April 20, 2000

Robert Cohen
560 Oradell Ave.
Oradell, NJ 07649

Re: Docket No. 99P-4613

Dear Mr. Cohen:

This is the final response from FDA to your Citizen Petition dated October 21, 1999. The petition concerns Posilac®, a recombinant bovine growth hormone (rbGH) product, sponsored by the Monsanto Corporation (Monsanto) and approved for marketing by the Food and Drug Administration (FDA).

Prior to commercial distribution of any new animal drug for use in food-producing animals, a sponsor must prove its product is safe and effective when used as described in the proposed labeling of the drug. Effectiveness simply means that the product does what the labeling claims. Safety routinely covers the safety of the food products to humans, and safety to the target animals. In addition to these requirements, the sponsor must prove that they can consistently manufacture the drug to a specific purity, potency and quality.

Your petition was filed by the agency on October 26, 1999. The petition requested "that FDA rescind their approval of Monsanto's Posilac and immediately remove it from the market" based on "new evidence" that the product poses "serious health consequences for human consumers." The new evidence you cite is an article by Robert P. Heaney, et al. entitled "Dietary changes favorably affect bone remodeling in older adults" which appeared in the October 1999 issue of the *Journal of the American Dietetic Association*. You also raised issues in the petition regarding the effects of rbGH in cattle on the levels of IGF-I in milk, the purported carcinogenic effects of IGF-I in humans, and the effects of heat treatment on the presence of rbGH in milk.

You amended this petition by FAX on October 27, 1999, and on November 3, 1999. The first FAX contained, in addition to the original petition, a document entitled "Docket #99P-4613". The second FAX contained the original petition and a document entitled "Meeting agenda for November 19, 1999." These documents had identical text but different headings. The documents recounted the issues raised in the original petition and raised another one. The new issue was based on an article by B. N. Violand, et al. entitled "Isolation of *Escherichia coli* synthesized recombinant eukaryotic proteins that contain epsilon-N-acetyllysine" which appeared in the July 3, 1994 issue of *Protein Science* and related to the purity of the rbGH produced by Monsanto.

On December 2, 1999, you amended your petition by letter, raising an issue regarding FDA's interpretation of a 90-day toxicology study (conducted by Richard, Odaglia and Deslex) and raising a new issue that the study "actually lasted for 180 days" and that the FDA had either misinterpreted or failed to review the study.

Thus, as amended, your petition raised three primary issues in support of your request for withdrawal of Posilac. These are: 1) that a recently reported increase in serum levels of insulin-like growth factor-I (IGF-I) in humans following milk consumption represents absorption of dietary IGF-I, invalidating a basic premise of FDA's safety assessment and proving that IGF-I in milk represents a hazard to human health; 2) that Monsanto changed the manufacturing process for rbGH after the studies supporting the New Animal Drug Application (NADA) were completed, thereby invalidating the research used to support the approval; and 3) that the 90-day toxicology study and/or the information derived from the additional 90 days of the study demonstrate both that rbGH is absorbed and that it is not safe. Finally, as a minor point, you commented on the potential ability of rbGH to survive the pasteurization process and thus be available for absorption.

We have thoroughly reviewed the issues you raised in your petition. We believe that the arguments presented in your petition do not demonstrate any human food safety issue related to the use of Posilac. Therefore, your petition requesting withdrawal of the approval of Posilac is denied. The issues you raised are addressed below.

1) The safety of IGF-I.

One of your main areas of concern is that IGF-I in milk will result in IGF-I levels that are elevated in humans after they consume milk from cows supplemented with rbGH. The FDA has previously maintained and continues to maintain that levels of IGF-I in milk whether or not from rbGH supplemented cows are not significant when evaluated against the levels of IGF-I endogenously produced and present in humans. In support of your position, you cite the *Science* article by Juskevich and Guyer (August 24, 1990). From this article's review of experimental data, you conclude that when rbGH is injected into cows, levels of IGF-I in milk increase by 80%.

IGF-I is normally found in human plasma at concentrations much higher than those found in bovine milk (Schaff-Blass et al., 1984). The levels in human plasma range from a low in neonates of 14 ng/mL to a high of 686 ng/mL in late pubertal females. The mean values of IGF-I concentrations in human blood plasma are between 42-308 ng/mL. The total daily production of IGF-I (endogenously) in an adult is 10,000,000 ng/day (Guler, H.P., et al., 1989). Additionally, IGF-I is normally found in human breast milk in concentrations higher than those found in bovine milk. The IGF-I concentrations in human milk ranged between 13 and 40 ng/mL six to

eight weeks postpartum (Corps, A.N., et al., 1988) while the milk samples from 5 commercial dairy herds not supplemented with rbGH had a mean IGF-I concentration of 2.54 ng/mL (Juskevich & Guyer, 1990).

Reported percentage increases in IGF-I concentrations in milk of rbGH supplemented cows can be misleading because the levels of IGF-I in milk are so low prior to any increase. For example, a 1988 study (Torkelson et al., 1988) indicated that while IGF-I concentrations in milk of rbGH treated cows could be as much as two-fold higher (a 100% increase) than unsupplemented cows, the absolute increase was 2-3 ng/mL. The 80% increase in IGF-I levels you refer to in the petition falls in this same range of 2-3 ng/mL.

IGF-I is a normal but highly variable constituent of bovine milk with the concentration depending on the animal's state of lactation, nutritional status and age. While some studies indicate that levels of IGF-I may statistically increase in the milk of rbGH supplemented cows relative to unsupplemented cows, reported increases are still within the normal variation of IGF-I levels in milk. Therefore, while IGF-I levels in milk from rbGH supplemented cows has been considered to be elevated by some groups (WHO FAS 31, 1993), IGF-I levels in milk from rbGH supplemented cows do not differ from those of unsupplemented cows in general with regard to IGF-I.

You cite new evidence demonstrating that levels of IGF-I increase in the blood serum after humans consume milk. The new evidence is an article by Heaney, et al. published in October 1999. This article states that there is a 10% increase in serum IGF-I levels in the milk groups. (The milk groups consumed three servings of milk per day for 12 weeks.) However, the study reported in the article made no effort to identify whether the milk products consumed by the participants were from dairy farms that used rbGH treatment. Also, the actual levels of IGF-I in the consumed milk are not reported. Therefore, while this study reports an increase in serum IGF-I following increased milk consumption, it does not link this increase to rbGH supplementation of dairy cattle, nor does it demonstrate any dietary link to the serum IGF-I levels.

The IGF-I increase observed in this study by Heaney, et al. must be viewed in light of the total daily adult endogenous production of IGF-I, which is in the milligram range while daily levels of IGF-I consumed in milk (by three-glass-a-day milk drinkers) are in the microgram range (a thousand-fold difference). As detailed below, IGF-I in milk would alter plasma levels by less than 1%--even if the entire amount of IGF-I in 1.5 liters (three very large glasses--an 8 oz. glass is less than a ¼ liter) of milk were totally absorbed. Therefore, the 10% increase in serum IGF-I reported in this study cannot possibly be due directly to IGF-I absorption from milk.

Finally, we note a difference between the study by Heaney et al. and that of Storm et al. (1998). Storm, et al. reported no significant change in serum IGF-I levels over a 2 year period for women supplemented with milk. This study utilized three treatment groups: placebo, non-dietary calcium, and milk supplementation. The milk group of 20 volunteers consumed four 8-ounce glasses of milk every day for the duration of the trial.

The presence and the concentrations of IGF-I resulting from rbGH administration were addressed by the 1992 and 1998 meetings of the World Health Organization/Food and Agriculture Organization's Joint Expert Committee on Food Additives (JECFA). FDA scientists were invited to participate in these proceedings. At these independent scientific meetings of international experts on food safety, concerns about the biological significance of rbGH-induced increases of IGF-I levels in milk were thoroughly evaluated.

The 1992 JECFA expert committee reached the conclusion that any elevation of IGF-I levels in milk resulting from rbGH administration were not of any human health concern due the lack of significant oral absorption of IGF-I under normal physiologic circumstances in humans. The 1998 JECFA expert committee concluded purely on the basis of exposure that the amount of IGF-I in milk is insignificant compared to the production of IGF-I in people, (less than 0.09%). This amount, even if it all survived digestion (and there is insufficient credible evidence that it does), could not reasonably elevate human plasma levels by even 1%. The international experts, including those from the FDA, concluded that IGF-I levels in milk of rbGH supplemented cows do not produce a biologically significant or deleterious effect in people. This conclusion of safety is reinforced by the JECFA decision that an allowable daily intake (ADI) and maximum residual limits (MRL) in food are not needed for rbGH and that rbGH can be used without any appreciable risk to the health of consumers.

Your petition also asserts that there is a connection between increases in levels of IGF-I and cancer. In the last few years there have been several articles published in the scientific literature that have dealt with the incidence of breast, prostate, and lung cancer in association with elevated levels of IGF-I, which you have cited (Chan, J. M. et al., 1998; Hankinson, S.E., et al., 1998; Yu, H. et al., 1999). These articles postulate an epidemiological association between significant increases in the plasma levels of IGF-I and tumor appearance. None of the articles demonstrate a causal relationship between IGF-I and the appearance of tumors. It must be noted that while large percentage increases in IGF-I concentrations in human plasma are reported in association with some tumors, the authors of these articles do not reach the conclusion that IGF-I caused the tumors.

Among the growth factors, IGFs play a crucial role in regulating cell proliferation and differentiation. IGFs' mitogenic activity (activity on reproduction of the cell) is regulated by receptor binding, which is in turn facilitated by IGF-binding proteins. These articles note a possible dose-response relationship between increased risks of these cancers and elevated levels of IGF-I, but none of the three articles empirically demonstrates a causal relationship. The authors' state that increased IGF-I plasma levels may be part of the phenotype of certain types of cancer; thus, the cancerous cells themselves may promote IGF-I to maintain their accelerated cell cycle. That is, the increased IGF-I levels may be the result of the cancer, and characteristic of the cancer process, but not the cause.

While clearly debatable, if increased circulating IGF-I levels were assumed to increase cancer risk, then the interaction between IGF-I and its receptor and the binding proteins becomes important. The putative carcinogenic effects of IGF-I would then depend upon receptor

interaction. IGF-I is not directly genotoxic (i.e., it does not directly alter the DNA). The ligand-receptor complex is responsible for the increased DNA synthesis and the acceleration of the cell cycle seen in the presence of IGF-I. There is no experimental evidence that (1) oral rbGH impacts the level of circulating IGF-I, or the number of IGF receptors and (2) there is no evidence that rbGH increases the number of ligand-receptor complexes. Thus, at present there is no evidence linking rbGH to any increased cancer risks that might be due to increased IGF-I and IGF receptor interactions. We agree with your conclusion that bovine IGF-I and human IGF-I are indistinguishable, and we reiterate that with respect to potential effects in humans, the amount of IGF-I in milk from cows (regardless of possible rbGH supplementation) is insignificant compared to the endogenous production of IGF-I in people (less than 0.09%).

2) The manufacturing process for rbGH.

The FDA was, of course, fully aware of the modification of the N-terminal amino acid of rbGH with a methionine (the established name of the product, methionyl sometribove, reflects that knowledge). We recognize that you have not taken issue with the incorporation of the methionine, conceding that it is not a health concern because it does not interfere with the tertiary structure of the protein nor does it impact the biological activity of the protein. FDA was also aware of the potential for an acetylated lysine at position 144 as well as other positions as reported by Violand, et al. in 1994. The FDA was informed of the latter potential difference between natural bGH and the sponsor's rbGH in 1987, six years prior to approval. {We note that you submitted an FOIA request for documents from January 1, 1990 to the present (which we considered to be December 20, 1999, the date your FOIA request was filed) regarding the “five different amino acids created during the process of genetically engineering [Monsanto's] bovine growth hormone”. We had no documents from that time period and we so advised you in our FOIA response.}

With regard to the alteration of amino acid 144, from lysine to epsilon-acetyl-lysine, and similar potential acetylations elsewhere, we note that these are post-translational modifications to the molecule. In other words, once the protein molecule is formed by incorporation of the appropriate amino acids at the appropriate positions, that is, after both transcription and translation, some amino acids may be further modified by the addition of various functional groups. While with rbGH the concern is over acetylation, with other proteins post-translational modifications may include glycosylation and disulphide-bridge formation. Most types of post-translational modification (including acetylation) do not affect the overall tertiary structure of the protein and thus, do not affect the biological activity of the protein. However, depending on the chemical moiety being added to the amino acid component, it is possible that these post-translational modifications alter the net electrical charge of the protein.

When Monsanto informed the FDA in 1987 that a small percentage of their rbGH product contained modified amino acid components, they did so by reporting on the electrical charge states of the resultant proteins. We note, in passing, that amino acid modifications of this kind are probably not appropriately referred to as "freak amino acids" as you refer to them in your petition. Rather, acetylation is a recognized naturally occuring post-translational event in

proteins. In any event, only a small percentage of the total rbGH produced is post-translationally modified in this manner.

We also note that the N-terminal amino acid of natural bGH is either phenylalanine or alanine--both of these amino acids occur as natural variants. Another site for variation in the amino acid sequence that occurs in natural bGH is position 126 at which valine and leucine are both known to occur. Thus, natural bGH, itself, is not one substance but a group of molecularly similar substances demonstrating similar biological activity. In general, pituitary extracts yield a class of related growth hormone polypeptides that vary in charge, mass, and N-terminal amino acids (Breems, D.N. et al., 1985). This heterogeneity of composition does not alter biological function and, thus, does not create a public health concern.

You note that Jerome Moore's 1988 *Endocrinology* article, comparing recombinant human growth hormone with natural human growth hormone, "warned that a different amino acid in the middle of the sequence of a protein chain could have significant consequences, quite often disastrous." While we could find no evidence of such a warning in the Moore article, the FDA fully agrees that substitutions of specific amino acids in specific proteins have been shown to lead to deleterious effects on the function of the hormone in some cases. However, post-translational modifications and N-terminus modifications of bGH specifically have not been shown to have any adverse effect on the biological activity of the molecule. Moore, himself, demonstrated this with respect to two different recombinant human growth hormone forms: with an N-terminal methionine and without the methionine. Therefore, we believe that Moore's discussion has been misconstrued in your petition relative to the kinds of changes associated with rbGH.

As previously noted, post-translational acetylation results in a small change in the electrical charge of the rbGH molecule relative to non-acetylated rbGH. This charge difference allowed Monsanto to improve their manufacturing process by separating most of the acetylated rbGH from the non-acetylated rbGH. (The net charge on the molecule is reduced by one positive charge due to the binding of the neutralizing acetyl group to the positive lysine.) The acetylated rbGH has similar activity to non-acetylated rbGH on the basis of in vitro assays and the acetylated rbGH never represented more than a small portion of the total rbGH produced. The improved manufacturing procedure resulted in rbGH that is of a purity comparable to that achieved in protein products approved for use in humans.

From statements in your Citizen Petition it appears that you believe that Monsanto satisfactorily "fixed" the problem of post-translational acetylation, but that in doing so the sponsor invalidated seven years of research because the pivotal studies supporting the approval utilized rbGH which was produced prior to the "fix". We assume your argument is that the rbGH used in the studies is not the same rbGH that FDA approved.

As stated earlier, FDA was aware of the change in the manufacturing process prior to its approval of the product and believed that the change did not result in a different product such that the research with the product prior to the manufacturing change was invalid. We note that

during the new animal drug development process, it is the usual case that sponsors make continued improvements in the manufacturing process. If those changes result in only biologically inconsequential variations, we consider the products to be the same.

However, to reaffirm that the conclusion we reached in this case was correct, we re-examined information previously submitted by Monsanto to their Investigational New Animal Drug file, to their manufacturing chemistry Master File, and to their New Animal Drug Application to support the approval of the rbGH product. We also made a site visit to the sponsor to examine batch records. These records are not required to be submitted to the new animal drug files.

Based on our examination, we reaffirmed our conclusion that the manufacturing changes resulted in only biologically inconsequential variations in the product used in the safety and effectiveness studies and, therefore, the rbGH product we approved is the same as the product used in the studies.

3) The fate and effects of bGH in milk

You cite the *Science* paper by Juskevich and Guyer and challenge the article's conclusion that pasteurization at 162°F for thirty minutes destroys bGH. You claim that the heat treatment in the study cited in the *Science* article, which produced only a 19% decrease, was not truly "pasteurization" because pasteurization at the temperature used in the study typically occurs for only 15 seconds. A review of the definition of pasteurization appearing in the Code of Federal Regulations (21 CFR 1240.61) defines pasteurization as the process of heating every particle of milk and milk product in properly designed and operated equipment to one of the temperatures given in the following table and holding it at or above that temperature for at least the corresponding time.

Temperature	Time
145° F (63° C)	30 minutes
161° F (72° C)	15 seconds
191° F (89° C)	1 second
194° F (90° C)	0.5 seconds
201° F (94° C)	0.1 seconds
204° F (96° C)	0.05 seconds
212° F (100° C)	0.01 seconds

The Agency considers all approaches in 21 CFR 1240.61 equivalent in pasteurizing a product. These alternative definitions of pasteurization only address the minimal time duration. While there is no indication that the heating process can not last longer and still be considered "pasteurization", we concede that it may have been more appropriate to refer to the process utilized in the referenced study as simply "heating" of the milk at the specified temperature for the specified duration.

Like most dietary proteins, rbGH is degraded by digestive enzymes in the gastrointestinal tract and not absorbed intact. *In vitro* studies on the metabolism of rbGH demonstrate that digestive enzymes readily cleave the molecule (Heiman & Harris, 1989). The progressive cleavage of peptide bonds results in the loss of biological activity because both the C- and N-termini and appropriate tertiary structure are required for receptor binding. *In vivo* studies confirm that proteolytic fragments of rbGH produced by digestive enzyme cleavage have no biological activity (Hammond, B.G., et al., 1990). In fact, one of the most common techniques for studying the primary structure of proteins is trypsin cleavage. In this technique, the protein molecule is treated with a proteolytic enzyme commonly found in the human digestive tract, trypsin, and the peptide bonds of the protein are broken down such that the amino acid sequence may be determined.

Your concern regarding the potential oral activity of rbGH leads to the issue you raised with respect to the "Three-Month (90-day) Oral Toxicity Study of Sometribove in the Rat." This study is the same 180-day study that you claim the FDA has not evaluated. This is a study of complex design and FDA welcomes the opportunity to clarify both the design of the study and its interpretation. The following discussion will address the full study.

In this study from Monsanto, conducted by Richard, Odaglia and Deslex, Charles River VAF rats (30 /sex/treatment group) were administered rbGH by oral gavage (0, 0.1, 0.5, 5 and 50 mg/kg bw) or subcutaneous injection (1 mg/kg bw) once daily for 90 days. Clinical observations, morbidity, mortality, body weights, and feed consumption were recorded for all rats. Upon cessation of drug treatment, 15 rats/sex/treatment group were necropsied to determine toxicology endpoints, including ophthalmology, hematology, clinical chemistry, urinalysis, pathology and histopathology.

The remaining 15 rats/sex/treatment group were used for the blood collection/reversibility groups. Blood samples were obtained from all of these rats prior to drug administration. Of these animals, blood samples were collected from 10 rats/sex/treatment group during week 7 of treatment and at week 14 (upon cessation of drug treatment). These rats were then removed from the study and destroyed without necropsy. The remaining 5 rats/sex/treatment group (the reversibility group) were also bled at week 14, and then on study week 28 (recovery week 14). At study week 28, following the collection of blood and recording of body weight and feed consumption, these animals were destroyed without necropsy.

Data collected for the 10 rats/sex/treatment group that made up the blood collection experimental group were limited to serum rbGH antibody concentration. Data collected for the 5 rats/sex/treatment group that made up the reversibility experimental group were limited to body weights, body weight change, feed consumption, and serum rbGH antibody concentration.

Thyroid cysts were observed in the gross and histopathological examinations in all treatment groups, including the positive and negative control groups. Neither the frequency nor severity of the cysts was attributable to rbGH treatment. Similarly, a prostatitis was observed in animals from all treatment groups including the positive and negative control groups and again this was

not attributable to rbGH treatment. No adverse effects of rbGH were observed in any animals of the toxicology group.

There were no statistically or biologically significant effects of daily oral rbGH in clinical observations, body weights, body weight change or feed consumption, either during treatment or during the recovery phases. Daily administration of subcutaneous rbGH significantly increased body weights, body weight change, organ weights, and feed consumption in the toxicology experimental group. However, body weights, body weight change, and feed consumption following subcutaneous administration returned to the "base-line" levels found in the control group animal values over the course of the recovery period.

Administration of subcutaneous or oral rbGH resulted in a significant increase in plasma antibody concentration. One out of 30 rats (male and female) receiving 0.1 mg/kg bw oral rbGH per day, 6/30 rats receiving 5 mg/kg bw, and 9/30 rats receiving 50 mg/kg bw per day had a measurable plasma antibody response following 90 days of continuous oral treatment. Twenty-seven out of 28 rats showed a significant antibody response following 90 days of subcutaneous rbGH injection (1 mg/kg bw). Fourteen weeks after cessation of rbGH administration, 9/10 rats of the subcutaneous rbGH treatment group, and 2/10 rats of the 50 mg/kg bw oral rbGH treatment group had measurable titers.

The immunological assay could not distinguish between an antibody response to intact rbGH or fragments of rbGH. It was also not possible to distinguish between antibodies produced in response to absorbed rbGH or gastrointestinal rbGH. While further interpretation of the plasma antibody response data is not possible, we reiterate that no adverse effects of rbGH were observed following 90 continuous days of oral administration or following an additional 90 days of recovery after the cessation of drug administration.

4) Conclusion

For the reasons stated above, FDA denies your Citizen Petition requesting withdrawal of the approval of the New Animal Drug Application providing for the marketing of Posilac by Monsanto.

Sincerely yours,

/s/

Dennis E. Baker
Associate Commissioner
for Regulatory Affairs

cc: HFA-305 (Docket 99P-4613)

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