ENZON, Inc.

030-33128

June 29, 1998

U.S. Nuclear Regulatory Commission
Region I
475 Allendale Road
King of Prussia, PA 19406-1415

RE: License Renewal **Control Number 125676**

Dear Licensing Administrator:

In order to complete the needed documentation for renewal of the Radioactive Materials License No. 29-30031-01 issued to Enzon, Inc., I have enclosed the credentials of myself as Radiation Safety Officer for Enzon. My letter of May 4, 1998 informed you of our correct mailing address.

There will be **no changes** in the terms and specifications of our requested license for renewal.

The license renewal fee of $640 has been forwarded to Brenda Brown of the NRC.

Correspondence should be addressed to:

David Filpula, Ph.D.
Radiation Safety Officer
Enzon, Inc.
20 Kingsbridge Road
Piscataway, New Jersey 08854-3969

Tel: 732-980-4941
FAX: 732-885-2950

Sincerely,

David Filpula

David Filpula, Ph.D.
Radiation Safety Officer and
Director, Molecular Genetics

125865

NMSS/RGNI MATERIALS-002

REC'D IN LAT JUL 13 1998

OFFICIAL RECORD COPY ML 10

JUN 30 1998

RE: Radioactive Materials License Renewal **Control Number 125676**

Statement of Credentials and Experience of David Filpula for the position of Radiation Safety Officer of Enzon, Inc.

I have over twenty years of experience with the safe handling of the radioactive isotopes included in our license. During my doctoral training at the University of Minnesota, St. Paul, I recieved formal training in radiation safety both in accredited classes and in my own research which required techniques such as autoradiography and liquid scintillation counting using isotopes including ^{3}H and ^{32}P in quantities of 0.1 mCi to 10 mCi.

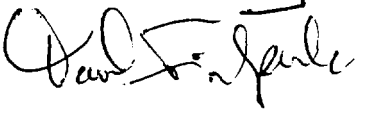
My postdoctoral research at Stanford University and the University of California, San Diego employed also 1 mCi to 10 mCi quantities of ^{125}I, ^{32}P, ^{14}C, ^{35}S, and ^{3}H isotopes for our research.

At Genex Corporation of Gaithersburg, Maryland and presently at Enzon, Inc., my laboratory continued to use the above five radioisotopes in the 0.1 mCi to 10 mCi range.

During 1992-1993, I served as the Radiation Safety Officer of Genex Corporation and recieved practical training in all aspects of isotope safety, inventory, monitoring, and disposal of the above isotopes in quantities of 100 mCi. Presently at Enzon, I am serving as RSO and monitor the safe use of radioisotopes at our facility. During the past year, our usage of radioisotopes has been minimal. During the past 6 months, we purchased only 3 mCi of an ^{35}S labeled compound.

I have enclosed my C.V. as additional documentation of my credentials.

Name: David Ray Filpula

Signature: 

DAVID R. FILPULA, Ph.D.

SPECIALIZATION

Molecular biologist with over ten years of managerial and research experience in industrial and pharmaceutical biotechnology.

EDUCATION

Ph.D. Biochemistry (1978) University of Minnesota (Dr. James A. Fuchs, thesis advisor)
B.S. Biology (1974 - summa cum laude) University of Minnesota

EXPERIENCE

1995-Present	Director, Molecular Genetics and Fermentation Department, Enzon, Inc., Piscataway, New Jersey
1994-1995	Acting Director, Tumor Biology and Molecular Genetics Department, Enzon, Inc., Piscataway, New Jersey
1993-1994	Principal Scientist, Molecular Genetics Department, Enzon, Inc., Piscataway, New Jersey
1991-1993	Principal Scientist, Protein Engineering Department, Enzon Labs, Inc. (formerly Genex Corporation) Gaithersburg, Maryland
1990-1991	Corporate Research Scientist, Molecular Genetics Department, Genex Corporation, Gaithersburg, Maryland
1987-1990	Principal Research Scientist, Biochemical Genetics Department, Genex Corporation, Gaithersburg, Maryland
1983-1987	Senior Research Scientist, DNA Chemistry Department, Genex Corporation, Gaithersburg, Maryland
1981-1983	Postgraduate Research Biochemist in the laboratory of Dr. Theodore Friedmann, Department of Pediatrics, University of California, San Diego, California
1981 (Aug)	Visiting Research Scientist in the laboratory of Dr. Frederick Sanger, Medical Research Council, Cambridge, England
1980-1981	Postdoctoral Fellow in the laboratory of Dr. Theodore Friedmann, Department of Pediatrics, University of California, San Diego, California
1978-1980	Postdoctoral Fellow in the laboratory of Dr. David Korn, Department of Pathology, Stanford University, Stanford, California

ACCOMPLISHMENTS

- Director of Molecular Genetics and Fermentation at Enzon, Inc. Department activities include development and scale-up of a recombinant E. coli production process for the existing nonrecombinant Enzon pharmaceutical ONCASPAR® (PEG-asparaginase); cGMP manufacturing from baculovirus/insect cells of LYSODASE™ (PEG-glucocerebrosidase) for current Phase I clinical trial in Gaucher patients; recombinant expression, protein purification, formulation, and pharmacological evaluation of PEG-arginine deiminase for use as an anti-melanoma therapeutic (in collaboration with the laboratory of Dr. Lloyd Old, MSKCC); development of Pichia pastoris as

an expression system for targeted human lysosomal enzymes and single-chain antibodies; original research in identification of the gene responsible for an arginine auxotrophy in melanomas; protein engineering of hemoglobin; and viral validation studies for PEG-Hemoglobin.

- Acting Director of Tumor Biology and Molecular Genetics at Enzon, Inc. Department activities include human xenografts and other rodent tumor models; evaluation of cytotoxic drugs and immune modulation in tumor growth and metastasis; bioassays and immunoassays; cell surface and signal transduction directed drugs; tumor radiosensitization; hybridoma production; gene delivery; protein engineering.

- Group Leader for Genex and Enzon molecular genetics section. Managerial responsibilities in recruitment and training; experimental design and innovation; purchasing and laboratory renovation; cGMP facility design; research meetings and preparation of reports, patents, and journal articles.

- Principal Investigator for five extramural grants funded by NCI, NIAID, NIGMS, NIDR and NSF through Small Business Innovation Research program including research on antibody engineering, novel catalysts, recombinant protein adhesives, isolation of gene for a chemotherapeutic protein, and engineering of protein-protein interactions.

- Isolated and characterized cDNA and genomic clones from mammalian, invertebrate, yeast, viral, protozoan and bacterial sources for diverse biotechnological applications, including protein pharmaceuticals, gene therapy, wound healing, diagnostic products, food processing, detergents, affinity matrices, fine chemicals, host/vector development, and vaccines. Over twenty independent full length gene sequence entries in the GENBANK/EMBL data bases.

- Designed and expressed synthetic and modified genes in prokaryotic and eukaryotic expression systems, including *E. coli* and yeast hosts, phage display systems, mammalian cell culture, and baculovirus/insect cell system.

- Active involvement in Genex single-chain antibody program with genetic construction, purification and in vitro and in vivo assay of ten distinct SCA (sFv) proteins derived from monoclonal antibody variable domains.

- Participant in the development, IND preparation and cGMP manufacturing at Enzon of an anti-tumor CC49 sFv protein for the 1995 clinical trial at Memorial Sloan-Kettering Cancer Center.

- Co-discoverer of the primary structure of the marine mussel adhesive protein and the bioadhesive functional requirements *in vitro*, using protein prepared from recombinant yeast.

- Member of research and development team for Genex (Pharmacia) GammaBind^{R}G products for antibody purification based on engineered streptococcal protein G. Conducted additional research on bacterial and mammalian immunoglobulin receptors.

- Co-inventor on patent applications pertaining to Genex and Enzon protein engineering, enzyme therapy, gene therapy, bioseparations, protein adhesive, and single-chain antibody technologies including issued US Patents No. 5,049,504; No. 5,202,236; No. 5,202,256.

- Manager of Genex DNA sequencing and DNA synthesis sections and Director of successful Genex contract DNA sequencing business.

- Developed a novel screening method for protein-protein interactions of two cloned gene products. Participant in Genex and Enzon protein engineering projects aimed at redesign of enzyme stability and kinetic parameters, and the modification of protein immunogenicity.

DAVID R. FILPULA, Ph.D.

- Conducted collaborative research with three industrial research teams on the development of a potential coccidial vaccine, using cloned and expressed antigen-encoding genes.

- Active in Genex research programs sponsored by major European and Japanese pharmaceutical companies for isolation and expression of genes for targeted human blood proteins.

- Administrative responsibilities as Patent Committee member, Corporate Radiation Safety Officer, IND and DMF preparation team member, Institutional Biosafety Committee Chairman, curator of corporate strain collection, invited reviewer for scientific journals, and manager of research collaborations with academic and private institutions.

- Graduate and postdoctoral research in enzymology, gene expression, cell culture, DNA replication, protein purification and characterization, virology, and gene therapy.

- Chairman and seminar speaker "Antibody Engineering in Microbes," ASM 1992 General Meeting; Invited symposium speaker, "Use of Biotechnology to Improve the Quality of Life," ASM 1991 General Meeting; Invited speaker, "Vectors for Cloning the Immune Response," 1990 Banbury Conference; Invited speaker, "On the Cutting Edge of Drug Delivery Systems," SRI 1994 Conference. Invited speaker, "Emerging Technologies in Cancer Research', 1996 Annual Retreat on Cancer Research in New Jersey and recipient of a Gallo Award from the Cancer Institute of New Jersey for outstanding cancer research.

PUBLICATIONS

Filpula, D., McGuire, J. and Whitlow, M. (1996) Production of single-chain Fv monomers and multimers, In Antibody Engineering: A Practical Approach (J. McCafferty, H. Hoogenboom, and D.J. Chiswell, eds.; Oxford University Press, Oxford, UK) pp. 253-268

Lee, T. K., Rollence, M. L., Hallberg, P. L., Oelkuct, M. S., Dodd, S. W., Nagle, J. W., and Filpula, D. R. (1995) Production of engineered IgM-binding single-chain antibodies in Escherichia coli, J. Industr. Microbiol. 14:371-376.

Filpula, D., Rollence, M., Essig, N., Nagle, J., Achari, A., and Lee, T. (1995) Engineering of immunoglobulin Fc and single-chain Fv proteins in Escherichia coli, In Antibody Expression and Engineering (H. Y. Wang and T. Imanaka, eds.; American Chemical Society, Washington, DC) pp. 70-85.

Whitlow, M., Filpula, D., Rollence, M.L., Feng, S.-L., and Wood, J.F. (1994) Multivalent Fvs: Characterization of single-chain Fv oligomers and preparation of a bispecific Fv. Protein Engng. 7:1017-1026.

Fisher, L.W., McBride, O.W., Filpula, D., Ibaraki, K., and Young, M.F. (1994) Human drebrin (DBN1): cDNA sequence, mRNA tissue distribution and chromosomal localization. Neurosci. Res. Comm. 14:35-42.

Whitlow, M., Bell, B.A., Feng, S.-L., Filpula, D., Hardman, K.D., Hubert, S.L., Rollence, M.L., Wood, J.F., Schott, M.E., Milenic, D.E., Yokota, T., and Schlom, J. (1993) An improved linker for single-chain Fv with reduced aggregation and enhanced proteolytic stability. Protein Engng. 6:989-995.

Whitlow, M. and Filpula, D. (1993) Single-chain Fvs, In A Practical Approach to Tumour Immunobiology, Characterization and Manipulation of Host Antibody Responses (G. Gallagher, R. C. Rees & C. W. Reynolds, eds.; Oxford University Press, Oxford, UK) pp. 279-291.

Milenic, D.E., Yokota, T., Filpula, D.R., Finkelman, M.A.J., Dodd, S.W., Wood, J.F., Whitlow, M., Snoy, P., and Schlom, J. (1991) Construction, binding properties, metabolism, and tumor targeting of a single-chain Fv derived from the pancarcinoma monoclonal antibody CC49. Cancer Res. 51:6363-6371.

DAVID R. FILPULA, Ph.D.

Gronenborn, A.M., Filpula, D.R., Essig, N.Z., Achari, A., Whitlow, M., Wingfield, P.T., and Clore, G.M. (1991) A novel, highly stable fold of the immunoglobulin binding domain of Streptococcal protein G. Science 253:657-661.

Whitlow, M. and Filpula, D. (1991) Single-chain Fv proteins and their fusion proteins. Methods, Companion Methods Enzymol. 2:97-105.

Gibbs, R.A., Posner, B.A., Filpula, D.R., Dodd, S.W., Finkelman, M.A., Lee, T.K., Wroble, M., Whitlow, M., and Benkovic, S.J. (1991) Construction and characterization of a single-chain catalytic antibody. Proc. Natl. Acad. Sci. USA 88:4001-4004.

Filpula, D.R., Lee, S.-M., Link, R.P., Strausberg, S.L., and Strausberg, R.L. (1990) Structural and functional repetition in a marine mussel adhesive protein. Biotechnol. Prog. 6: 171-177.

Fahnestock, S.R., Alexander, P., Filpula, D., and Nagle, J. (1990) Structure and evolution of the streptococcal genes encoding Protein G, In Bacterial Immunoglobulin-Binding Proteins, Vol. 1 (M.D.P. Boyle, ed.; Academic Press, Inc., San Diego) pp. 133-148.

Inana, G., Chambers, C., Hotta, Y., Inouye, L., Filpula, D., Pulford, S., and Shiono, T. (1989) Point mutation affecting processing of the ornithine aminotransferase precursor protein in gyrate atrophy. J. Biol. Chem. 264:17432-17436.

Vasantha, N., and Filpula, D. (1989) Expression of bovine pancreatic ribonuclease A coded by a synthetic gene in Bacillus subtilis. Gene 76:53-60.

Strausberg, R.L., Anderson, D.M., Filpula, D., Finkelman, M., Link, R., McCandliss, R., Orndorff, S.A., Strausberg, S.L., and Wei, T. (1989) Development of a microbial system for production of mussel adhesive protein, In Adhesives from Renewable Resources (R.W. Hemingway, A.H. Conner, and S.J. Branham, eds.; American Chemical Society, Washington, DC) pp. 453-464.

Vaslet, C.A., Strausberg, R.L., Sykes, A., Levy, A., and Filpula, D. (1988) cDNA and genomic cloning of yeast phenylalanine ammonia-lyase genes reveal genomic intron deletions. Nucleic Acids Res. 16:11382.

Filpula, D., Vaslet, C.A., Levy, A., Sykes, A., and Strausberg, R.L. (1988) Nucleotide sequence of gene for phenylalanine ammonia-lyase from Rhodotorula rubra. Nucleic Acids Res. 16:11381.

Filpula, D., Nagle, J.W., Pulford, S., and Anderson, D.M. (1988) Sequence of L-asparaginase gene from Erwinia chrysanthemi NCPPB 1125. Nucleic Acids Res. 16:10385.

Rollence, M.L., Filpula, D., Pantoliano, M.W., and Bryan, P.N. (1988) Engineering thermostability in subtilisin BPN' by in vitro mutagenesis. CRC Crit. Rev. Biotechnol. 8:217-224.

Tenover, F.C., Filpula, D., Phillips, K.L., and Plorde, J. (1988) Cloning and sequencing of a gene encoding an aminoglycoside 6'-N-acetyltransferase from an R factor of Citrobacter diversus. J. Bacteriol. 170:471-473.

Filpula, D., Alexander, P., and Fahnestock, S.R. (1987) Nucleotide sequence of the protein G gene from Streptococcus GX7805, and comparison to previously reported sequences. Nucleic Acids Res. 15:7210.

Fahnestock, S.R., Alexander P., Nagle, J., and Filpula, D. (1986) Gene for an immunoglobulin-binding protein from a group G Streptococcus. J. Bacteriol. 167:870-880.

Filpula, D., Ally, A.H., and Nagle, J. (1986) Complete nucleotide sequence of a native plasmid of Brevibacterium lactofermentum. Nucleic Acids Res. 14:5114.

DAVID R. FILPULA, Ph.D.

Nickerson, J.M., Wawrousek, E.F., Borras, T., Hawkins, J.W., Norman, B., Filpula, D.R., Nagle, J.W., Ally, A.H., and Piatigorsky, J. (1986) Sequence of the chicken δ2 crystallin gene and its intergenic spaces: Extreme homology with the δ1 crystallin gene. J. Biol. Chem. 261:552-557.

Beman, V., Filpula, D., Herber, W., Bibb, M., and Katz, E. (1985) The nucleotide sequence of the tyrosinase gene from Streptomyces antibioticus and characterization of the gene product. Gene 37:101-110.

Krieg, T.M., Schafer, M.P., Cheng, C.K., Filpula, D., Flaherty, P., Steinert, P.M., and Roop, D.R. (1985) Organization of a type I keratin gene: Evidence for evolution of intermediate filaments from a common ancestral gene. J. Biol. Chem. 260:5867-5870.

Vasantha, N., Thompson, L.D., Rhodes, C., Banner, C., Nagle, J., and Filpula, D. (1984) Genes for alkaline protease and neutral protease from Bacillus amyloliquefaciens contain a large open reading frame between the regions coding for signal sequence and for mature protein. J. Bacteriol. 159:811-819.

Tamashiro, J.C., Filpula, D., Friedmann, T., and Spector, D.H. (1984) Structure of the heterogeneous L-S junction region of human cytomegalovirus strain AD169 DNA. J. Virol. 52:541-548.

Woods, R.A., Roberts, D.G., Stein, D.S., and Filpula, D. (1984) Adenine phosphoribosyltransferase mutants in Saccharomyces cerevisiae. J. Gen. Microbiol. 130:2629-2637.

Vasantha, N., Rhodes, C.S., Thompson, L.D., Banner, C.D.B., and Filpula, D. (1984) Cloning of a serine protease gene from Bacillus amyloliquefaciens and its expression in Bacillus subtilis. In Genetics and Biotechnology of Bacilli (A.T. Ganesan and J. Hoch eds.; Academic Press, NY) pp. 163-172.

Spector, D.H., McDonough, S.H., Staprans, S.I., Tamashiro, J.C., Friedmann, T., and Filpula, D. (1984) Organization and expression of the repeats at the termini and L-S junction of human cytomegalovirus strain AD169. In Herpes virus, UCLA symposia on molecular and cellular biology, New Series, Vol. 21. (F. Rapp, ed.; Alan R. Liss Inc., NY) pp. 441-454.

Friedmann, T., Esty, A., Filpula, D., Doolittle, R., and Jolly, D. (1983) Characterization of an expressible human HPRT cDNA, In Recombinant DNA Applications to Human Disease. Banbury Report 14. Session II: Specific Disease Entities (C.T. Caskey, and R.L. White, eds.; Cold Spring Laboratory) pp. 91-96.

Woods, R.A., Roberts, D.G., Friedmann, T., Jolly, D., and Filpula, D. (1983) Hypoxanthine: guanine phosphoribosyltransferase mutants in Saccharomyces cerevisiae. Mol. Gen. Genet. 191:407-412.

Jolly, D.J., Okayama, H., Berg, P., Esty, A.C., Filpula, D., Bohlen, P., Johnson, G.G., Shively, J.E., Hunkapillar, T., and Friedmann, T. (1983) Isolation and characterization of a full-length expressible cDNA for human hypoxanthine phosphoribosytransferase. Proc. Natl. Acad. Sci. USA 80:477-481.

Filpula, D., Fisher, P.A., and Korn, D. (1982) DNA polymerase α: common polypeptide core structure of three enzyme forms from human KB cells. J. Biol. Chem. 257:2029-2040.

Filpula, D., and Fuchs, J.A. (1979) Increased synthesis of ribonucleotide reductase after deoxyribonucleic acid inhibition in various species of bacteria. J. Bacteriol. 139:694-696.

Filpula, D., and Fuchs, J.A. (1978) Regulation of the synthesis of ribonucleoside diphosphate reductase in Escherichia coli: Specific activity of the enzyme in relationship to perturbations of deoxyribonucleic acid replication. J. Bacteriol. 135:429-435.

DAVID R. FILPULA, Ph.D.

Filpula, D., and Fuchs, J.A. (1977) Regulation of ribonucleoside diphosphate reductase synthesis in Escherichia coli: Increased synthesis as a result of inhibition of deoxyribonucleic acid synthesis. J. Bacteriol. 130:107-113.

RE: Radioactive Materials License Renewal **Control Number 125676**

Statement of Credentials and Experience of David Filpula for the position of Radiation Safety Officer of Enzon, Inc.

I have over twenty years of experience with the safe handling of the radioactive isotopes included in our license. During my doctoral training at the University of Minnesota, St. Paul, I recieved formal training in radiation safety both in accredited classes and in my own research which required techniques such as autoradiography and liquid scintillation counting using isotopes including ^{3}H and ^{32}P in quantities of 0.1 mCi to 10 mCi.

My postdoctoral research at Stanford University and the University of California, San Diego employed also 1 mCi to 10 mCi quantities of ^{125}I, ^{32}P, ^{14}C, ^{35}S, and ^{3}H isotopes for our research.

At Genex Corporation of Gaithersburg, Maryland and presently at Enzon, Inc., my laboratory continued to use the above five radioisotopes in the 0.1 mCi to 10 mCi range.

During 1992-1993, I served as the Radiation Safety Officer of Genex Corporation and recieved practical training in all aspects of isotope safety, inventory, monitoring, and disposal of the above isotopes in quantities of 100 mCi. Presently at Enzon, I am serving as RSO and monitor the safe use of radioisotopes at our facility. During the past year, our usage of radioisotopes has been minimal. During the past 6 months, we purchased only 3 mCi of an ^{35}S labeled compound.

I have enclosed my C.V. as additional documentation of my credentials.

Name: David Ray Filpula
Birthdate:
SSN#:
Signature: