UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

AMGEN INC., Plaintiff, v. F. HOFFMANN-LA ROCHE LTD., a Swiss Company, ROCHE DIAGNOSTICS GmbH, a German Company and HOFFMANN-LA ROCHE INC., a New Jersey Corporation, Defendants.	)	Civil Action No.: 05-12237 WGY

DECLARATION OF RALPH A. BRADSHAW, Ph.D. IN SUPPORT OF AMGEN INC.'S MOTION FOR SUMMARY JUDGMENT OF NO OBVIOUSNESS-TYPE DOUBLE PATENTING

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I. INTRODUCTION

I, Ralph A. Bradshaw, declare that:

1. I have been retained by counsel for Amgen Inc. as an expert witness in this action. If called to testify as to the truth of the matters stated herein, I could and would do so competently.

2. I am submitting this declaration in support of Amgen's Motion for Summary Judgment of No Obviousness-Type Double Patenting. I have been asked to give expert opinions and testimony regarding erythropoietin purification techniques, including those disclosed in U.S. Patent Nos. 5,547,933 ("the '933 patent"), 5,621,080 ("the '080 patent"), 5,756,349 ("the '349 patent"), 5,955,422 ("the '422 patent"), 5,441,868 ("the '868 patent"), 5,618,698 ("the '698 patent"), and 4,667,016 ("the Lai '016 patent"). For the reasons explained below, it is my opinion that the particular erythropoietin purification process that is the subject of the claims of the Lai '016 patent, including claim 10, would not have been obvious to one of ordinary skill in the art in 1983-1984.

II. QUALIFICATIONS

3. A copy of my curriculum vitae, reflecting my professional experience, affiliations, and work is attached as Exhibit A.

4. I am a Professor Emeritus in the Department of Physiology & Biophysics at the University of California at Irvine. I am also a Professor (Recall) in the Department of Pharmaceutical Chemistry at the University of California, San Francisco. Additionally, I serve as the Deputy Director of Mass Spectrometry Facility at the University of California, San Francisco. During the nearly 40 years I have been a faculty member, I have trained more than 20 students, advised dozens more and lectured to thousands of medical and graduate students in

areas of molecular and cell biology. Most of the students who did their research in my laboratory now hold positions in either academia or industry.

5. In addition to fulfilling my duties at the Irvine and San Francisco campuses of the University of California, I am a Co-Editor and Associate Editor of Molecular & Cellular Proteomics and Growth Factor, respectively. Regarding Molecular & Cellular Proteomics, I was also the publication's founding Editor-in-Chief. In addition to these journals, I currently serve on the Editorial Boards of the journals Biotechnology and Applied Biochemistry, Cancer Communications/Oncology Research, and IN VITRO Rapid Communications in Cell Biology. In the past, I have served as an Associate Editor of the Journal of Biological Chemistry and of Protein Science and as Editor-in-Chief of Trends in Biochemical Sciences. Over the course of my career, I have served on a total of seventeen Editorial Boards of academic journals.

6. I am (or have been) a member of a total of eighteen scientific societies, including the American Chemical Society, the American Society for Biochemistry and Molecular Biology, the Endocrine Society, the American Society for Cell Biology, the Society for Neuroscience, the International Society of Neurochemistry, the American Peptide Society, the Protein Society, the Association of Biomolecular Resource Facilities and the American Society of Bone and Mineral Research. I am an elected fellow of the AAAS and I also have served as the President of the Federation of American Societies of Experimental Biology, the Treasurer of the American Society for Biochemistry and Molecular Biology, and was the founding President of the Protein Society.

7. I have organized over three dozen national and international meetings on the structure and function of proteins, with a particular emphasis on growth factors. In addition, I have served on the Board of Directors of the Keystone Symposia on Molecular and Cell Biology

for the past ten years and in the capacity of Treasurer for nine of those years. I have also been a member of the U. S. National Committee on Biochemistry of the National Academy of Sciences-National Research Counsel for almost ten years, serving as that Committee's Chairman for four years.

8. Finally, I have served on a number of international advisory committees including the Hagedorn Institute in Denmark and the Australian Health and Medical Research Strategic Review Committee, a group that was charged with reviewing all of that country's biomedical research. In addition, I served on the Executive Committee of the International Union of Biochemistry and Molecular Biology for six years.

9. I received my undergraduate degree in chemistry in 1962 from Colby College. I was awarded my doctorate degree in biochemistry from Duke University in 1966. I have edited twelve scientific treatises and books and am the author or co-author of more than 300 articles or reviews and more than 150 abstracts in the area of protein chemistry and cell biology.

10. In the course of my over 40 years of research experience, I have isolated, characterized and/or determined the sequence of some fifty proteins, including several polypeptide growth factors and glycoproteins. I determined the first amino acid sequence of a growth factor (nerve growth factor) and I have contributed substantially to the development of this field.

11. Before making this declaration, I reviewed the documents cited herein, including the following:

- The specification and claims of Dr. Lin's '933, '080, '422, '349, '868, and '698 patents (Exhibit B);[1]

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[1] I have been informed that these patents share a common specification. For convenience, Exhibit B includes the specification of the '933 patent but only the claims of the other patents.

- The specification and claims of the Lai '016 patent (Exhibit C); and

In forming my opinions, I additionally have relied upon the knowledge, training, and experience that I have acquired during my over 40 years as a protein chemist.

12. For my analysis and testimony, I am being paid my usual consulting rate of $300.00 per hour.

III. BACKGROUND REGARDING PROTEIN PURIFICATION

13. Protein chemical studies include the elucidation of the structure/function relationship of a protein. In more recent times, protein chemistry has evolved to include the field of proteomics, a field that deals with the relationship between proteins in a sample and their functions (as compared to the reductionist approach — reducing samples to their component parts prior to considering their function).

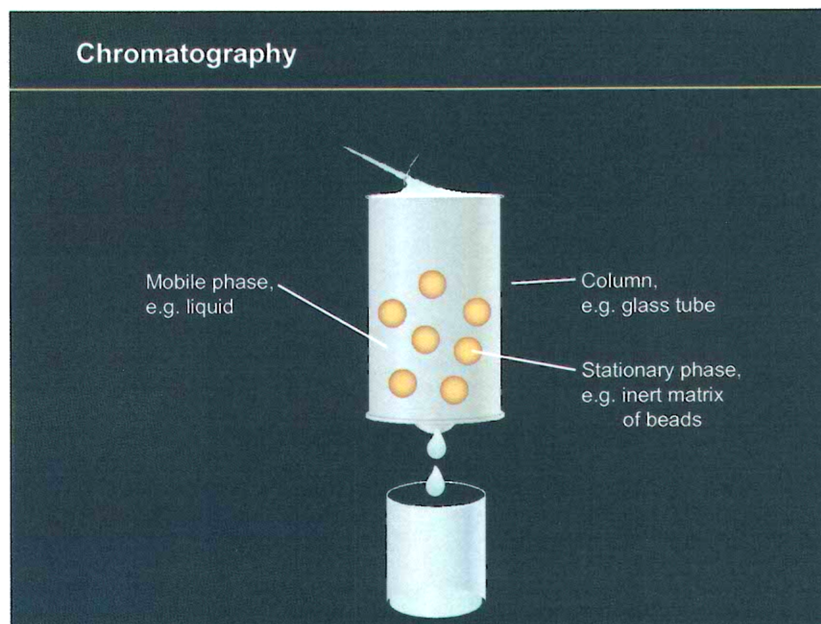
14. In order to eliminate the confounding contributions of other entities, proteins are usually isolated before their structure/function relationships are elucidated. The types and number of procedures needed to purify a particular protein from a sample will depend on what other materials are present, the amount of the desired protein, and the level of purity of that protein in the original sample. For example, generally, it is easier to purify a desired protein from starting material that is made up of about 50% of that desired protein than starting material that only has minute quantities of the protein.

15. Protein purification methodology is usually grouped into three categories: crude, refined, and affinity/super-refined procedures. The boundaries between these three categories are arbitrarily drawn and depending on the protocol, any given procedure within that protocol may be categorized as crude or refined, or refined or super-refined.

16. Typically, when attempting to purify a protein from natural materials, crude procedures are initially used to eliminate the presence of large molecules, such as components of the extracellular matrix and DNA. "Crude" procedures, such as precipitation and concentration steps, also often precipitate or concentrate a sample with a salt, such as ammonium sulfate, or an organic solvent, such as ethanol. Crude procedures can also include steps that eliminate precipitated or finely suspended material from a sample. These types of materials are generally removed using centrifugation or filtration procedures. The specific crude procedure or series of procedures to be used for any given protein will depend on the characteristics of that protein.

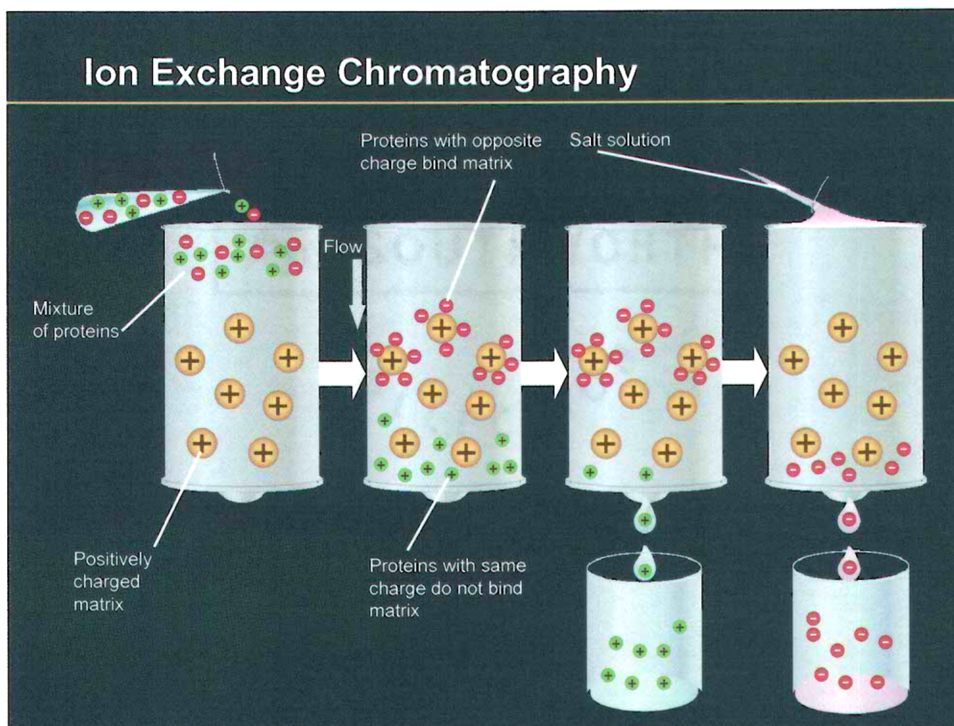
17. "Refined" steps typically include, but are not limited to, chromatographic and electrophoretic procedures. Unlike the procedures initially employed in treating crude samples, which largely depend on the behavior of a protein under certain conditions (and are mostly dependent on basic characteristics), the behavior of a protein in a refined procedure, or combination of procedures, tends to be also influenced by what other materials may be present in the sample (*i.e.*, the materials from which the protein must be separated).

18. Generally speaking, chromatographic procedures involve the separation of molecules using two physically distinguishable components — a mobile phase (a phase that moves) and a stationary phase (a phase that remains associated with an inert matrix). Molecules are separated based on how they are distributed between these two phases. For protein separations, the inert matrix is usually prepared in a column for convenience. Chromatographic procedures include ion exchange chromatography and high performance liquid chromatography.



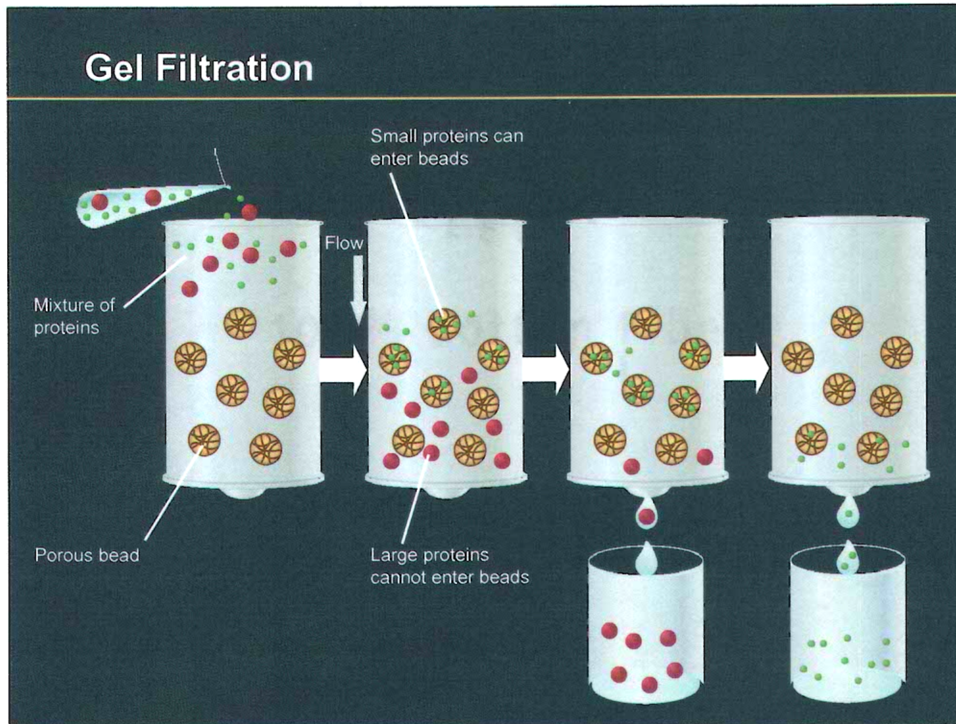
19. In ion exchange chromatography, a methodology developed in the 1950s, the matrix bears chemically bound charged groups to which ions in a sample can electrostatically interact. Depending on the charge of the matrix, molecules in a sample will reversibly interact with the matrix. In addition to its charge, how a molecule interacts on passage through the column will also depend on the porosity of the matrix used, pH, ionic strength and temperature.

20. Set forth below is a general diagram reflecting the principles and steps involved in ion exchange chromatography.



21. Of particular note, a desired molecule may either flow through the matrix (leaving at least some the undesired components of a sample on the matrix), or may interact to the matrix, allowing at least some of the undesired components to flow through the matrix. In the usual situation, some contaminants will not be bound while others are. In either case, the overall conditions of the chromatography experiment can then be manipulated so that the once-bound molecule can be released for collection.

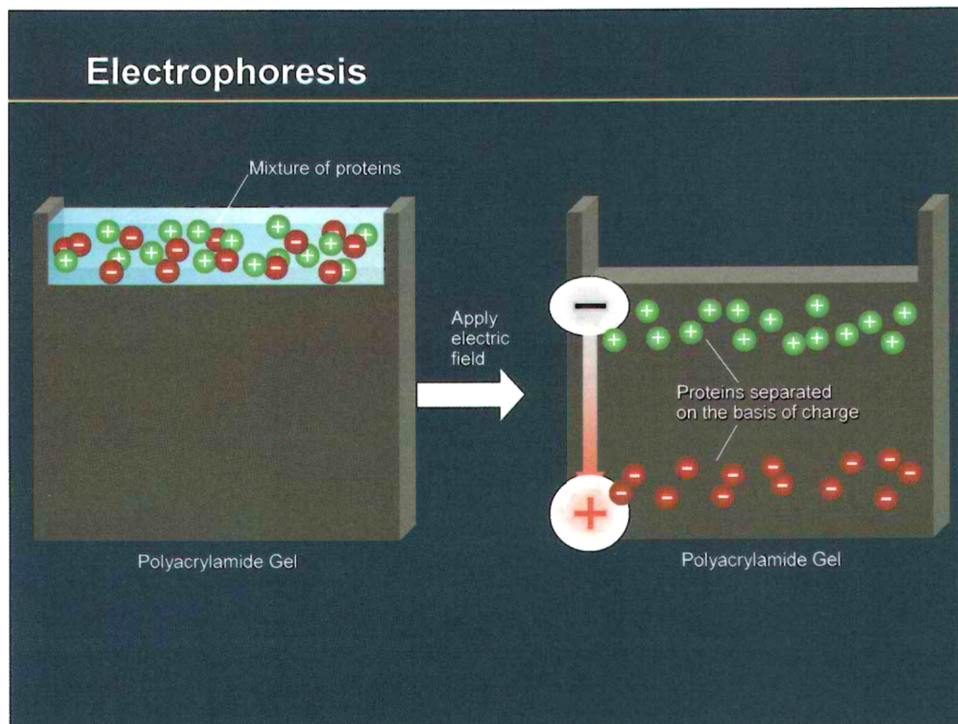
22. A second column methodology is gel filtration. Like ion exchange chromatography, gel filtration was also developed in the 1950s. In gel filtration, there is theoretically no interaction with the matrix. Instead, filtering is the process by which separation occurs. Below is a general schematic explaining the principles and steps involved in the gel filtration procedure.



23. Since their development in the 1950s, the general concepts underlying the two procedures have remained basically the same. However, in the intervening years, there have been improvements and refinements in the matrices used, and in various aspects of how samples and columns are managed. The primary focus today, however, is directed to being able to separate or detect smaller and smaller quantities of molecules in a sample. These improvements and refinements did not fundamentally change the concepts underlying the chromatography and gel filtration procedures.

24. Electrophoresis involves the separation of charged particles by exposing them to an electric field. Unlike ion exchange chromatography and gel filtration, which are typically conducted using columns and inert matrices, electrophoresis techniques are more often conducted using a flat gel. Gels useful in electrophoretic techniques include starch (the original "gel"), polyacrylamide, agarose, and agarose-acrylamide.

25. In electrophoresis, a protein's rate of movement will depend on its charge characteristics. If the matrix becomes limiting (e.g., the holes in the matrix are too small, as compared to the protein), the protein's molecular weight and shape may also have effect.



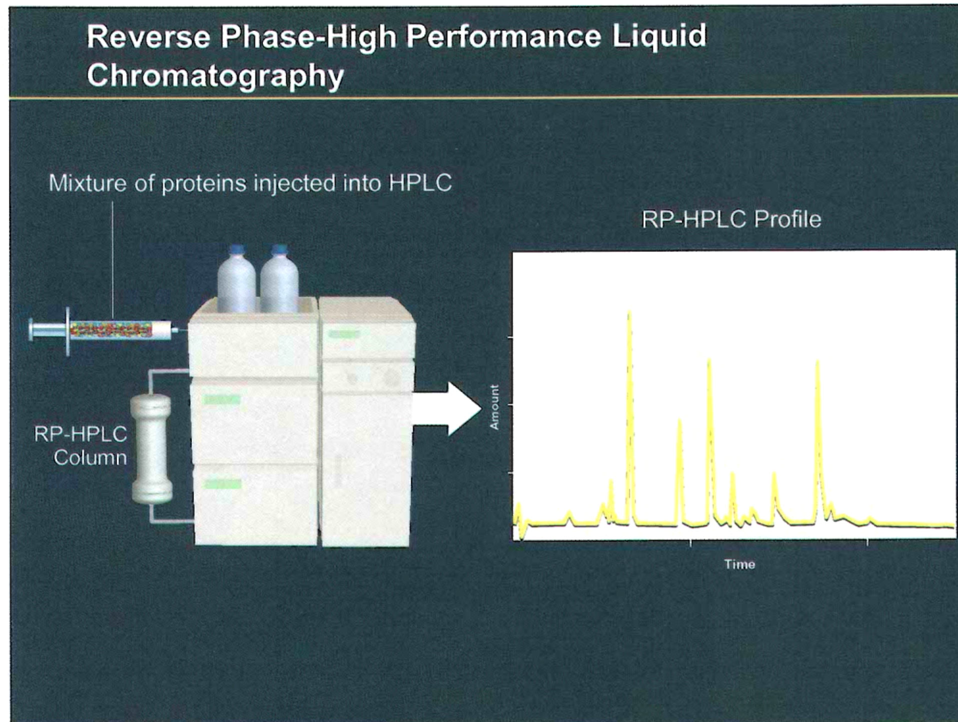
26. As in chromatography, developments in electrophoretic methods since the 1950s have often focused on being able to detect and separate molecules from smaller and smaller samples.

27. When using refined purification procedures such as electrophoresis and chromatography to isolate proteins from cell culture media or natural sources, an ordinarily skilled person in 1983-1984 understood generally that crude purification steps must be performed first. Otherwise, unwanted molecules in the sample (for example, DNA, extracellular matrix, or particulate matter) will likely interfere with the ability of subsequent refined methods

to separate molecules. In lay terms, if the samples, containing these unwanted molecules, are put on a column, the undesirable molecules will "gunk-up" the columns, making it difficult or impossible to properly develop the column, and potentially rendering the column unusable in the future. At the same time, this will likely disrupt or destroy the resolving power of the column.

28. Affinity/super-refined procedures include both affinity and highly resolving methodologies. An example of an affinity procedure is immunoaffinity chromatography, which involves the use of antibodies that are usually covalently bound to a matrix. Antibodies recognize specific epitopes on a protein or group of proteins having similar structures and bind to them. Components of a sample can then be separated based on whether they bind to the antibody.

29. An example of a procedure using high resolving matrices is reverse phase-high performance liquid chromatography (RP-HPLC). The use of this technique can be categorized as having extraordinarily high resolving power (its ability to separate molecules). As a procedure, it would fall within the gray area that I mentioned above between refined and super-refined procedures depending on the material to be isolated, the material from which the desired material must be separated, and its placement in the overall purification protocol. RP-HPLC may be run manually using a small column, or it can be automated, using specifically designed machinery.



30. The usual goal of protein purification is to purify a protein to homogeneity — a state where the protein is free from all other contaminants. Although theoretically possible, this level of purification is practically very difficult to achieve, in part because of the resolving powers of available procedures. The realization that this goal is rarely if ever achieved has resulted in large part from improved techniques to detect the presence of even the minutest quantities of a contaminant in a sample. Thus, protein chemists generally refer to the terms "apparent homogeneity" or "substantially homogeneous" instead. These terms reflect the reality of protein chemistry. "Apparent homogeneity," generally speaking, refers to the fact that the sample is deemed to be free from the presence of detectable contaminants. As I use the term, "substantial homogeneity" refers to a preparation, which may contain some small, acceptable level of contaminants.

IV. IN 1983-1984, THE ERYTHROPOIETIN PURIFICATION PROCESS OF LAI '016 CLAIM 10 WOULD NOT HAVE BEEN OBVIOUS TO ONE OF ORDINARY SKILL IN THE ART OVER ANY CLAIM IN DR. LIN'S PATENTS-IN-SUIT

31. The specification of Dr. Lin's patents-in-suit discloses prior art purification methods, such as the technique described by Miyake *et al.*, that one of ordinary skill in the art in 1983-1984 could have used to purify recombinant erythropoietin to apparent homogeneity. *See, e.g.*, '933 Patent at 6:60-7:62 (Exhibit B).

32. Based on my review of the Lai '016 patent (Exhibit C), it appears to me that Drs. Lai and Strickland built upon Dr. Lin's teachings and the Miyake *et al.* procedure in an effort to develop a more efficient and economical way to purify human recombinant erythropoietin from cell culture media for use in a formulation.

33. In my opinion, the Lai '016 patent claims, including claim 10, are directed to a new and non-obvious combination — a specific process comprising a defined set of steps. In the anion exchange step, material is loaded at a neutral pH and the column then washed with a urea solution at a pH of 4.5. The acidic urea wash was added to facilitate the separation of proteases from EPO and thus minimize the formation of EPO fragments (as set forth in the Lai '016 patent). To my knowledge, the resulting combination was and is an improvement over any of the processes previously described in the art. The ordinarily skilled person would not necessarily have found Dr. Lai and Dr. Strickland's process intuitive. One of ordinary skill in the art would not have thought to use a urea wash to separate proteases from the EPO because urea was not typically used for this purpose and a contaminant in urea was known to cause carbamylation of proteins in alkaline conditions. Drs. Strickland and Lai avoided the carbamylation issue by conducting the step under acidic conditions. For at least these reasons, it is my opinion that one of ordinary skill in the art in 1983-1984 would have found the erythropoietin purification process

claimed in the Lai '016 patent claims, including claim 10, to be non-obvious over any of the claims in Dr. Lin's '933, '422, '349, '868, and '698 patents.

34. The United States Patent Office apparently also considered the erythropoietin purification process claimed in the Lai '016 patent to be new and not obvious, because it granted a patent to Drs. Lai and Strickland for their invention.

35. I declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both (18 U.S.C. § 1001).

Executed this 7th day of June, 2007.

RALPH A. BRADSHAW, PH.D.