Mr. Alan Blake  
Yorktown Technologies, LP  
Austin, TX 78759

RE: Safety Assessment of Fluorescent Proteins

Dear Mr. Blake:

You have asked me to analyze and comment on the toxicity of fluorescent proteins that are commonly used in transgenic organisms. Specifically, your company plans to create and sell through retail channels transgenic ornamental fish containing genes, which express such fluorescent proteins, including green fluorescent protein (GFP) and red reef coral fluorescent protein (RFP).

The following analysis is based on the currently available information on the ecological and toxicological aspects of fluorescent proteins and transgenic organisms expressing such fluorescent proteins, as well as my knowledge of the fluorescent protein literature and scientific research in this area.

My own experience with fluorescent proteins and scientific and technical experience is summarized in the attached C.V. In brief, I have a Ph.D in Molecular Biology from the University of California, San Diego. I have been actively involved in scientific research on fluorescent proteins for approximately five years, and subsequently involved in intellectual property and business development aspects of the commercialization of fluorescent proteins for approximately three years. During this time I published several research articles on fluorescent proteins. I am also an inventor on a number of significant US patents directed to fluorescent proteins.

The available data summarized below suggests that fluorescent proteins are a low toxicological risk and are very unlikely to represent a health risk to either consumers or other organisms. Since fluorescent proteins naturally and harmlessly occur in the marine ecosystem, their presence in transgenic ornamental fish is unlikely to cause harm to the ecosystem.

I. Fluorescent Proteins have been widely used without apparent toxic effects

Fluorescent proteins have been widely used and successfully expressed in a wide range of cell types without toxic side effects.


Stable cell lines have been successfully obtained from virtually every cell type and organism so far tested, and transgenic animals that constitutively express fluorescent proteins have been reported as being healthy.

- Higashijima et al. (1997) High frequency generation of transgenic zebra fish which reliably express GFP in whole muscles or the whole body by using promoters of zebra fish origin. Dev. Biol. 192 289-299.


- Tsai et al. (2001) Uniform GFP-expression in transgenic medaka (Oryzias latipes) at the F0 generation. Transgenic Research 10 303-315.


Accordingly, the available data suggests that fluorescent proteins do not exert significant toxicological effects at the sub-cellular, or whole animal level.

II. **Fluorescent Proteins Are Already Present in the Food Chain**

Fluorescent proteins are widely dispersed in the natural ecosystem and are found naturally in many oceanic species. Fluorescent proteins are particularly common within the phylum Cnidaria that includes medusae (jellyfish), sea anemones, corals, marine hydroids, fresh water hydras, and sea fans.


Accordingly, it is concluded that any fluorescent proteins added to the ecosystem through genetically modified fish would be of minor influence because fluorescent proteins are already widely present and harmless in the ecosystem and within the marine food chain.

III. **Fluorescent Proteins Do Not Appear to Share Significant Homology to Known Allergens**

Sequence analysis of fluorescent proteins reveals that these proteins do not share significant homology in amino acid sequence with any known food allergens.


Further, direct analysis of the effect of recombinant GFP fed to rats demonstrated no adverse effects of oral GFP administration suggesting that that GFP is not an allergen or irritant to the digestive tract.


IV. Fluorescent Proteins Would Be Predicted to Act Like Other Proteins and Undergo Rapid Digestion in the Gut

Fluorescent proteins are comprised of naturally occurring amino acids, which would be predicted to be rapidly degraded in the gut.


Direct analysis of the effect of fluorescent proteins on animals fed recombinantly expressed fluorescent proteins, or diets containing transgenic canola expressing fluorescent proteins, demonstrates that such treatments do not affect the growth, food intake, relative weight of intestine or other organs, or the activities of hepatic enzymes in the serum.

VI. CONCLUSION

It is concluded that there is no basis for believing that fluorescent protein expression in transgenic fish would represent a toxicological risk, to either the environment or consumers, if the fish should enter the ecosystem.

Sincerely,

[Signature]

Andrew Cubitt, Ph.D
BIOGRAPHICAL SKETCH AND BIBLIOGRAPHY

Andrew Bryan Cubitt

Education

<table>
<thead>
<tr>
<th>Institution and Location</th>
<th>Degree</th>
<th>Conferred</th>
<th>Field of Study</th>
</tr>
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<tbody>
<tr>
<td>University of Birmingham, UK</td>
<td>B.Sc</td>
<td>1984</td>
<td>Medical Biochemistry</td>
</tr>
<tr>
<td>University of Sheffield, UK</td>
<td>Ph.D.</td>
<td>1987</td>
<td>Biochemistry</td>
</tr>
</tbody>
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Professional Qualifications

U.S. Patent Agent Registration No. 45,452 1999

SBIR New Technology review member 1999 to 2002

Research and Professional Experience

2002 to present

X-Ceptor Therapeutics Inc. Associate Director, Intellectual Property

1999 to 2002

Aurora Biosciences Corporation, Senior Manager.
Responsible for the review, preparation and analysis of new technologies and patent applications. Responsible for overall management and worldwide prosecution of biotechnology patents at Aurora (approximately 120 pending applications). Responsible for technical and IP analysis of third IP and freedom to operate analysis for internal use and commercialization. Review of technology and IP issues for in-licensing. Response for internal IP due diligence analysis for potential M & A activities.

1998 to 1999

Aurora Biosciences Corporation, Manager, Technology and Intellectual Property
Prosecute biotechnology patent applications with review of General Counsel. Oversees technology transfer into and out of Aurora. Oversight and coordination of intellectual property databases. Develop external academic and internal networks to enable successful biotechnology IP acquisition and dissemination of intellectual information for internal review. Provide technical support for external licensing into and out of Aurora. Review of public disclosures.

1996 to 1998

Aurora Biosciences Corporation, Principal Biochemist
Responsible for the development of fluorescence based biochemical and cell based assays for internal and external research projects. Supervision and training responsibilities for technicians. Responsible for the development of improved green fluorescent mutants, and subsequent licensing. Provide technical support for IP counsel.

1994 to 1996

Howard Hughes Medical Institute, UCSD, Project Scientist.
Worked on the use of rational design and random mutagenesis of GFP to produce brighter and more spectrally distinguishable mutants in laboratory of Roger Tsien. Characterization of GFP photochemistry, chromophore formation and spectral properties. Initiated development of novel biosensors based on GFP fluorescence. Optimization of the production and purification of GFP for X-ray crystallography and NMR studies.

1990 to 1994

Department of Biology, UCSD, Post-Doctoral Research Fellow
Molecular genetic analysis of signal transduction pathways in the developmental regulation of Dictyostelium in the laboratory of Richard Firtel. Evaluated the effect of gene disruptions of G-alpha subunits on developmental progression on phospholipid metabolism, including the regulation of phospholipases A, C and D. Used expression of Apo-aequorin as a novel method to measure for the first time calcium measurements throughout the developmental progression of the multicellular stages of Dictyostelium.

1993 to 1994

Marine Biological Laboratory, Visiting Scientist
Collaborated on the measurement of calcium levels using aequorin expression in Dictyostelium using the ultra low level light imaging equipment in the laboratory of Lionel Jaffe and cell lines created at UCSD. Demonstrated for the first time waves of calcium moving through all multicellular stages of development. Established a correlation between developmental fate and free cytosolic calcium levels.

1987 to 1990

Cornell University Medical College, New York, post-doctoral fellow
Analysis of phospholipase C activity and the control of inositol lipid synthesis in rat GH3 cells in the laboratory of Marvin Gershengorn. Identified, characterized and purified a phosphatidylinositol synthetase enzyme activity from GH3 cells. Established a link between the size of the hormone responsive pool of inositol lipids and the rate of resynthesis of lipids during TRH stimulated phospholipid hydrolysis.

Publications

Seventeen journal articles: journals represented include Nature (2), Science (1), J. Biological Chemistry (2), Development (2) and Biochemical J. (6). Three invited reviews


**Patents**


9. Pending Fluorescent Protein sensors of post-translational modifications. Inventor Cubitt, A. B.