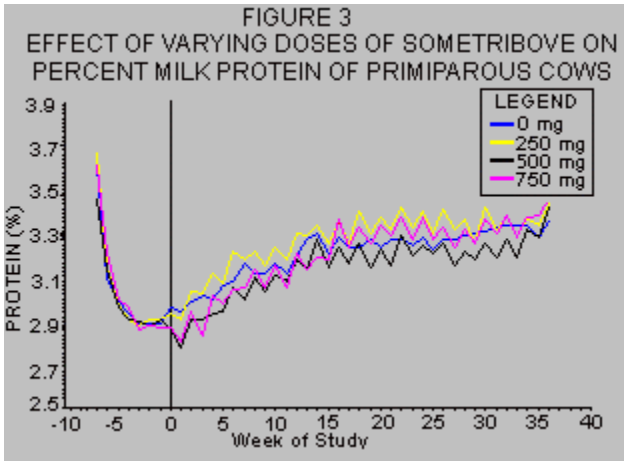


Figure 4
EFFECT OF VARYING DOSES OF SOMETRIBOVE ON PERCENT MILK PROTEIN OF MULTIPAROUS COWS

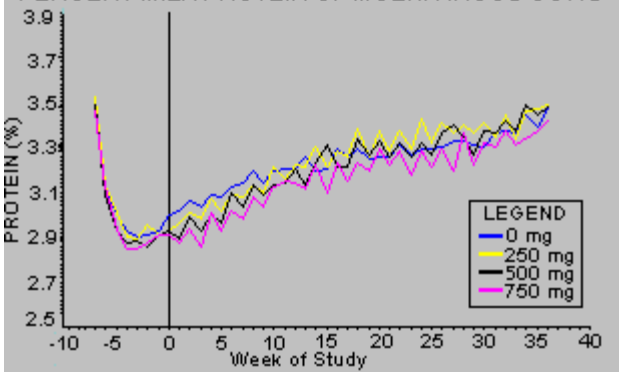


FIGURE 5
EFFECT OF VARYING DOSES OF SOMETRIBOVE ON PERCENT MILK LACTOSE OF PRIMIPAROUS COWS

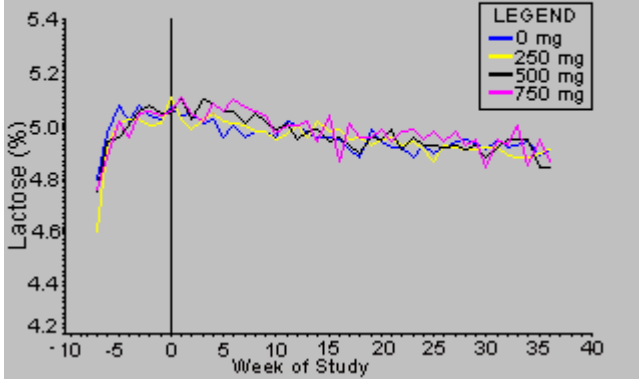


Figure 6
EFFECT OF VARYING DOSES OF SOMETRIBOVE ON PERCENT MILK LACTOSE OF MULTIPAROUS COWS

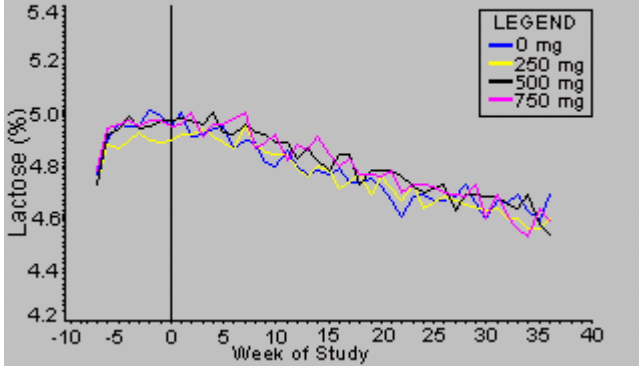


Figure 7
EFFECT OF VARYING DOSES OF SOMETRIBOVE ON DRY MATTER INTAKE OF PRIMIPAROUS COWS

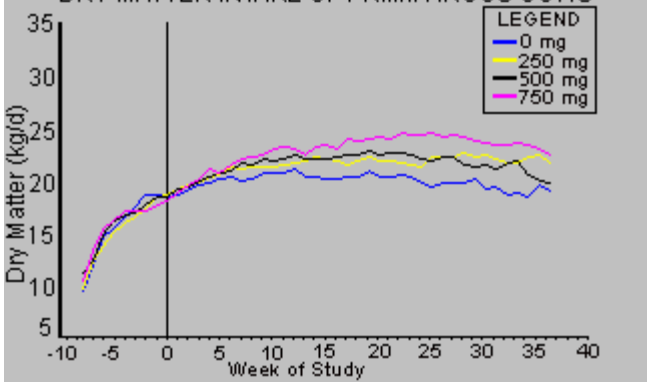


Figure 8
EFFECT OF VARYING DOSES OF SOMETRIBOVE ON ACTUAL MILK OF PRIMIPAROUS COWS

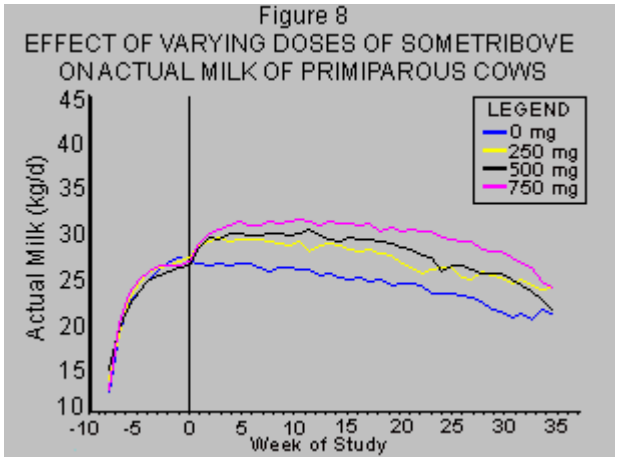
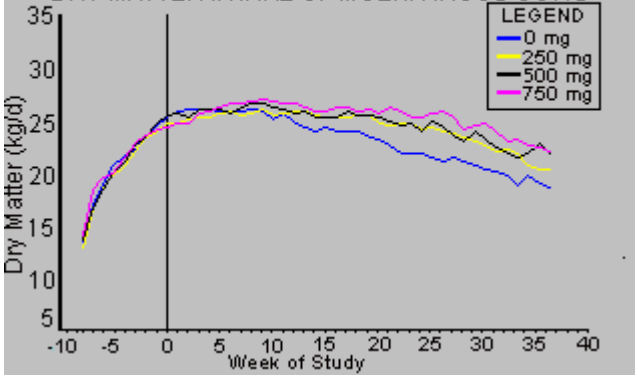


Figure 9
EFFECT OF VARYING DOSES OF SOMETRIBOVE ON DRY MATTER INTAKE OF MULTIPAROUS COWS



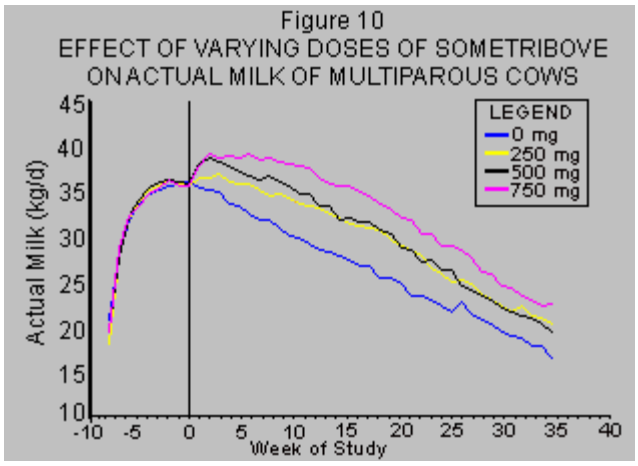


Figure 10
EFFECT OF VARYING DOSES OF SOMETRIBOVE
ON ACTUAL MILK OF MULTIPAROUS COWS
LEGEND
0 mg
250 mg
500 mg
750 mg
Actual Milk (kg/d)
45
40
35
30
25
20
15
10
-10
-5
0
5
10
15
20
25
30
35
40
Week of Study