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Report to Congressional Requesters

August 1992

RECOMBINANT BOVINE GROWTH HORMONE

FDA Approval Should Be Withheld Until the Mastitis Issue Is Resolved

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Program Evaluation and
Methodology Division

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The Honorable Patrick J. Leahy
Chairman, Committee on Agriculture, Nutrition,
and Forestry
United States Senate

The Honorable Richard G. Lugar
Ranking Minority Member
Committee on Agriculture, Nutrition, and Forestry
United States Senate

The Honorable John Conyers, Jr.
Chairman, Committee on Government Operations
House of Representatives

The Honorable Ted Weiss
Chairman, Subcommittee on Human Resources
and Intergovernmental Relations
Committee on Government Operations
House of Representatives

The Honorable Thomas A. Daschle
United States Senate

The Honorable Albert Gore, Jr.
United States Senate

The Honorable Herb Kohl
United States Senate

The Honorable David R. Obey
House of Representatives

The Honorable James H. Scheuer
House of Representatives

You asked us to evaluate the thoroughness of the investigational review of the bovine growth hormone products submitted to the Food and Drug Administration (FDA) for its approval. The safety and efficacy of recombinant bovine growth hormone (rBGH) products developed to

increase cow milk production have been questioned by a number of individuals and groups.[1] Some have noted that the introduction of a biotechnologically engineered product into the food supply of the American consumer could be a threat to human health, while others are concerned about the animal safety effects and efficacy of the drug. Proponents of the use of rBGH believe that it will increase the quantity of milk produced by cows without endangering humans or animals. We have completed our evaluation of the rBGH review and present within this report our findings, conclusions, and recommendations.[2]

Background

The FDA has approved the use of rBGH for research only, and has also allowed the sale and consumption of milk and beef products from rBGH-treated cows during the investigational process. If approved for commercial use, this biotechnologically engineered animal drug will be allowed to gain widespread adoption within the U.S. marketplace.

In its naturally occurring state, BGH is a protein produced in the pituitary gland of all cattle. It is a somatotropin, or growth hormone, that helps to coordinate how energy from feed is normally allocated within a cow's body to meet its physical needs and to produce milk. BGH can be produced synthetically using recombinant DNA technology. Since both natural BGH (nBGH) and rBGH have been shown in several studies to increase milk production in cattle, rBGH is being introduced to improve the efficiency and lower the cost of milk production.

Drugs for use in animals are approved by FDA's Center for Veterinary Medicine by way of two distinct applications. Sponsors must first submit to the Center an investigational new animal drug application that outlines the way they will conduct their investigational research in the areas of human food safety, animal safety, and drug efficacy. After this application has been approved and the studies have been completed, the sponsor then requests final approval to market the drug by submitting a new animal drug application. This second application consists of a compilation of certain investigational studies—known as pivotal studies—that have been

[1]In this report, we use three terms associated with bovine growth hormone. Recombinant bovine growth hormone (rBGH) is a biotechnologically engineered product, some formulations of which are under product review by the FDA. Natural bovine growth hormone (nBGH) is a natural bovine hormone. The term BGH is used generically in discussions that apply to both recombinant and natural BGH.

[2]The terms "rBGH review" or "rBGH investigational review" are used throughout this report to mean both the investigational work conducted by rBGH product sponsors and the work conducted by the FDA in reviewing the rBGH drug applications.

completed by the sponsors to show the safety and efficacy of the proposed product.

The Center makes two basic decisions during its review. First, early in the investigational phase, it determines whether food products from the target animal are safe for human consumption. This conclusion allows marketing of food products from tested animals during the investigational phase. Second, and later in the investigational phase, decisions are made as to (1) whether the drug is effective for its intended use, (2) whether it is safe for the target animal, (3) whether the drug production process can reliably generate a product that meets the exact formulation proposed, and (4) whether the drug poses an environmental threat.

A sponsor of a new animal drug will often first submit protocols (research designs) to the FDA's Center for Veterinary Medicine for the pivotal studies it will be conducting to demonstrate that the drug is safe and effective. The sponsor is under no obligation to submit the protocols to the Center before conducting the pivotal studies, but it is often in the sponsor's best interest to obtain the Center's view on study designs in advance. The Center reviews the protocols and makes comments as to whether the designs of the pivotal studies are acceptable and whether the studies will be helpful in determining whether or not to approve the drug.

The sponsor then conducts pivotal studies in the areas of human food safety, animal safety, and drug efficacy and is required to submit all of the resulting data and information to FDA's Center for Veterinary Medicine for review. In almost all cases, the Center sends the sponsor an "incomplete letter" detailing inadequacies in a study or raising questions about its findings. The Center reviews follow well-established FDA guidelines—both procedural and technical—in the areas of human food safety, animal safety, and drug efficacy. Specific studies are required by FDA to ensure production and environmental viability.

The process of addressing the original and subsequent questions or issues raised by FDA's Center continues until it is satisfied that the studies adequately address all the human food safety, animal safety, and drug efficacy points pertinent to the new animal drug application. This process is the one currently being used for the review of rBGH, and as of June 30, 1992, rBGH products have not received final approval by the FDA.

Objectives, Scope, and Methodology

Objectives

In examining the completeness or thoroughness of FDA's review of rBGH in the areas of human food safety, animal safety, and drug efficacy, we posed four evaluation questions.[3]

- First, what are the FDA guidelines that are relevant for the investigational review of rBGH products?
- Second, did the rBGH investigational review meet the FDA guidelines?
- Third, what are the implications of any gaps or other problems discovered?
- And finally, how can the FDA animal drug review process be improved?

Scope

Our evaluation focused on four rBGH products, independently developed by four different drug manufacturers, that were submitted to the FDA for approval. Any one of these investigational new animal drugs could receive FDA approval after its review. We examined all FDA documentation submitted by the sponsors, which included 16 animal safety protocols, 18 drug efficacy protocols, 20 pivotal safety study summaries, 25 pivotal efficacy studies, and raw data for the four products. The research conclusions submitted to the FDA are contained in eight summary studies, which we also reviewed. Our findings and conclusions, especially in the area of human food safety, are relevant only for the rBGH products that are currently under review by FDA. Other rBGH products have been developed but have not been submitted to FDA for approval.

Methodology

Question 1

What are the FDA guidelines that are relevant for the investigational review of rBGH products? We determined which FDA research guidelines in the areas of human food safety, animal safety, and drug efficacy were relevant to rBGH by (1) interviewing FDA officials, (2) interviewing independent outside experts, (3) performing literature and document reviews, and (4) convening a panel of experts on the risk to humans of BGH (see appendix I

[3]The vast majority of the concerns raised about the possible approval of rBGH have concerned human food safety, animal safety, and drug efficacy issues. Consequently, we limited our evaluation to these areas and did not examine the FDA review in the drug production and environmental viability areas.

for human safety panel). The panel was specifically asked to help us define the human health and safety risks associated with BGH. We used this information to determine whether conclusions that certain guidelines need not be investigated were correct.

Question 2

Did the rBGH investigational review meet the guidelines? We used the set of FDA guidelines identified by answering question 1 as a basis to determine the completeness and thoroughness of the rBGH investigational review. First, we compared the guidelines to the protocols and pivotal study summaries submitted for the rBGH products. This resulted in the identification of a number of information gaps; that is, guidelines that were not addressed in either the protocols or the pivotal study summaries. To ascertain whether these guidelines were truly omitted or had, in fact, figured in the raw data submitted with the study summaries, we reviewed the raw data as well, looking for those guidelines that a second expert panel defined as critical to the validity of an investigational review (see appendix I for animal safety and drug efficacy panel).

Question 3

What are the implications of any gaps or other problems discovered? Here, we summarized our conclusions concerning the rBGH studies' conformance to the FDA research guidelines and any problematic conclusions reached in the studies. Thus, we assessed whether the rBGH products required further investigation before approval, based upon the thoroughness of the review as well as the results of the pivotal studies.

Question 4

How can the FDA animal drug review process be improved? From the rBGH cases, we identified problems in FDA's review process as a whole. Whether idiosyncratic or generalized, these problems could be important indicators of potential weaknesses in the way the safety and efficacy of new animal drugs are currently established.

Our evaluation was conducted to determine the completeness of the rBGH review. We examined protocols, pivotal studies, raw data, and correspondence between rBGH sponsors and the FDA regarding their drug applications. We conducted our review in accordance with generally accepted government auditing standards between July 1990 and December 1991.

Oral Activity of rBGH

A number of the human food safety concerns raised about the use of rBGH have been dismissed on the basis that rBGH is orally inactive and species-specific. Another concern that has been raised is whether insulin growth factor I (IGF-I) is elevated in milk that is produced from rBGH-treated cows and whether there is an associated human health risk. At the time we conducted our evaluation, the scientific consensus was that rBGH is orally inactive and cannot bind to human receptors, and that IGF-I does not pose a human health risk at the levels found in milk produced by rBGH-treated cows. The work of our expert panel on human safety and the conclusions of a National Institutes of Health Technology Assessment Conference on rBGH (cited later in this report) have supported these conclusions. Consequently, we have not conducted a methodological evaluation of the work conducted in these areas.

Results in Brief

Among the three research areas we evaluated—human food safety, animal safety, and drug efficacy—we found that for all three, the major critical FDA review guidelines were addressed. However, with regard to human food safety, we found a critical consideration that was not—but should have been—part of FDA's established research review: that is, the identification and evaluation of indirect human food safety risks that result from animal health effects caused by the use of the animal drug. These risks are not covered by the FDA guidelines and have not been addressed in the rBGH case. Their importance, however, could be considerable for rBGH. In effect, the increased milk production in cows from the rBGH treatment has triggered an increase in their incidence of mastitis, which would often be treated with antibiotics. As a consequence, higher levels of antibiotic residues in milk and beef could result.

Concern exists now about whether antibiotic levels in milk are already too high from present antibiotic usage and how well these levels are monitored.[4] Nevertheless, there has been no examination of whether rBGH use will increase antibiotic levels in milk or beef beyond that which currently exist and, if so, to what degree those levels are acceptable.

We also found that food products from the rBGH-treated animals were commercially processed and sold to consumers without any labeling noting their origin. The FDA does not require the labeling of food products derived from animals involved in drug treatment trials.

[4]See Food Safety and Quality: FDA Strategy Needed to Address Animal Drug Residues in Milk (GAO/RCED 92-209, Aug. 5, 1992).

GAO's Analysis

Relevant FDA Research Guidelines

We identified many human food safety, animal safety, and drug efficacy issues and guidelines that were pertinent to the review of rBGH. To understand which FDA guidelines were applicable in the human food safety area, we examined a spectrum of human risk issues that had been raised about rBGH over time, and then convened an expert panel to advise us on which of these issues were valid. We then reached our conclusions on rBGH human health risks and which FDA food safety guidelines needed to be addressed.

Animal safety and drug efficacy guidelines were obtained through a review of internal FDA documents pertaining to the investigational review of new animal drugs. A second expert panel reviewed the set of animal safety and drug efficacy guidelines we had identified to advise us on which were critical to the validity of investigational studies in these two areas. We ended with a set of 151 guidelines, covering the three investigational areas, that we used to evaluate the completeness of the rBGH review: 13 in the area of human food safety, 46 for animal safety, and 92 for drug efficacy. A detailed presentation of issues and guidelines is provided in appendix II.

Extent to Which the Guidelines Were Addressed

Human Food Safety

Our review showed that the rBGH products that are currently under review are orally inactive and species-specific.[5] Orally inactive means that rBGH is not absorbed orally in humans; its chemical compounds are broken down by the digestive system and are inactive. Regarding species specificity, somatotropins from nonprimate species, including BGH, are inactive in humans. The structure of BGH is significantly different from that of human growth hormone and, thus, cannot bind to human receptors to initiate any biological activity. This is why, in the case of immature digestive systems (babies and newborns) and dysfunctional digestive systems (adults) where hormone absorption may take place, there is no human risk. Thus, rBGH, itself, is not a harmful residue. But since rBGH as a potential residue is not a direct human food safety risk, the research guidelines that exist are, for the

[5]It should be noted that simply because an rBGH formulation approximates nBGH, this does not necessarily imply that the formulation is orally inactive. In the case of the four products submitted to FDA for approval, related sponsors conducted toxicity tests that demonstrated oral inactivity.

most part, irrelevant. That is, both the sponsors and FDA assured themselves that the consensus of existing scientific information is such that additional research on human safety risks is unnecessary. (Appendix III contains a more complete discussion of our findings in this area.)

Animal Safety

Regarding animal safety, we determined that most of the FDA guidelines were addressed in the protocols or pivotal study summaries. There were, however, apparent information gaps regarding reproductive issues such as teratogenic and embryotoxic effects as well as fertility rates of offspring. Given that without conclusions in these areas the rBGH animal safety studies would be incomplete, we examined the raw data submitted with the pivotal studies and found that, in fact, all of the critical guidelines had been addressed.

Mastitis studies are not routine in an animal safety review. However, because of their pertinence here, the FDA established mastitis guidelines specifically for the rBGH case. These were developed after the rBGH products were under FDA review when mastitis was recognized as a potential problem. Again, after a review of the raw data submitted with the studies, we determined that all critical mastitis guidelines had been addressed.

Drug Efficacy

In the area of drug efficacy, the sponsors are required to address not only treatment or production results, but also some associated animal safety issues. After examining the raw data files, we found once again that all critical drug efficacy guidelines had been addressed in the research review of rBGH.

Conclusions

We have concluded that all critical FDA research guidelines were followed in the investigational review of rBGH products. Where some guidelines were not addressed, these were not threats to the validity of the pivotal study conclusions.

Possible Human Food Safety Issue

Concerning the indirect (nonresidue) human food safety issue that is neither reflected in FDA guidelines nor addressed in the rBGH review, we have concluded that rBGH treatment does increase the incidence of mastitis in cows. We have two bases for our conclusion. First are the results of studies that were submitted to the FDA. The specific data, however, are proprietary and cannot be presented in our report. Second is a published

report (discussed in appendix III) which, although focused on the incidence of mastitis as a function of the natural production level of cows, demonstrates that rBGH treatment does increase mastitis.[6] In comparing the treatment and control groups, the number of cows experiencing mastitis was approximately 33 percent higher in the treatment group (28 percent versus 21.2 percent), while the incidence of mastitis was also greater in the treatment group (0.415 cases per cow versus 0.361 cases per cow). The National Institutes of Health Technology Assessment Conference panel that was convened in December 1990 to specifically examine the risks of rBGH also raised a concern about the mastitis issue.[7]

The problem here is that the increased incidence of mastitis in cows treated with rBGH could possibly lead to the increased use of antibiotics, which, in turn, might raise the level of antibiotics found in milk and beef. We noted in a previous report that given the lack of actual testing conducted, we cannot conclude at present that the nation's milk supply has not already been contaminated by antibiotics beyond acceptable levels.[8] Yet there has been no effort by either the drug sponsors or FDA to determine whether there may be higher antibiotic levels in milk associated with rBGH treatment and whether they would be acceptable from a human food safety viewpoint.

In sum, for the existing research guidelines required by FDA, we found that the review was thorough and complete. However, we also found that a gap exists, both in the research performed and in FDA's review of it, because the guidelines themselves failed to include a potentially critical area for human food safety, and no research has examined this area.

Process Issues

The FDA protocol review process as reflected by the rBGH cases showed limited emphasis on the completeness of the protocols submitted by the rBGH sponsors. Adherence to FDA's pivotal study guidelines was rarely reflected in most of the submitted protocols.

[6]Neil Craven, "Milk Production and Mastitis Susceptibility: Genetic Relationships and Influence of Bovine Somatotropin Treatment," Mammites de Vaches Laitieres, paper presented at the conference of the Societe Francaise de Buatrie, Paris, Dec. 18-19, 1991 (Toulouse: Polygone, 1992).

[7]NIH, Technology Assessment Conference, "Statement on Bovine Somatotropin," Washington, D.C., Dec. 5-7, 1990.

[8]See Food Safety and Quality: FDA Surveys Not Adequate to Demonstrate Safety of Milk Supply (GAO/RCED-91-26, Nov. 1, 1990).

Again, as indicated above, there was no requirement within the FDA guidelines to examine indirect effects such as those of antibiotic-treated mastitis or antibiotic levels in milk and their potential effect on human health. In talking to FDA officials, we learned that indirect effects were not required to be examined in the investigational drug review process.

Labeling issues also arose from our review. Milk and beef products from rBGH-treated cows were not labeled as such during the investigational research phase of the review, even though they were being marketed. We determined that this was not required by the FDA for any investigational new animal drug. Consequently, consumers have had no way of knowing if food products were derived from animals being treated with investigational drugs. In the case of rBGH products, we have no basis to believe that the safety of the current milk supply has been compromised.

Finally, we found no systematic tracking procedure whereby FDA could monitor which firm and which drug dose form had met FDA guidelines or the specific health or safety issues underlying the guidelines. Yet, the lack of such a systematic tracking process compromises both the efficiency and effectiveness of FDA's drug review process (a more complete discussion of our findings for process problems is provided in appendix IV).

Recommendations

Based upon these findings, we recommend that the Commissioner of Food and Drugs take the following actions:

- Examine the indirect effects of rBGH specific to rBGH products—before approval—to answer specific questions about its safety for human food consumption. That is, given the incidence of mastitis occurring in cows treated with rBGH, the FDA should study the degree to which antibiotics must be used to treat these cows and the incremental effects of rBGH treatment on the nation's milk and beef supply.
- Discontinue the marketing of food products from rBGH-tested animals until the potential risk concerning increased antibiotic levels has been evaluated.

As more general measures, the Commissioner should

- Study the feasibility of labeling food products derived from animals being tested with drugs so as to provide the public with information concerning the nature of such products.

- Avoid potentially dangerous shortfalls of information in human food safety reviews of animal drugs by ensuring that indirect risks are explicitly considered and examined.

Agency Comments

As requested by your offices, we did not ask for official comments from FDA regarding this report. However, we did discuss this report with agency officials, who generally agreed with our findings. The officials were concerned, however, about the issue of labeling food products derived from animals being used to test new drugs in investigational trials. They said that this is an issue that needs to be addressed, but were concerned that the recombinant bovine growth hormone review was an inappropriate case to use to raise the issue, since their conclusion is that rBGH residue does not represent a human food safety risk.

Although we agree that rBGH does not appear to represent a direct human food safety risk, we do not believe this obviates the need to address the labeling issue. First, we are concerned about the possible indirect risk of antibiotic levels. In addition, we believe the public should have the right to know which food products have been produced from animals being tested with investigational drugs. Consequently, we disagree with FDA on this point.

As we arranged with your offices, we plan no further distribution of this report until 30 days from its date of issue, unless you publicly announce its contents earlier. We will then send copies to the Commissioner of Food and Drugs and to other interested parties. We will also make copies available to others upon request.

If you have any questions or would like additional information, please call me at (202) 275-1854 or Kwai-Cheung Chan, Director of Program Evaluation in Physical Systems Areas, at (202) 275-3092. Other major contributors are listed in appendix XV.

Eleanor Chelimsky
Assistant Comptroller General

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Abbreviations

BGH	Bovine growth hormone
DNA	Deoxyribonucleic acid
FCM	Fat corrected milk
FDA	Food and Drug Administration
GAO	General Accounting Office
GLP	Good laboratory practices
IGF-I	Insulin growth factor I
IMI	Intramammary infection
nBGH	Natural bovine growth hormone
NE	Net energy
NIH	National Institutes of Health
rBGH	Recombinant bovine growth hormone

Expert Panel Members

Human Safety

Dr. Charles Capin, D.V.M., Ph.D.
Chair, Department of Veterinary Pathobiology
Ohio State University, Ohio

Dr. Gary Flamm, Ph.D.
Biological Chemistry - Consultant
Flamm Associates
Reston, Va.

Dr. Joseph Levy, M.D.
Chief, Division of Pediatric Gastroenterology and Nutrition
New York Hospital, Cornell Medical Center, N.Y.

Dr. Maria New, M.D.
Chair, Department of Pediatrics
New York Hospital, Cornell Medical Center, N.Y.

Dr. Martin Sonenberg, M.D., Ph.D.
Sloan-Kettering Memorial Cancer Center, N.Y.

Animal Safety and Drug Efficacy

Dr. Robert Bremel, Ph.D.
Department of Dairy Science
University of Wisconsin at Madison, Wisc.

Dr. Joe Hogan, Ph.D.
Research Scientist
Department of Dairy Science
Ohio State University at Wooster, Ohio

Dr. Harold Farris, Ph.D.
Associate Vice President for Research Compliance
Clemson University, S.C.

Guideline Requirements for New Animal Drug Review

Our analysis of the review of rBGH was based on whether FDA research guidelines were followed during the investigational research phase for three of five areas investigated by FDA. We identified and carefully enumerated the human food safety, animal safety, and drug efficacy issues and guidelines to determine the extent to which they were addressed. Mastitis guidelines were also addressed in our review.

This first set of findings answers our first evaluation question, which is, What are the FDA guidelines that are relevant for the investigational review of rBGH products?

Human Food Safety Issues

There are 10 issues raised by the scientific community and public interest groups that needed to be reflected in the review of rBGH. (These are presented in appendix III.) Areas of concern involve human biological activity, oral activity, rBGH activity in babies and newborns, rBGH interaction in impaired adult systems, components of rBGH that may be active in humans, interaction with and production of insulin growth factor I (IGF-I) variation in drug formulas, milk composition, secondary health effects, and differences between natural and recombinant bovine growth hormone.

Human Food Safety Guidelines

The human food safety guidelines are found in FDA's General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals (September 1986). These guidelines are the principal basis for the FDA review of human health-related animal drug studies, and six, in particular, form the basis for the human food safety review of rBGH products.

First, the "Guideline for Metabolism Studies and for Selection of Residues for Toxicological Testing" requires that the sponsor develop information on the amount, persistence, and chemical nature of the total residue in the edible products of treated target animals. Second, the "Guideline for Toxicological Testing" helps to define the biological effects of the sponsored compound and its quantitative limits. Third, the "Guideline for Threshold Assessment" describes how FDA uses information to determine whether chronic bioassays are necessary to resolve questions concerning the potential carcinogenicity of a compound. Fourth, the "Guideline for Establishing a Tolerance" is used to determine the type and duration of toxicity testing required and establishes the concentration of marker residue permitted in the target tissue of a treated animal. Fifth, the "Guideline for Approval of a Method of Analysis for Residues" defines

requirements under which the sponsor proposes an acceptable analytical method (either chemical or biological) capable of reliably measuring the marker residue to ensure that the total residue of toxicological concern is not exceeded. Lastly, the "Guideline for Establishing a Withdrawal Period" describes a procedure for establishing a period in which food products must be held before being sold commercially. This is based on a statistical tolerance limit procedure.

There are also statutory and regulatory requirements (21 U.S.C. 512 and 21 C.F.R. 511) that bear on the animal drug approval process and need to be reflected in human food safety studies. They deal with such issues as drug safety, efficacy, and drug labeling.

Animal Safety Guidelines

The animal safety guidelines are those contained in the general "Target Animal Safety Guideline" developed by the FDA (see appendix V). This guideline gives both descriptive and substantive guidance on how the sponsors are to carry out their animal safety studies. Areas of concern include drug tolerance tests, identification of maximum dose levels, route of administration, study design, animal observations, reproductive studies, tissue irritation studies, physical examinations, and statements on good laboratory practices and test animals.

Specifically, the guidelines require that the sponsors induce toxicity in the animals to test for drug tolerance; give multiple dose levels to find the most effective level at which the proposed drug might work; perform pathologic tests for signs of toxicity; record weights of animals and feed and water consumption; evaluate fertility of the cows in the study; record estrous cycle, conception rates, and abortions; and perform gross and histologic examinations on tissues and organs.

Guidelines pertaining to mastitis issues can be found in the "Protocol for the Evaluation of Mastitis in Efficacy Studies of Bovine Somatotropin and Production Drugs in Dairy Cattle" (see appendix VI). This is an internal FDA document written specifically to address mastitis issues that arose during the rBGH review. Seventeen guidelines were developed, which include topics such as sampling schedules, techniques and storage, microbiological procedures, contaminated samples, diagnosis of quarter infection status, clinical status of quarters, summary of intramammary infection (IMI) and clinical mastitis data, and milk somatic cell counts.

All of this work is necessary to ensure that the proposed drug is safe for the test animal. These guidelines must be followed if studies that are submitted to the FDA by the sponsors are to be deemed acceptable.

Drug Efficacy Guidelines

The drug efficacy guidelines that we used for our evaluation are found in the "Technical Assistance Document for Efficacy Studies of Bovine Somatotropin in Lactating Dairy Cows" (see appendix VII). The general guideline includes milk weight analysis, extrapolation of milk weights, fat corrected milk (FCM) yields, feed efficiency, dose titration, testing of herds, treatment regimen, analysis of unsalable and salable milk, data collection for the lactation period, body weights and body condition scores, recording of daily temperature and humidity, blinding and accountability rules, nutrition factors, reproduction, herd-breeding practices, milk analysis, general health observations, design and analysis techniques, dry off and removal rules, and statistical considerations.

Specifically, the drug efficacy studies are required to provide a vast array of information concerning the efficacious use of the test product. Daily milk yields and feed intake help to determine the feed efficiency findings; primiparous and multiparous cows are used to analyze any difference in how the cows react to the test drug; body condition scores are taken to help discern any possible problems in the health of the cow; pregnancy rates, services per cow, length of lactation, and number of abortions are required for the herd-breeding analysis; and blocking procedures are incorporated to highlight differences in the milk production levels of the cows.

Guideline Conformance

This appendix provides a detailed answer to our second evaluation question, which is, Did the rBGH investigational review meet the FDA guidelines? Thus, it characterizes the completeness of the FDA's review of the four rBGH formulations with regard to whether the protocols, pivotal studies, raw data, and correspondence between the FDA and the sponsors addressed the critical guidelines identified for human food safety, animal safety, and drug efficacy.

Human Food Safety Issues

The following summarizes our experts' consensus about the human food safety issues raised by the scientific community and the public concerning the four rBGH formulations. These issues are discussed because they provide the scientific basis concerning which guideline, regulatory, and statutory requirements in the human food safety area need to be addressed.

Human Biological Activity

The recombinant BGH formulations are not active in humans. The protein hormone has no effect in the human species (that is, it is species-specific). Thus, there are no general human health concerns associated with rBGH as a food residue.

Oral Activity

Neither the rBGH formulations nor nBGH are active when administered orally. The human digestive tract breaks down the BGH protein hormone molecule into its chemical components and peptides, thereby rendering the molecule inactive.

BGH Activity in Babies and Newborns

Even though babies and newborns are more likely to experience absorption of protein and hormones that escape digestion, they absorb only trace amounts intact. Infant formula preparation destroys the BGH. Consequently, the rBGH formulations pose no food residue risks to babies and newborns.

BGH in Impaired Adults

Dysfunctional digestive systems may not break down the BGH hormone as effectively as the healthy adult system, and trace amounts theoretically may be absorbed. However, because the rBGH formulations are orally inactive and species-specific, these trace amounts would have no effect.

Components of BGH May Be Active in Humans

There is no convincing data that fragments of nBGH or the rBGH formulations are biologically active in humans when administered orally. There is no evidence, either, that the rBGH formulations will produce unique peptide fragments that have biological effects.

Effects on IGF-I

Insulin growth factor I does increase in milk from cows treated with the rBGH formulations, but increased amounts may not pose a risk. Insulin growth factor I is not a toxic contaminant and also is natural to the human system. The NIH panel recommended that further research be done in this area.

Variations in Drug Formulations

Though the rBGH formulations submitted by the drug sponsors for FDA approval are different, all four have been found to be orally inactive through toxicological testing. The methionyl-rBGH product has no different effect than nBGH, even though it has additional amino acid components. Thus, the concern about human health effects associated with different formulations has been dismissed because there is no evidence of any effects.

Milk Composition

The milk composition and nutritional value of milk from rBGH-treated cows is essentially the same as milk from untreated cows. BGH levels in milk may be elevated but remain within the normal range. There are no significantly increased levels of fat. This issue, thus, is not a health concern.

Secondary Health Effects

Secondary food safety effects—those that are not associated specifically with residues of the treatment drug—are important to consider. One secondary health effect—the increased use of antibiotics to treat mastitis—may pose a human health risk. This was one concern raised at the NIH Technology Assessment Conference on the rBGH formulations.

Differences Between nBGH and rBGH

There is no assay to distinguish between nBGH and the rBGH formulations. There are no significant chemical differences between nBGH and the rBGH formulations submitted to FDA for approval even though the amino acid structures are somewhat different. Toxicity tests have shown that even though there is variation in chemical structure between nBGH and the rBGH formulations submitted for FDA approval, the latter pose no human health threat.

Human Food Safety Guidelines

Here we present how the human health conclusions discussed above affect the human food safety guidelines that needed to be addressed.

Metabolism Studies for Selection of Residues for Toxicological Testing

Tests were conducted to determine the oral activity of the rBGH formulations. FDA's review of the sponsors' human food safety studies determined that the rBGH formulations were not orally active in humans. This coincides with expert conclusions. Consequently, the FDA determined that metabolism studies were irrelevant since there would be no harmful residues from the rBGH formulations. We concur with this conclusion.

Toxicological Testing

The review of the past human food safety studies determined that there was no risk associated with intermittent or chronic exposure of people because of the oral inactivity of the rBGH formulations' residues. Experts concur with this conclusion. Consequently, there was no need for toxicological testing of the formulations, although toxicologic testing was conducted. We did not evaluate the methodological adequacy of the toxicological testing.

Threshold Assessment

The review of earlier human food safety studies determined that the "Guideline for Threshold Assessment" was not applicable as the rBGH formulations were shown to have no potential for carcinogenic effects. Experts agreed with this conclusion as well. No threshold assessments were deemed necessary. We agree with this conclusion.

Establishing Tolerances

The review concluded that there were no harmful residues from the rBGH formulations because they are orally inactive, and thus, the "Guideline for Establishing a Tolerance" was not applicable. As a result of its species specificity, the rBGH being reviewed could not bond to any receptor in humans. Experts also agree with this conclusion. We, too, concur with the conclusion that establishing tolerances for the formulations was unnecessary.

Approval of a Method of Analysis for Residues

The review concluded that the "Guideline for Approval of a Method of Analysis for Residues" was not needed because of the oral inactivity of the rBGH formulations. Concerns about measuring marker residues and total residues were irrelevant. We agree.

Establishing a Withdrawal Period

The review determined that no residue concentration was involved for the rBGH formulations as a result of their oral inactivity. In accordance with the "Guideline for Establishing a Withdrawal Period," a decision was made by the FDA to set a withdrawal for food products at zero (0) days. We concur.

Proposed Guideline: Chemistry Testing

This was a recommended guideline for proteins on a case-by-case basis. Where it could be demonstrated that the protein in question was not orally active, tissue residues would not be a human food safety concern. Since the rBGH formulations are not orally active, chemical testing was not required. We concur.

Human Food Safety Statutory and Regulatory Requirements

Under section 512(a)(1) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b(a)(1)), a new animal drug is deemed unsafe unless there is an approved application on file and the drug, labeling, and use conform to the approved application. FDA is still in the process of reviewing the sponsors' applications for their rBGH products and is evaluating the drugs and their labeling.

In determining whether to approve a new animal drug application, FDA is required (21 U.S.C. 360b(d)(2)) to consider a number of factors including the following:

- "the probable consumption of such drug and of any substance formed in or on food because of the use of such drug. . .";
- "the cumulative effect on man or animal of such drug, taking into account any chemically or pharmacologically related substance. . ."; and
- "safety factors which in the opinion of experts . . . are appropriate for the use of animal experimentation data. . . ."

We determined that the above factors have been addressed in the investigational phase of the rBGH review, with the exception of antibiotic levels that may be associated with increased mastitis due to rBGH use. During this review, FDA either requested or reviewed several studies to address safety issues as they pertain to the above requirements. This included the physicochemical makeup of the product. The FDA requested that the firms submit the chemical composition of their product for agency review.

The FDA reviewed the scientific literature on growth hormones and used this research as part of its evidence to support its decision that rBGH was

safe for humans. All four sponsors submitted rat studies to the FDA showing that their rBGH formulations were orally inactive. Administration of the rBGH formulations to rats did not cause any statistically significant changes or adverse effects when compared to controls for the levels at which residues would occur.

Also, IGF-I studies were conducted by the sponsors as a result of an FDA request that all four of them address the potential for oral activity of IGF-I in humans. Some firms had completed these tests as of August 1989. The FDA concluded that IGF-I administered orally is biologically inactive. The FDA determined that the difference in IGF-I levels in milk from untreated and from treated cows was insignificant. Lastly, the FDA requested documentation on the purity of the drug compound from the sponsors.

Subsection 511.1(b) of title 21, Code of Federal Regulations, outlines a number of conditions that must be adhered to by sponsors of new animal drugs who are seeking to use them for clinical investigations. We examined two of these conditions, which were technical in nature.

Paragraph 511.1(b)(5) outlines when products from animals treated with investigational new animal drugs may be authorized for human consumption. Sponsors must show that consumption of food derived from animals treated at the maximum levels with the minimum withdrawal period will not be "inconsistent" with the goals of public health. Also, there must be evidence that "food . . . does not contain drug residues or metabolites."[1] We determined that these requirements were met. The FDA's review of rat studies concluded that the rBGH formulations were orally inactive and resulted in no residues. Thus, residues did not compromise public health.

Subparagraph 511.1(b)(8)(iv) prohibits sponsors from representing the new animal drug as safe or effective for the purposes for which it is under investigation. We determined that this requirement was not met because sponsors have already made public pronouncements attesting to the safety of rBGH even though it has not yet received FDA approval. The FDA also has made inappropriate statements regarding the safety of rBGH. The agency

[1]A third condition, which we did not examine because it is administrative in nature, requires the sponsor to submit information regarding the name and location of the packing plant where the animals are processed.

has taken steps to address this deficiency as noted in a recent Department of Health and Human Services Inspector General's report.[2]

The Center for Veterinary Medicine internal memo 19 requests that sponsors submit a reliable assay method for detecting drug residues in edible tissues of treated animals. The FDA determined that this requirement was not applicable because no assay can distinguish between nBGH and the rBGH formulations. An assay for IGF-I was approved for one of the sponsors.

In summary, all human food safety guidelines have either been addressed in the rBGH review or were irrelevant. The one issue we have identified is a related concern about increased antibiotic levels in milk and beef owing to the increased incidence of mastitis in rBGH-treated cows. This issue is discussed in the animal safety section that follows.

Animal Safety Guidelines

The following discussion of the animal safety review is divided into two parts: an evaluation of the submitted protocols and, then, an evaluation of the pivotal study summaries, which illustrate where in the review process specific guidelines were or were not addressed.

Comparison Between the Guidelines and the Protocols

Our conclusions about which animal safety guidelines were met in the protocols is provided in appendix VIII. The following is a summarization of the technical areas of the guidelines that we concluded were not met in the protocols.

Drug Tolerance

The drug tolerance test characterizes, under controlled conditions, the target animal response to a toxic dose of a drug. The protocols did not address the exclusive use of target animals and the assurance that only the market formula of the drug was used.

Maximum Dose Levels

The objectives of the toxicity studies in target animal species are to document the safety of the drug product for the target animal under conditions of recommended use and to highlight the signs and effects associated with the toxicity of the drug product. If five times the maximum recommended drug use level, or less, is toxic to the test animals, the

[2]See Department of Health and Human Services, Office of Inspector General, "Audit of Issues Related to the Food and Drug Administration Review of Bovine Somatotropin," Feb. 1992.

studies should document the maximum dose level of the drug product that causes no obvious adverse effects to animal health or production. The protocols did not emphasize or reflect the identification of the maximum dose level with no ill effects.

Route of Administration

Route of administration should be the proposed route, or routes, that will appear on the label. The protocols did not emphasize administration of the tested drug for the recommended route.

Study Design

In designing toxicity experiments, consideration should be given to historical data on use of the drug. A literature search should be conducted and combined with results of any preliminary experiments to determine the possible areas of drug toxicity. Studies should be conducted in healthy ruminants representative of the species and class of ruminants for which the drug is intended. In regard to the protocols, no literature searches were evident and using specific classes of ruminants as a representative species was not discussed.

Reproductive Studies

Studies should be conducted on both sexes to evaluate possible drug effects on fertility in the target species. Fertility issues, reproductive data, and information on conception and abortion rates were not addressed completely in most of the protocols and FDA comments. Protocols did not mention if male offspring were tested for effects of the rBGH formulations on their neonatal and postnatal development. The FDA did not comment on this missing information.

Tissue Irritation

Studies for injectable drugs should establish the time it requires for the tissues surrounding an injection site to return to an acceptable condition. Several protocols did not state that injection site exams would be performed. The FDA comments did not address this issue.

Physical Examinations

An examination should be conducted for the purpose of detecting any abnormalities that may be drug-related. The protocols did not emphasize physical exams to detect abnormalities.

Mastitis

The mastitis guidelines were developed after the protocols for the rBGH research had been prepared. Consequently, no protocols reflected mastitis guidelines.

Comparison Between the Guidelines and the Pivotal Study Summaries

Our initial review of the protocols resulted in the identification of several problem areas. We extended our evaluation to pivotal study summaries to determine if these same problems or other, new problems existed. Our detailed conclusions are presented in appendix IX. After reviewing the sponsors' pivotal study summaries, we found that the number of problem areas was reduced. However, four problem areas still existed.

Maximum Dose Levels

The pivotal study summaries did not emphasize the identification of maximum dose levels with no ill effects.

Study Design

The pivotal studies did not emphasize or reflect required literature searches or that the proposed administration route should be that which was entered on the tested drug's label.

Reproductive Studies

Fertility issues and reproductive studies on both sexes were not addressed completely in most of the pivotal study summaries and FDA comments. Summaries did not mention if male offspring were tested for effects of the rBGH formulations on their neonatal and postnatal development. The FDA did not conclude that this was missing information. However, after reviewing the raw data developed for the pivotal studies, we determined that all critical areas had been addressed.

Mastitis

In the case of mastitis, the investigational research conducted by the sponsors was included in both the animal safety and drug efficacy studies. For the purpose of our report, we have combined our findings into this section under animal safety. We found that most of the mastitis guidelines had not been addressed in the pivotal study summaries (see appendix X).

Comparison Between the Guidelines and the Raw Data

As mentioned in the methodology section of this report, after determining that there were animal safety guidelines that did not appear to have been addressed in the pivotal study summaries, we took an additional step in our evaluation. We first had an expert panel review these specific guidelines that we determined had not been reflected in the pivotal study summaries to determine which were critical to the validity of the rBGH animal safety and mastitis reviews. Afterward, we reviewed the raw data supporting the pivotal study summaries to determine if these requirements had been met but simply not reflected in the summaries submitted to FDA.

In the area of animal safety, the expert panel determined that none of the outstanding guidelines were critical to the validity of the studies. But in the mastitis area, the expert panel determined that there were six guidelines that were critical to the validity of the studies. They were: (1) status at end of trial; (2) status at dry off; (3) status of calving; (4) all quarters sampled 7-14 days before trial entry; (5) resample within 10 days if different status than last sample; and (6) summary of rate of intramammary infection (IMI), duration of IMI, prevalence of IMI, incidence of clinical mastitis, and severity of clinical cases. In examining the raw data, we found that every one of these critical requirements had been met in the mastitis studies (see appendix XI).

Summary Conclusion

In spite of information gaps in both protocols and pivotal study summaries, our examination of the raw data developed for the pivotal studies leads us to conclude that all critical animal safety guidelines were addressed in the rBGH review.

Drug Efficacy Guidelines

Our review of drug efficacy guidelines was also divided into an assessment of the protocols and pivotal studies so that we could discern where in the FDA review process specific guidelines were or were not addressed.

Comparison Between the Guidelines and the Protocols

After analyzing the protocols and the FDA review of them, we found numerous drug efficacy guidelines that had not been addressed. Our detailed conclusions are presented in appendix XII. As highlighted in the appendix, most drug efficacy guidelines were not reflected in the submitted protocols.

Comparison Between the Guidelines and the Pivotal Study Summaries

We found that the problems in the drug efficacy protocols continued into the pivotal study summaries. (Our detailed conclusions are provided in appendix XIII.) Treatment regimen, reproductive issues, and design and analysis were not addressed. Such issues as starting times of treatment, use of reproductive aids, and blocking procedures were also not addressed. Other key areas in which we found deficiencies included blinding and accountability of the drug and nutrition and feed efficiency issues.

Comparison Between the Guidelines and the Raw Data

As in the case of animal safety and mastitis guidelines, we extended our evaluation of the completeness of the rBGH drug efficacy review to determine if the guidelines were met but simply not reflected in the pivotal study summaries. We reviewed the raw data developed for the studies. (Appendix XIV presents our findings.) We found that all critical drug efficacy guidelines were addressed in the pivotal studies.

Summary Conclusion

Although we found information gaps in both the protocols and pivotal study summaries, we determined by examining the raw data developed for the studies that all critical drug efficacy research guidelines were addressed.

Conclusions

We found that all critical guidelines for a valid animal safety, mastitis, and drug efficacy research review of the four rBGH products currently submitted for approval to FDA were met. In our review of the rBGH research study conclusions, however, we found one result that reflects a serious shortcoming. As we noted above, the research review of the rBGH formulations as a human food safety residue risk was thorough. However, the preliminary conclusion of the animal safety studies is that the incidence of mastitis is increased for animals being treated with rBGH versus control animals.

There are two sources of information that support the conclusion that increased mastitis incidence is associated with treatment of the rBGH formulations. First, during our review of the studies that had been submitted to FDA for the four rBGH products seeking approval, we noted that the treatment groups had a consistently higher incidence of mastitis than the control groups. The proprietary nature of the information prevents us from providing the actual data from the studies. Second, the most comprehensive published study examining the increase in mastitis for rBGH-treated cows has shown that, for the trials conducted by one of the sponsors in the United States and Europe, there was an increase both in the number of cows experiencing mastitis and in the incidence of mastitis between the control and treatment groups.[3] For the cows that experienced mastitis during the trials, 87 of 410 cows experienced mastitis in the control group (21.2 percent) while 120 of 429 cows (28 percent) experienced mastitis in the treatment group. For incidence of mastitis, 148

[3]Neil Craven, "Milk Production and Mastitis Susceptibility: Genetic Relationships and Influence of Bovine Somatotropin Treatment," Mammites de Vaches Laitieres (Toulouse: Polygone, 1992).

discrete cases were identified in the control group and 178 cases in the treatment group. On a normalized basis, this results in an incidence rate of 0.361 cases per cow in the control group and 0.415 cases per cow in the treatment group. The increased incidence of mastitis raises one concern about the possibility of increased use of antibiotics to treat the mastitis and the possibility of increased levels of antibiotics occurring in milk and beef products.

Looking again at the rBGH review, we did not find any guidelines that required the evaluation of the rBGH formulations as an indirect or secondary human food safety risk; that is, as an animal drug that causes an animal health effect (mastitis) that is treated by a chemical agent (antibiotics) that, in turn, makes its way into the food supply. This is a shortcoming in the FDA animal drug review approach.

Another concern is whether the possibility of increased antibiotic levels in milk and beef products poses a risk that would affect the approval of rBGH products. A recent GAO report has concluded that the current testing methods are not adequate for determining the extent to which milk is contaminated by antibiotics beyond acceptable levels.[4]

Given this conclusion and a concern about the extent to which milk antibiotic contamination is occurring, we believe the sponsors should determine through research whether the use of the rBGH formulations results in increased levels of antibiotic residues in milk. None of the research conducted for rBGH approval has addressed this concern.

[4]Food Safety and Quality: FDA Surveys Not Adequate to Demonstrate Safety of Milk Supply (GAO/RCED-91-26, Nov. 1, 1990).

Process Issues

In this appendix, we discuss our fourth evaluation question: How can the FDA animal drug review process be improved? These conclusions reflect only our evaluation of the rBGH review. However, they may suggest more general areas of weaknesses in the overall FDA review process.

FDA Protocol Review

Protocols and the FDA's review of them define for new animal drug approval what and how data will be collected in support of an application. The pivotal study is performed to demonstrate the safety and efficacy of the proposed animal drug. We found that in the rBGH case, many guidelines were not reflected either in the protocols or in the study summaries for animal safety and drug efficacy.

The FDA did not note that numerous guidelines were missing from the protocols and studies. Protocol reviews were conducted at different levels of specificity and thoroughness as were reviews on the pivotal studies. FDA's comments only sporadically obligated or reminded the firms to follow important guidelines. We found that FDA did not seem to assign much importance to the thoroughness of the protocol review or the protocols themselves.

Systematic Tracking Procedure

We did not observe any systematic tracking procedure within FDA to monitor which firm and which dose form met guidelines or addressed specific health or safety issues underlying the guidelines. Nor is there a way to determine what information has already been provided by a firm and which pieces of information or analysis for the new animal drug application are missing. Such a roadmap would permit the agency to monitor and manage the application process and thus ensure that all needed information would form the basis of an FDA decision.

Human Food Safety Review

The human food safety guidelines focus on identifying and monitoring primary drug (residue) risks in animal by-products. The research and possible health impacts of antibiotics in cows treated with rBGH suggest that indirect nonresidue risks may also need to be emphasized and explicitly addressed in an FDA human food safety review for animal drugs. This also raises the question of whether human consumption of food products should be allowed before animal safety studies have been completed.

FDA Authorization of Food Product Consumption

The FDA approved the commercial sale of milk and beef from rBGH-treated cows, deciding that the milk was safe for humans to drink. This was consistent with current FDA regulations and is similar to procedures used for other new animal drug applications. There is public concern surrounding the authorization of food products from animals that are still under an investigational drug status.

This issue is based on the concern of persons who do not want to unknowingly drink milk or eat beef from treated cows before the FDA has completed its review of a new animal drug. The public would not normally be aware it is consuming the by-products of this investigational drug under the current process unless sponsors chose to make their studies and applications public.

Currently, the decision to authorize commerical sale of products from test animals is taken when human safety concerns have been addressed. This practice should be reevaluated in light of possible secondary effects, which may not arise until animal safety and drug efficacy test results have been submitted by the sponsors. This is especially pertinent given the above example of rBGH and the issue of possible secondary health effects associated with increased mastitis and antibiotic levels found in milk from rBGH-treated cows.

Animal Safety Guidelines

Guidelines

1.Use only target animals.

2.Induce toxicity and record clinical signs.

3.Pathologic and histologic data collected.

4.Market formula of drug used.

5. If drug is for long-term use, should administer 10X maximum dose for up to 21 days.

6. Tests should be conducted only on healthy animals (at least 4 cows required).

7. Identify maximum dose level with no ill effects.

8. Statement on good laboratory practices.

9. Administration by recommended route.

10.Conduct literature search.

11. Conduct multiple dose level studies.

12. Use ruminants as representative species.

13. Administer drug for at least 6 weeks if for long-term use (Complete Animal Safety Study).

14. Dosing regime should include 0, 1X, 3X, 5X levels.

15. Proposed route of administration should be that on the label.

16. Evaluation should include weight, feed/water consumption.

17. Pathologic tests on all animals that show signs of toxicity should be done.

18. Gross pathologic exams on randomly selected cows should be performed.

19. Reproductive studies should be conducted on both sexes.

20. Fertility study should emphasize estrous cycle, mating, conception rate, and gonadal function.

21. Teratogenic and embryotoxic effects should be determined.

22. Effects of drug on labor and delivery, abortion, and neonatal viability should be examined.

23.Evaluate fertility of both sexes.

24. Injection site exams should be performed.

25. Studies should be conducted on healthy cows.

26. Physical exams to detect abnormalities should be performed.

27. Gross exams for pathologic lesions and organ weights should be complete.

28. Histological exams on tissues should be done.

29. Clinical pathologic tests should be conducted.

Mastitis Guidelines

Guidelines

1. Infection status of all quarters of all cows determined before study.

2. Infection status determined at intervals not longer than 60 days while on trial.

3. Infection status determined at end of trial.

4. Infection status determined at drying off.

5. Infection status determined at the calving following the lactation in which the drug was tested.

6. All quarters should be sampled 7-14 days before trial entry.

7. When culture results differ from status determined for the quarter at last sampling, then quarter must be resampled in duplicate within 10 days.

8. All cows should be observed twice daily for evidence of clinical mastitis by forestripping.

9. Samples should be refrigerated within 15 minutes for transport to a laboratory.

10. When culture of sample will not occur within 24 hours, sample should be frozen for storage and transport. Frozen samples should be cultured within 7 days.

11. Laboratory tests should be sufficient to identify numerous microorganisms.

12. Clinical mastitis data should be summarized separately from intramammary infection data.

13. Cause of clinical mastitis summarized under four categories: single pathogen, mixed infection, clinical—no isolation, clinical—contaminated.

14. Clinical severity codes should be used and reported.

15. Summary data should be obtained for rate of intramammary infection (IMI), duration of IMI, prevalence of IMI, incidence of clinical mastitis, severity of clinical cases.

16. Data on somatic cell counts should be obtained for each cow, at least once per month.

17. All somatic cell count determinations should be converted to a log scale or log score.

Drug Efficacy Guidelines

Guidelines

A. Incidence of mastitis was evaluated.

B. Data collected under field conditions.

1. Milk weights recorded once every 7 days.

1A. Milk weights taken for 1 day (24 hours).

2. Milk production determined/expressed as 3.5 FCM per day.

3. Milk weights from total weight collected, divided by number of days in treatment.

4. If BGH administered weekly or less, daily milk weights required.

5. If cows dried before 305 days, milk records extrapolated to 305 days.

6A. If dried off due to low production, completing 2/3 of treatment, extrapolation not permitted.

6B. Actual FCM will be divided by expected number of days at 305 days.

7. FCM not to be adjusted to mature equivalence.

8. Weekly treatment means of milk and FCM yields to be plotted at each site and data pooled.

9. Feed efficiency—ratio of FCM per NE intake, corrected for body weight changes, over treatment period.

10. Total FCM produced is to be divided by NE intake, over time period.

11. Dried off before 305 days: total FCM divided by NE intake over time till dry off.

12. Body weights taken every 4 weeks.

13. Change in body weight: subtract body weight at initiation from body weight at end of period.

14. Factor of 5.12 Mcal per kg gain/4.92 Mcal loss in body weight used to correct feed efficiency.

15. Control and three nonzero levels for groups.

16. Maximum effective dose: highest level of BGH above which no significant improvement occurs.

17. Linear plateau or polynomial analysis models should be used.

18. Minimum of three herds from different geographical areas.

19. High- and low-producing and multiparous and primiparous cows to be used.

20. Variation at which cows are started on treatment should be no greater than 7 days.

20A. Treatment starts at dry off or 400th day of lactation.

21. Control animal should receive an equivalent injection.

22. Start/finish time for daily milking in same order.

23. Cow should remain on BGH until 400th day of lactation or dry off.

24. Drying off should occur between 45 and 60 days before parturition or if milk falls below a certain level or when last cow is off BGH.

25. Nonpregnant cows or cows with long open period: should be on treatment until 400 days or when milk falls below certain level or when last cow is off BGH.

26. Pretreatment average based on 2 weeks' milk production.

27. Unsalable milk included in milk production totals.

28. Total weight of unsalable milk recorded for each cow.

29A. Salable FCM averaged and summarized for each group or site.

29B. Weekly treatment means plotted at each site.

30. Data for entire lactation collected: FCM production, for each treatment.

31. Data for entire lactation collected: feed efficiency, for each treatment.

32. Cows having over 305 days lactation--parameters: average FCM per day, feed efficiency, unsalable FCM, number of disease treatment days.

33A. Body weights for correction of feed efficiency and animal health, by using accurate scales.

33B. Body condition scores taken by same person.

34. Measurements on each cow in three herds plus trials.

35. Measurement every 4 weeks, pretreatment through dry off.

36. Monthly treatment means plotted for each site.

37. Temperature and humidity recorded daily for each site.

38. Individuals blinded to treatments or dose levels.

39. Drug accountability: injection route, storage, record of use.

40A. Monitoring by sponsor to be thorough.

40B. Instruction well described; protocols and standard operating procedures at each site.

41. Feeds sampled once per week, pooled/analyzed once per month.

42. Feed levels determined: dry matter, crude protein, calcium, phosphorus, and acid detergent fiber.

43. NE content estimated and calculations reported.

44A. Daily feed intake per cow once each 7 days, in one herd.

44B. Feed efficiency: three herds with feed refusals/weigh backs recorded.

45A. NE intake per cow once per week (from beginning of treatment to termination).

45B. Weekly means plotted over time at each site, data pooled over all sites.

46A. NE calculation: NE balance = NE intake – (milk NE + maintenance NE).

46B. Weekly means plotted, NE balance.

46C. Maintenance allowance increased 20 percent for first lactation.

47. Feed efficiency: corrected for changes in body weight.

48. Requirements for maintenance and milk production met: NE, protein, calcium, and phosphorus.

49. Pool of sires used consistently/randomly for all treatment groups.

50A. Medications to aid reproductive performance must not cover up potential reproductive problems.

50B. Reproductive agents not to be used before treatment.

51A. If treatment given at 70 days postcalving, should rebreed.

51B. If not pregnant at 65 days of BGH treatment: use approved therapeutic aids.

51C. Comparison among treatments: reproductive performance at end of 1st 65 days and end of 2nd 65 days.

52A. If treatment less than 70 days: no agents should be used until 135 days postcalving.

52B. Reproductive aids may be used: 135-200 days postcalving. Evaluate at begining and end.

53. Milk progesterone assays used through lactation for cycling. Cannot be used to aid in breeding until periods mentioned above.

54. Criteria for reproductive aids made in advance for each herd. Used consistently and documented.

55. Situations require use of hormonal therapy. Not for use to treat metritis.

56. Herd breeding and policy recorded.

57. Breeding: report heat-monitoring methods and personnel.

58. Records include any use of aids for reproductive performance.

59. Compare: reproductive performance to rest of herd and with past years.

60. Describe special medical conditions that could influence reproductive efficiency.

61. Describe special environmental conditions that could influence reproductive efficiency.

62. Reproductive performance characterized, for heifers and multiparous cows.

63A. Abortion up to 7 days before expected calving: not considered parturition.

63B. Parturition is delivery of dead calf 7 days before expected calving.

64. Average/compare between groups: pregnancy rate, conception rate, number of live births per cow.

65. Average/compare between pregnant and nonpregnant: days to 1st heat, days to 1st insemination, average number of days between services, services per cow, length of lactation.

66. Average/compare for pregnant cows: service per conception, days open, number of abortions., number of stillbirths, length of gestation, calving interval, days carried calf.

67. Cows open to be physically examined to determine problem.

68. Aborted fetuses, stillborn calves, placentas: should be necropsied.

69. Calving should be scored using numerical codes (1 to 5).

70A. Body weights of all calves taken at birth.

70B. Heifer calves weighed at 2 and 4 weeks of age.

71A. Aliquot milk pooled proportional to milk yield from 24-hour period: protein, fat, somatic cells.

71B. Somatic cells log transform before averaging, means converted back to actual counts, per cow.

72A. Level of phosphorus, calcium, lactose: 3-4 times during lactation.

72B. Milk analysis, method/source reported.

72C. Milk from each cow in one herd analyzed for BGH for 3 times.

73. Health problems recorded: observations, treatment decisions, doses, methods of administration.

74. Physical exams recommended.

75. Injection site scale: normal milk, infected, major reaction.

76. Breeds to be blocked.

77. Primiparous and multiparous blocked separately.

78. Blocks filled with homogenous pretreatment FCM groups.

79. No more than 20 lb. spread of pretreatment FCM in a block.

80. Blocks formed within 6-8 weeks of cows entry into block.

81. Randomization for each block determined in advance.

82. Evaluate differences in FCM production: pretreatment and lactation number.

83. Missing data noted and cause.

84. If cow removed after 2/3 of treatment: extrapolate production to 305-day lactation (or delete).

85. Feed efficiency: base on actual data if 2/3 period collected.

86. Dried off before 305 days: data extrapolated to 305 days.

87. If dry off due to low production: no extrapolation. FCM divided by expected days of treatment.

88. Cow removed due to illness: all data deleted and reason.

89. If variances heterogenous among herds: weighted analysis.

90. If error variances different for primiparous and multiparous: separate analysis.

91. Is treatment response dependent on level of pretreatment production.

92. Average pretreatment FCM production by dose levels discounted if $P > 0.05$.

Comparison Between the Guidelines and the Protocols on Animal Safety[a]

Guideline	Drug Tolerance[b] Met	Drug Tolerance[b] Partly met	Drug Tolerance[b] Not met	Animal Safety[b] Met	Animal Safety[b] Partly met	Animal Safety[b] Not met
Drug tolerance tests						
1. Use only target animals[c]	0	4	0	d	d	d
2. Induce toxicity and record clinical signs	4	0	0	d	d	d
3. Pathologic and histologic data collected	2	1	1	d	d	d
4. Market formula of drug used[c]	1	3	0	d	d	d
5. If long-term use, up to 10X maximum dose; 21 days	4	0	0	d	d	d
6. Test only healthy animals, up to four cows[c]	1	1	2	d	d	d
Maximum dose levels						
7. Identify maximum dose level with no ill effects[c]	0	0	4	1	0	11
Good laboratory practices						
8. Statement on GLPs	4	0	0	11	0	1
Route						
9. Administration by recommended route[c]	1	3	0	4	8	0
Study design						
10. Literature search[c]	d	d	d	0	0	12
11. Multiple dose levels done	d	d	d	11	0	1
12. Use ruminants as representative species[c]	d	d	d	2	0	0
13. Complete Animal Safety Study, at least 6 weeks	d	d	d	12	0	0
14. Complete Animal Safety Study dose levels: 0, 1X, 3X, 5X	d	d	d	7	0	5
15. Proposed route should be that on label[c]	d	d	d	1	11	0
Observations						
16. Weight, feed, water consumption noted	d	d	d	11	1	0
17. Pathologic tests on all that show signs of toxicity and randomly selected	d	d	d	11	1	0
18. Gross pathologic exams on preselected cows	d	d	d	10	2	0
Reproductive studies						
19. Reproductive studies on both sexes[c]	d	d	d	4	7	1
20. Fertility—estrous cycle, mating, conception rate, gonadal function[c]	d	d	d	1	10	1
21. Teratogenic and embryotoxic effects[c]	d	d	d	5	5	2
22. Labor/delivery, abortion, neonatal viability examined	d	d	d	6	5	1
23. Evaluate fertility of both sexes[c]	d	d	d	1	9	2
Tissue irritation						
24. Injection site exams[c]	d	d	d	6	2	4
Test animals						
25. Studies should be conducted on healthy cows[c]	d	d	d	2	2	8

(continued)

Guideline	Drug Tolerance[b] Met	Drug Tolerance[b] Partly met	Drug Tolerance[b] Not met	Animal Safety[b] Met	Animal Safety[b] Partly met	Animal Safety[b] Not met
Physical examinations						
26. Physical exams to detect abnormalities[ce]	[d]	[d]	[d]	4	7	1
27. Gross exams for pathologic lesions and organ weights	[d]	[d]	[d]	10	0	2
28. Histological exams on tissues[f]	[d]	[d]	[d]	10	0	2
29. Clinical pathologic exams[g]	[d]	[d]	[d]	11	0	1

[a]There are two major Target Animal Safety Studies. The Drug Tolerance Study characterizes, under controlled conditions, the target animal response to a toxic dose of a drug. The Complete Animal Safety Study documents the safety of the drug and monitors safety issues such as reproduction, disease, and the local effects on the animal when given the investigational drug.

[b]The Drug Tolerance Study guidelines are found in 1-9. The Animal Safety Study guidelines are found in 7-29. (Both types of studies require guidelines 7-9.) There were 4 protocols for Drug Tolerance and 12 protocols for Complete Animal Safety.

[c]These guidelines are those that we determined were not fully addressed, because more than 50 percent of the results were either only partially met or not met at all.

[d]Not applicable.

[e]Ocular, equilibrium, muscular disturbance, appetite, injection site, gastrointestinal, cardiovascular, respiratory, and behavior.

[f]Pituitary gland, thyroid gland, kidneys, adrenal glands, heart, liver, and stomach.

[g]Blood and serum chemistries, hematology, urinalysis, and fecal examination.

Comparison Between the Guidelines and the Pivotal Studies on Animal Safety[a]

	Drug Tolerance[b]			Animal Safety[b]		
Guideline	**Met**	**Partly met**	**Not met**	**Met**	**Partly met**	**Not met**
Drug tolerance tests						
1. Use only target animals	4	0	0	c	c	c
2. Induce toxicity and record clinical signs	3	0	1	c	c	c
3. Pathologic and histologic data collected	3	1	0	c	c	c
4. Market formula of drug used	3	1	0	c	c	c
5. If long-term use, up to 10X maximum dose; 21 days	4	0	0	c	c	c
6. Test only healthy animals, up to four cows	2	1	1	c	c	c
Maximum dose levels						
7. Identify maximum dose level with no ill effects[d]	1	0	3	2	0	2
Good laboratory practices						
8. Statement on GLPs	4	0	0	4	0	0
Route						
9. Administration by recommended route	4	0	0	3	1	0
Study design						
10. Literature search[d]	c	c	c	0	0	4
11. Multiple dose levels done	c	c	c	4	0	0
12. Use ruminants as representative species	c	c	c	4	0	0
13. Complete Animal Safety Study, at least 6 weeks	c	c	c	4	0	0
14. Complete Animal Safety Study dose levels: 0, 1X, 3X, 5X	c	c	c	4	0	0
15. Proposed route should be that on label[d]	c	c	c	1	3	0
Observations						
16. Weight, feed, water consumption noted	c	c	c	4	0	0
17. Pathologic tests on all that show signs of toxicity and randomly selected	c	c	c	4	0	0
18. Gross pathologic exams on preselected cows	c	c	c	4	0	0
Reproductive studies						
19. Reproductive studies on both sexes[d]	c	c	c	1	3	0
20. Fertility—estrous cycle, mating, conception rate, gonadal function	c	c	c	3	1	0
21. Teratogenic and embryotoxic effects	c	c	c	2	2	0
22. Labor/delivery, abortion, neonatal viability examined	c	c	c	4	0	0
23. Evaluate fertility of both sexes[d]	c	c	c	1	3	0
Tissue irritation						
24. Injection site exams	c	c	c	4	0	0
Test animals						
25. Studies should be conducted on healthy cows	c	c	c	4	0	0

(continued)

Guideline	Drug Tolerance[b] Met	Drug Tolerance[b] Partly met	Drug Tolerance[b] Not met	Animal Safety[b] Met	Animal Safety[b] Partly met	Animal Safety[b] Not met
Physical examinations						
26. Physical exams to detect abnormalities[e]	[c]	[c]	[c]	3	1	0
27. Gross exams for pathologic lesions and organ weights	[c]	[c]	[c]	4	0	0
28. Histological exams on tissues[f]	[c]	[c]	[c]	4	0	0
29. Clinical pathologic exams[g]	[c]	[c]	[c]	4	0	0

[a]There are two major Target Animal Safety Studies. The Drug Tolerance Study characterizes, under controlled conditions, the target animal response to a toxic dose of a drug. The Complete Animal Safety Study documents the safety of the drug and monitors safety issues such as reproduction, disease, and the local effects on the animal when given the investigational drug.

[b]The Drug Tolerance Study guidelines are found in 1-9. The Animal Safety Study guidelines are found in 7-29. (Both types of studies require guidelines 7-9.) There were 4 protocols for Drug Tolerance and 12 protocols for Complete Animal Safety.

[c]Not applicable.

[d]These guidelines are those that we determined were not fully addressed, because more than 50 percent of the results were either only partially met or not met at all.

[e]Ocular, equilibrium, muscular disturbance, appetite, injection site, gastrointestinal, cardiovascular, respiratory, and behavior.

[f]Pituitary gland, thyroid gland, kidneys, adrenal glands, heart, liver, and stomach.

[g]Blood and serum chemistries, hematology, urinalysis, and fecal examination.

Comparison Between the Guidelines and Pivotal Studies on Mastitis[a]

Guideline	Met	Partly met	Not met
1. Infection status before trial	4	0	0
2. Status every 60 days	3	0	1
3. Status at end of trial[b]	0	0	4
4. Status at dry off[b]	1	0	3
5. Status at calving[b]	0	0	4
6. All quarters sampled 7-14 days before entry[b]	0	1	3
7. If different status than last sample, must resample within 10 days[b]	0	0	4
8. Observed twice daily for clinical mastitis by forestripping[b]	0	1	3
9. Samples refrigerated within 15 minutes for transport to laboratory[b]	0	0	4
10. Sample frozen if not to be cultured within 24 hours; cultured within 7 days[b]	0	0	4
11. Laboratory tests should identify numerous microorganisms[b]	0	2	2
12. Intramammary infection summarized separately from clinical mastitis[b]	1	0	3
13. Cause of clinical mastitis summarized under four categories[b]	0	0	4
14. Use clinical severity code[b]	0	0	4
15. Summary of rate of IMI, duration of IMI, prevalence of IMI, incidence of clinical mastitis, severity of clinical cases[b]	1	3	0
16. Data on somatic cell count for each cow, once per month	4	0	0
17. All somatic cell counts converted to log scale or log score	4	0	0

[a]There were four pivotal studies on mastitis.

[b]These guidelines are those that we determined were not fully addressed, because more than 50 percent of the results were either only partially met or not met at all.

Critical Guidelines on Mastitis and Raw Data Conclusions

Guideline	Result
1. Infection status before trial	Data from individual cow records showed status at pretreatment time period
3. Status at end of trial	Recorded in individual cow records
5. Status at calving	Recorded in individual cow records
6. All quarters sampled 7-14 days before entry	Recorded in pretreatment records
7. If different status than last sample, must resample within 10 days	Records show resampling and dates done
8. Observed twice daily for clinical mastitis by forestripping	Found in raw data
15. Summary of rate of IMI, duration of IMI, prevalence of IMI, incidence of clinical mastitis, severity of clinical cases	Most information found in individual records or could be extrapolated from existing records

Comparison Between the Guidelines and the Protocols on Drug Efficacy[a]

Guideline	Met	Partly met	Not met
General			
A. Incidence of mastitis was evaluated[b]	8	0	10
B. Data were collected under field conditions[b]	2	0	16
Milk weights			
1. Milk weights were recorded once every 7 days	14	0	4
1A. Milk weights were taken for 1 day (24 hours)	13	0	5
2. Milk production determined, expressed as 3.5 percent FCM per day[b]	7	1	10
3. Milk weights from total weight collected, and divided by number of days in treatment[b]	5	0	13
4. If BGH administered weekly or less, daily milk weights required[b]	7	1	6
Extrapolation of weights			
5. If cows dried before 305 days, milk records are extrapolated to 305 days[b]	2	0	16
6A. If dried off due to low production and completed 2/3 of treatment, extrapolation of weights is not permitted[b]	1	0	17
6B. Actual FCM will be divided by expected number of days on treatment at 305 days[b]	2	1	15
Fat corrected milk yields			
7. FCM not to be adjusted to mature equivalence[b]	2	0	16
8. Weekly treatment means of milk and FCM yields are to be plotted at each site and data pooled[b]	4	4	10
Feed efficiency[c]			
9. Feed efficiency = ratio of FCM per NE intake, corrected for body weight changes, over treatment period[b]	1	2	7
10. Total FCM produced divided by NE intake, over time period[b]	1	1	8
11. If dried off before 305 days, total FCM divided by NE intake over time till dry off[b]	2	0	8
12. Body weights every 4 weeks	5	4	1
13. Change in body weight = subtract body weight at initiation from body weight at end of period[b]	0	2	8
14. Factor of 5.12 Mcal per kg gain/4.92 Mcal loss in body weight used to correct feed efficiency[b]	0	0	10
Dose titration			
15. Control and three nonzero dose levels for groups[b]	7	8	3
16. Maximum effective dose[b]	2	3	13
17. Linear plateau or polynomial analysis models to be used[b]	2	1	15
Herd testing			
18. Minimum of three herds from different geographical areas	9	3	6
19. High- and low-producing multiparous and primiparous cows to be used[b]	4	5	9
Treatment regimen			
20. Variation at which cows are started on treatment should be no greater than 7 days[b]	5	1	12
20A. Treatment starts at dry off or 400th day lactation[b]	2	1	15
21. Control animal should receive an equivalent injection	12	4	2

(continued)

Guideline	Met	Partly met	Not met
BGH treatment			
22. Start/finish time for daily milking in same order[b]	3	7	8
23. Cow should remain on BGH until 400th day or dry off[b]	1	6	11
24. Drying off should occur between 45 and 60 days before parturition, or milk falls below a certain level, or last cow is off BGH[b]	3	2	13
25. Nonpregnant or cows with long open period: on treatment until 400 days, or milk falls below certain level, or last cow is off BGH[b]	5	2	11
26. Pretreatment, average based on 2 weeks' milk production	10	1	7
Unsalable and salable milk			
27. Unsalable milk included in milk production totals[b]	5	0	13
28. Total weight of unsalable milk recorded for each cow[b]	5	1	12
29A. Salable FCM averaged and summarized for each group or site[b]	2	1	15
29B. Weekly treatment means plotted at each site[b]	2	0	16
Data collection for lactation			
30. Data for lactation collected: FCM production, each treatment	9	3	6
31. Data for entire lactation collected: feed efficiency, each treatment	6	2	2
32. Cows having over 305 days lactation—parameters: average FCM per day, feed efficiency, unsalable FCM, number of disease treatment days[b]	0	0	18
Body weights			
33A. Body weights for correction of feed efficiency/animal health using scales[b]	2	8	8
33B. Body condition scores taken by same person[b]	1	2	15
34. Measurements on each cow in three herds plus trials[b]	2	3	5
35. Measurement every 4 weeks, pretreatment through dry off	13	0	5
36. Monthly treatment means plotted for each site[b]	1	2	15
Temperature and humidity			
37. Temperature and humidity recorded daily each site[b]	7	0	11
Blinding and accountability			
38. Individuals are blinded to treatments or dose levels[b]	7	0	11
39. Drug accountability: injection route, storage, record of use monitored[b]	3	8	7
40A. Monitoring by sponsor to be thorough[b]	3	1	14
40B. Instructions well described, protocols and standard operating procedures at each site[b]	2	1	15
Nutrition			
41. Feeds sampled once per week, pooled/analyzed once per month[b]	5	6	7

(continued)

Guideline	Met	Partly met	Not met
42. Feed levels determined: dry matter, crude protein, calcium, phosphorus, and acid detergent fiber[b]	7	1	10
43. NE content estimated and calculations reported[b]	5	1	12
44A. Daily feed intake per cow once each 7 days, in one herd	9	4	5
44B. Feed efficiency: three herds with feed refusals/weigh backs recorded[b]	2	0	8
45A. NE intake per cow once per week (from beginning of treatment to termination)[b]	0	7	11
45B. Weekly means plotted over time at each site, data pooled over all sites[b]	0	1	17
46A. NE balance = NE intake – (milk NE + maintenance NE)[b]	1	3	14
46B. Weekly means plotted, NE balance[b]	0	0	18
46C. Maintenance allowance increased 20 percent for 1st lactation[b]	0	0	18
47. Feed efficiency: corrected for changes in body weight[b]	1	0	9
48. Requirement for maintenance and milk production met: NE, protein, calcium, and phosphorus[b]	1	2	15
Reproduction			
49. Pool of sires used consistently or randomly for all treatment groups[b]	1	1	16
50A. Medications to aid reproductive performance must not cover up potential reproductive problems[b]	1	1	16
50B. Reproductive agents are not to be used before treatment[b]	3	0	15
51A. If treatment at 70 days postcalving, rebreed cow[b]	2	1	15
51B. If not pregnant at 65 days of BGH treatment, use approved therapeutic aids[b]	2	0	16
51C. Comparison among treatments: reproductive performance at end of 1st 65 days and end of 2nd 65 days[b]	1	0	17
52A. If treatment is less than 70 days, no agents should be used until 135 days postcalving[b]	0	1	17
52B. Reproductive aids may be used 135-200 days postcalving; evaluate at beginning and end[b]	1	0	17
53. Milk progesterone assays should be used through lactation for cycling; they cannot be used to aid in breeding until the periods mentioned above[b]	2	1	15
54. Criteria for use of reproductive aids made in advance for each herd; should be used consistently and documented[b]	0	2	16
55. State situations that require use of hormonal therapy: not for use to treat metritis[b]	0	1	17
Herd breeding			
56. Herd breeding and policy recorded[b]	1	2	15
57. Breeding: report heat-monitoring methods and personnel[b]	1	1	16
58. Records include any use of aids for reproductive performance[b]	0	1	17
59. Compare reproductive performance to rest of herd and with past years[b]	1	0	17
60. Describe special medical conditions that could influence reproductive efficiency[b]	1	0	17

(continued)

Guideline	Met	Partly met	Not met
61. Describe special environmental conditions that could influence reproductive efficiency[b]	1	0	17
62. Reproductive performance characterized for heifers and multiparous cows[b]	0	3	15
63A. Abortion up to 7 days before expected calving; noted as no parturition[b]	1	0	17
63B. Parturition is delivery of dead calf 7 days before expected calving[b]	1	0	17
64. Average and compare between groups: pregnancy rate, conception rate, number of live births per cow[b]	0	6	12
65. Average and compare between pregnant and nonpregnant: days to 1st heat, days to 1st insemination, average number of days between services, services per cow, length of lactation[b]	2	1	15
66. Average and compare for pregnant cows: service per conception, days open, number of abortions, number of stillbirths, length of gestation, calving interval, days carried calf[b]	1	0	17
67. Cows open are to be physically examined to determine problem[b]	3	1	14
68. Aborted fetuses, stillborn calves, placentas: should be necropsied[b]	4	1	13
69. Calving should be scored using numerical codes (1 to 5)[b]	1	0	17
70A. Body weights of all calves at birth should be recorded[b]	8	2	8
70B. Heifer calves weighed at 2 and 4 weeks of age[b]	5	0	13
Milk analysis			
71A. Aliquot milk pooled to be proportional to milk yield from 24-hour period: include protein, fat, somatic cells[b]	1	0	17
71B. Somatic cell counts: log transform before averaging, means should be converted back to actual counts, per cow[b]	0	0	18
72A. Level of phosphorus, calcium, lactose: 3-4 times during lactation[b]	2	3	13
72B. Milk analysis, method and source should be reported[b]	3	1	14
72C. Milk from each cow in one herd analyzed for BGH 3 times[b]	2	0	16
General health			
73. Health problems recorded: observe, treatment decisions, doses, methods of administration[b]	7	7	4
74. Physical exams recommended[b]	6	5	7
75. Injection site scale: normal,irritated, infected, major reaction[b]	4	9	5
Design and analysis			
76. Breeds are to be blocked[b]	1	0	17
77. Primiparous and multiparous should be blocked separately[b]	6	2	10
78. Blocks should be filled with homogenous pretreatment FCM groups[b]	6	1	11
79. No more than 20 lb. spread of pretreatment FCM in a block[b]	4	0	14

(continued)

Guideline	Met	Partly met	Not met
80. Blocks should be formed within 6-8 weeks from cows' entry into block[b]	1	1	16
Removal and dry off			
81. Randomization for each block determined in advance[b]	3	1	14
82. Evaluate differences in FCM production: pretreatment level and lactation number[b]	0	3	15
83. Missing data are to be noted as well as probable cause[b]	4	0	14
84. If cow is removed after 2/3 of treatment, production may be extrapolated to 305-day lactation (or delete)[b]	1	0	17
85. Feed efficiency: based on actual data if 2/3 of treatment period is collected[b]	1	0	9
86. Dried off before 305 days: data will be extrapolated to 305 days[b]	2	0	16
87. If dry due to low production, no extrapolation to 305 days; observed FCM to be divided by expected days on treatment at 305th day[b]	1	0	17
88. Cow removed due to illness: all data deleted and reason[b]	1	1	16
Statistical considerations			
89. If variances are heterogenous among herds, weighted analysis should be performed[b]	2	0	16
90. If error variances different for primiparous and multiparous, separate analysis should be conducted[b]	0	0	18
91. Data evaluated to determine if response is dependent on level of pretreatment production[b]	3	0	15
92. Average pretreatment FCM production by dose levels discounted if $P > 0.05$[b]	2	1	15

[a]There were 18 protocols on drug efficacy.

[b]These guidelines are those that we determined were not fully addressed, because more than 50 percent of the results were either only partially met or not met at all.

[c]Guidelines 9-14, 31, 34, 44B, 47, and 85 related to feed efficiency issues. These were relevant in 10 protocols that were to support claims of feed efficiency.

Comparison Between the Guidelines and the Pivotal Studies on Drug Efficacy[a]

Guideline	Met	Partly met	Not met
A. Incidence of mastitis was evaluated	4	0	0
B. Data were collected under field conditions	4	0	0
Milk weights			
1. Milk weights were recorded once every 7 days	4	0	0
1A. Milk weights were taken for 1 day (24 hours)	4	0	0
2. Milk production determined, expressed as 3.5 percent FCM per day	4	0	0
3. Milk weights from total weight collected, and divided by number of days in treatment	3	0	1
4. If BGH administered weekly or less, daily milk weights required	4	0	0
Extrapolation of weights			
5. If cows dried before 305 days, milk records are extrapolated to 305 days[b]	0	2	2
6A. If dried off due to low production and completed 2/3 of treatment, extrapolation of weights is not permitted[b]	0	1	3
6B. Actual FCM will be divided by expected number of days on treatment at 305 days[b]	0	1	3
Fat corrected milk yields			
7. FCM not to be adjusted to mature equivalence[b]	0	0	4
8. Weekly treatment means of milk and FCM yields are to be plotted at each site and data pooled[b]	2	1	1
Feed efficiency[c]			
9. Feed efficiency = ratio of FCM per NE intake, corrected for body weight changes, over treatment period[b]	0	0	4
10. Total FCM produced divided by NE intake, over time period[b]	1	1	2
11. If dried off before 305 days, total FCM divided by NE intake over time till dry off[b]	1	0	3
12. Body weights every 4 weeks	3	0	1
13. Change in body weight = subtract body weight at initiation from body weight at end of period[b]	1	1	2
14. Factor of 5.12 Mcal per kg gain/4.92 Mcal loss in body weight used to correct feed. efficiency[b]	1	0	3
Dose titration			
15. Control and three nonzero dose levels for groups	4	0	0
16. Maximum effective dose: highest level of BGH above which no significant improvement occurs	4	0	0
17. Linear plateau or polynomial analysis models to be used	3	0	1

(continued)

Guideline	Met	Partly met	Not met
Herd testing			
18. Minimum of three herds from different geographical areas	4	0	0
19. High-and low-producing multiparous and primiparous cows to be used	4	0	0
Treatment regimen			
20. Variation at which cows are started on treatment should be no greater than 7 days[b]	2	0	2
20A. Treatment starts at dry off or 400th day lactation[b]	0	1	3
21. Control animal should receive an equivalent injection	4	0	0
BGH treatment			
22. Start/finish time for daily milking in same order[b]	1	0	3
23. Cow should remain on BGH until 400th day or dry off	2	2	0
24. Drying off should occur between 45 and 60 days before parturition, or milk falls below a certain level, or last cow is off BGH	4	0	0
25. Nonpregnant or cows with long open period: on treatment until 400 days, or milk falls below certain level, or last cow is off BGH	3	1	0
26. Pretreatment, average based on 2 weeks' milk production	4	0	0
Unsalable and salable milk			
27. Unsalable milk included in milk production totals[b]	2	0	2
28. Total weight of unsalable milk recorded for each cow	3	0	1
29A. Salable FCM averaged and summarized for each group or site[b]	1	1	2
29B. Weekly treatment means plotted each site[b]	1	1	2
Data collection for lactation			
30. Data for lactation collected: FCM production, each treatment	4	0	0
31. Data for entire lactation collected: feed efficiency, each treatment	2	0	2
32. Cows having over 305 days lactation—parameters: average FCM per day, feed efficiency, unsalable FCM, number of disease treatment days[b]	0	0	4
Body weights			
33A. Body weights for correction of feed efficiency/animal health using scales	2	2	0
33B. Body condition scores taken by same person[b]	1	3	0
34. Measurements on each cow in three herds plus trials	3	0	1
35. Measurement every 4 weeks, pretreatment through dry off	4	0	0
36. Monthly treatment means plotted for each site	2	2	0

(continued)

Guideline	Met	Partly met	Not met
Temperature and humidity			
37. Temperature and humidity recorded daily each site	3	1	0
Blinding and Accountability			
38. Individuals are blinded to treatments or dose levels	2	0	2
39. Drug accountability: injection route, storage, record of use monitored	2	1	1
40A. Monitoring by sponsor to be thorough[b]	1	0	3
40B. Instructions well described, protocols and standard operating procedures at each site[b]	1	0	3
Nutrition			
41. Feeds sampled once per week, pooled/analyzed once per month	2	2	0
42. Feed levels determined: dry matter, crude protein, calcium, phosphorus, and acid detergent fiber	2	1	1
43. NE content estimated and calculations reported	2	1	1
44A. Daily feed intake per cow once each 7 days, in one herd	4	0	0
44B. Feed efficiency: three herds with feed refusals/weigh backs recorded[b]	1	0	3
45A. NE intake per cow once per week (from beginning of treatment to termination)	3	1	0
45B. Weekly means plotted over time at each site, data pooled over all sites	2	0	2
46A. NE balance = NE intake – (milk NE + maintenance NE)	2	1	1
46B. Weekly means plotted, NE balance[b]	1	1	2
46C. Maintenance allowance increased 20 percent for 1st lactation[b]	1	0	3
47. Feed efficiency: corrected for changes in body weight	2	0	2
48. Requirement for maintenance and milk production met: NE, protein, calcium, and phosphorus	3	0	1
Reproduction			
49. Pool of sires used consistently or randomly for all treatment groups[b]	0	0	4
50A. Medications to aid reproductive performance must not cover up potential reproductive problems[b]	0	1	3
50B. Reproductive agents are not to be used before treatment[b]	0	0	4
51A. If treatment at 70 days postcalving, rebreed cow[b]	0	0	4
51B. If not pregnant at 65 days of BGH treatment, use approved therapeutic aids[b]	0	0	4
51C. Comparison among treatments: reproductive performance at end of 1st 65 days and end of 2nd 65 days[b]	0	0	4
52A. If treatment is less than 70 days, no agents should be used until 135 days, postcalving[b]	0	1	3

(continued)

Guideline	Met	Partly met	Not met
52B. Reproductive aids may be used 135- 200 days postcalving; evaluate at beginning and end[b]	0	0	4
53. Milk progesterone assays should be used through lactation for cycling; they cannot be used to aid in breeding until the periods mentioned above[b]	0	0	4
54. Criteria for use of reproductive aids made in advance for each herd; should be used consistently and documented[b]	0	1	3
55. State situations that require use of hormonal therapy; not for use to treat metritis[b]	0	0	4
Herd breeding			
56. Herd breeding and policy recorded[b]	0	1	3
57. Breeding: report heat-monitoring methods and personnel[b]	0	2	2
58. Records include any use of aids for reproductive performance[b]	0	0	4
59. Compare reproductive performance to rest of herd and with past years[b]	0	0	4
60. Describe special medical conditions that could influence reproductive efficiency[b]	0	0	4
61. Describe special environmental conditions that could influence reproductive efficiency[b]	0	0	4
62. Reproductive performance characterized for heifers and multiparous cows	2	0	2
63A. Abortion up to 7 days before expected calving: noted as no parturition[b]	0	0	4
63B. Parturition is delivery of dead calf 7 days before expected calving[b]	0	0	4
64. Average and compare between groups: pregnancy rate, conception rate, number of live births per cow[b]	1	3	0
65. Average and compare between pregnant and nonpregnant: days to 1st heat, days to 1st insemination, average number of days between services, services per cow, length of lactation[b]	0	4	0
66. Average and compare for pregnant cows: service per conception, days open, number of abortions, number of stillbirths, length of gestation, calving interval, days carried calf[b]	1	2	1
67. Cows open are to be physically examined to determine problem	2	0	2
68. Aborted fetuses, stillborn calves, placentas: should be necropsied	2	1	1
69. Calving should be scored using numerical codes (1 to 5)[b]	0	0	4
70A. Body weights of all calves at birth should be recorded	4	0	0
70B. Heifer calves weighed at 2 and 4 weeks of age	4	0	0
Milk analysis			
71A. Aliquot milk pooled to be proportional to milk yield from 24-hour period: include protein, fat, somatic cells[b]	0	0	4

(continued)

Guideline	Met	Partly met	Not met
71B. Somatic cell counts: log transform before averaging, means should be converted back to actual counts, per cow[b]	1	0	3
72A. Level of phosphorus, calcium, lactose: 3-4 times during lactation	3	1	0
72B. Milk analysis, method and source should be reported[b]	0	0	4
72C. Milk from each cow in one herd analyzed for BGH 3 times[b]	0	0	4
General health			
73. Health problems recorded: observe, treatment decisions, doses, methods of administration	2	1	1
74. Physical exams recommended	3	0	1
75. Injection site scale: normal, irritated, infected, major reaction	2	2	0
Design and analysis			
76. Breeds are to be blocked[b]	1	0	3
77. Primiparous and multiparous should be blocked separately	3	1	0
78. Blocks should be filled with homogenous pretreatment FCM groups[b]	1	1	2
79. No more than 20 lb. spread of pretreatment FCM in a block[b]	1	0	3
80. Blocks should be formed within 6-8 weeks from cows' entry into block[b]	3	0	1
Removal and dry off			
81. Randomization for each block determined in advance[b]	3	1	0
82. Evaluate differences in FCM production: pretreatment level and lactation number[b]	0	1	3
83. Missing data are to be noted as well as probable cause	2	0	2
84. If cow is removed after 2/3 of treatment, production may be extrapolated to 305-day lactation (or delete)	2	0	2
85. Feed efficiency: based on actual data if 2/3 of treatment period is collected[b]	0	0	4
86. Dried off before 305 days: data will be extrapolated to 305 days[b]	1	0	3
87. If dry due to low production, no extrapolation to 305 days; observed FCM to be divided by expected days on treatment at 305th day[b]	0	0	4
88. Cow removed due to illness: all data deleted and reason[b]	0	0	4
Statistical considerations			
89. If variances are heterogenous among herds, weighted analysis should be performed[b]	0	1	3
90. If error variances different for primiparous and multiparous, separate analysis should be conducted[b]	0	0	4

(continued)

Guideline	Met	Partly met	Not met
91. Data evaluated to determine if response is dependent on level of pretreatment production	2	0	2
92. Average pretreatment FCM production by dose levels discounted if P > 0.05[b]	1	0	3

[a]There were 18 protocols on drug efficacy.

[b]These guidelines are those that we determined were not fully addressed, because more than 50 percent of the results were either only partially met or not met at all.

[c]Guidelines 9-14, 31, 34, 44B, 47, and 85 related to feed efficiency issues. These were relevant in 10 protocols that were to support claims of feed efficiency.

Critical Guidelines and Raw Data Conclusions on Drug Efficacy

Guideline	Result
Extrapolation of weights	
5. If cows dried before 305 days, milk records are extrapolated to 305 days	Data showed cows that dried off before 305 days and their expected milk output at 305 days
6A. If dried off due to low production and competed 2/3 of treatment, extrapolation of weights is not permitted	No individual cow records indicated extrapolation of weights for cows that did not complete 2/3 of treatment
6B. Actual FCM will be divided by expected number of days on treatment at 305 days	Records did not show that this was done, but data that are available could give this information
Feed efficiency	
9. Feed efficiency = ratio of FCM per NE intake, corrected for body weight changes, over treatment period	Raw data explicitly used this formula for FCM scores
10. Total FCM produced is to be divided by NE intake, over time period	Raw data highlight that this was used in relevant computations
11. If dried off before 305 days, total FCM divided by NE intake over time till dry off	Records did not show that this was done, but data that are available could give this information
12. Body weights taken every 4 weeks	This was located in an internal protocol
13. Change in body weight = subtract body weight at initiation from body weight at end of period	Feed efficiency data allows one to compute this information
14. Factor of 5.12 Mcal per kg gain/4.92 Mcal loss in body weight used to correct feed efficiency	Part of 1978 Nutritional Requirements of Dairy Cattle; located in raw data
Unsalable milk	
29A. Salable FCM averaged and summarized for each group or site	Found in raw data files
29B. Weekly treatment means plotted at each site	Weekly means not kept, but could be obtained from existing data
Data collection for lactation	
32. Cows having over 305 days lactation—parameters: average FCM per day, feed efficiency, unsalable FCM, number of disease treatment days	All information found in raw data except for number of disease treatment days, but could be obtained from individual cow records
Nutrition	
44B. Feed efficiency: three herds with feed refusals/weigh backs recorded	Obtainable from herd records and individual cow data
46B. Weekly means plotted, NE balance	Not kept for weekly means but obtainable from cow records
46C. Maintenance allowance increased 20 percent for 1st lactation	Obtainable from individual cow records

(continued)

Guideline	Result
Reproduction	
51C. Comparison among treatments: reproductive performance at end of 1st 65 days and end of 2nd 65 days	Expert panel felt comparisons important, but 65 days not crucial; data compare treatment groups
Herd breeding	
58. Records include any use of aids for reproductive performance	Individual cow data showed medical records and aid usage
60. Compare reproductive performance to rest of herd and with past years	This was not done in summary form, but is obtainable from cow records
63A. Abortion up to 7 days before expected calving: noted as no parturition	No evidence that abortion noted differently
63B. Parturition is delivery of dead calf 7 days before expected calving	Records indicate this was followed
64. Average and compare between groups: pregnancy rate, conception rate, number of live births per cow	Found in raw data files
65. Average and compare between pregnant and nonpregnant: days to 1st heat, days to 1st insemination, average number of days between services, services per cow, length of lactation	Obtained from individual cow records
66. Average and compare for pregnant cows: service per conception, days open, number of abortions, number of stillbirths, length of gestation, calving interval, days carried calf	Obtained from individual cow records
Milk analysis	
71B. Somatic cell counts: log transform before averaging, means should be coverted back to actual counts per cow	Somatic cell counts recorded for cows; records show log transformation
72C. Milk from each cow in one herd analyzed for BGH 3 times	Followed Dairy Herd Improvement Association methods; found in cow data
Design and analysis	
76. Breeds are to be blocked	Only one breed used
77. Blocks should be filled with homogenous pretreatment FCM groups	Raw data broken down by lactation groups
79. No more than a 20 lb. spread of pretreatment FCM in a block	Found in raw data

(continued)

Guideline	Result
Removal and dry off	
82. Evaluate differences in FCM production: pretreatment level and lactation number	Obtained from individual cow records
85. Feed efficiency: based on actual data if 2/3 of treatment period is collected	Obtained from cow records (see feed efficiency discussion)
86. Dried off before 305 days: data will be extrapolated to 305 days	Obtained from cow records
87. If dry due to low production, no extrapolation to 305 days, observed FCM to be divided by expected days on treatment at 305 days	This was not recorded in summary form, but could be obtained from individual cow data
88. Cow removed due to illness, all data deleted and reason	Cow records show reason for removal and end of data collection
Statistical considerations	
89. If variances are heterogenous among herds, weighted analysis should be performed	Found in raw data
90. If error variances different for primparous and multiparous, separate analysis should be conducted	Separate analysis was performed; in raw data
92. Average pretreatment FCM production by dose levels discounted if $P > 0.05$	Obtained from raw data

Major Contributors to This Report

Program Evaluation and Methodology Division

Boris Kachura, Assistant Director
Kurt Kroemer, Project Manager
Elaine Vaurio, Project Manager

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