Utah Small Business Innovation Program

PROJECT SUMMARY

UTFC AWARD NO.

NAME OF FIRM

Protein Solutions, Inc.

ADDRESS

6009 Highland Drive
Salt Lake City, Utah 84121

PROJECT LEADER (NAME AND TITLE)

S. Winters, Vice President, Research and Development

TITLE OF PROJECT

NIGHT COLONY: The Living Night Light

TECHNICAL ABSTRACT (LIMIT TO 200 WORDS)

We propose to develop unique science education products which will motivate children (and their parents!) to observe and experience scientific phenomena. The product, NIGHT COLONY, will consist of a transparent container with a culture of bioluminescent marine microalgae. The startling bioluminescence (light production) is an intense blue color and occurs upon mechanical stimulation. A light tap on the container results in light production. The NIGHT COLONY product will include all needed materials and accessories to set up, maintain, and observe bioluminescence and to enhance the observer's science interests. The product is analogous to existing ANT FARM and Sea MONKEY products commonly sold in toy and science stores, museum gift shops, and via direct marketing, but will be far more exciting, more desirable, and more useful for science education purposes.

Additional products include NIGHT LAB -- a complete science education kit using bioluminescence. Preliminary market analyses indicate a projected annual sales of $5,000,000 after four years.

PSI is working with the Salt Lake City and Davis County school districts to use bioluminescence in their science curricula. We anticipate a market as large as $100M/year in the public and private education sector -- Grades K-12.

KEY WORDS TO IDENTIFY TECHNOLOGY (8 MAXIMUM)

Aquaculture, Bioluminescence, Science Education, Toys, Children's Products, Biotechnology.

POTENTIAL COMMERCIAL APPLICATIONS OF THE INNOVATION

Science Education, Children's Toys & Novelties

2-89

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IDENTIFICATION AND SIGNIFICANCE OF THE INNOVATION

Protein Solutions, Inc. (PSI) intends to develop, manufacture, and market a new line of educational, innovative toys, NIGHT LIFE TOYS, which use the phenomenon of bioluminescence to motivate and entertain children. This proposal specifically addresses the first product of this line, NIGHT COLONY, a living night light.

A new awareness is emerging in the literature and public opinion that the 90's will be a decade of increased emphasis on education. Most states have already adopted programs to enhance education at all levels, in both public and higher education. The National Science Foundation's budget for science education has increased dramatically in recent years. This will result in a significantly increased demand for products which have an educational component, including gifts and novelties for children and more serious products purchased by parents and teachers to enhance the education of their children and their students. PSI will be addressing this interest with products that stimulate a child's natural curiosity and eagerness to learn.

PSI is focused on the development and application of bioluminescence as an educational and product development pool. This is a unique technology which has never been utilized in educational products of toys. Children (and their parents!) are fascinated by bioluminescence. It is a new, "magical", and very stimulating experience for them.

NIGHT COLONY, a transparent container of bioluminescent marine micro-organisms which produce a brilliant blue light upon mechanical stimulation, is the first and most versatile NIGHT LIFE TOY. Two different marine microalgae have been identified as candidates for this product, Pyrocystis lunula and Pyrocystis noctiluca. These can be seen upon close scrutiny with the naked eye. With a small magnifying lens (provided with the product) they can be discovered and observed. With the use of a low power microscope (which will also be available through PSI), internal structures, cell division, and other behaviors may be observed. A fascinated child will want to take his "companions" to bed and explore their brilliant night light.

Successful development and manufacture of the NIGHT COLONY will provide a new, exciting introduction to the joy of discovery and exploration, and the impetus for developing questions and curiosity.

The National Council on Science and Technology Education recommends introduction to the nature of the scientific enterprise in our education curricula , including how science, mathematics and technology relate to one another and to the social system in general. Educational reform to integrate the various divisions of science is now recognized as a critical step which must be taken if the US is to compete with other nations[1]. NIGHT COLONY provides an excellent introduction to the interplay of biology, chemistry, physics, and environmental science.

Equally important, NIGHT COLONY, provides a teaching aid which may be used in classrooms from kindergarten through grade 12. Consider the fascination of the traditional Ant Farm used in classrooms throughout the country and extrapolate to the increased fun in observing organisms which produce their own light! PSI has had very positive responses from the Salt Lake City and Davis County school districts, and from the State Office of Education; there is strong interest in adding the exploration of bioluminescence into their curricula[8].

Thus, successful development and commercialization of the proposed NIGHT COLONY and other NIGHT LIFE TOYS will increase the interest and enthusiasm of children through the process of discovery, thereby enhancing science education. .

BACKGROUND, APPROACH AND ANTICIPATED RESULTS

Background

Flashing fireflies on summer evenings, glowing ocean surf, and other forms of natural bioluminescence have always been a target of curiosity. Ancient scientists Aristotle and Pliny the Elder studied bioluminescence but it was not until the 1670's that English chemist Robert Boyle described some of its fundamental properties. Although investigated for many years, biologists have been slow to exploit bioluminescence in the laboratory. Now scientists are using bioluminescence to study gene expression and developmental biology. Other applications in biology, medicine and agriculture are underway, including the arena of clinical medicine and diagnostics[2].

Nearly everyone who discovers and observes bioluminescence is impressed and motivated to see and learn more. In these times where children have their senses constantly stimulated to near exhaustion, bioluminescence is a relatively unknown, unexperienced phenomenon which can readily compete for a child's attention and interest.

Bioluminescence is the light produced by certain plants and animals. It is not only fascinating to observe, but can be packaged and discovered in ways which entertain a child while teaching him/her basic principles of biology, chemistry and physics via an integrated, multi-disciplinary approach to science education[3].

The so-called phosphorescence of the sea is due to the bioluminescence of dinoflagellates. The light emitted when the water is disturbed is characteristically emitted as a rapid flash which occurs upon stimulation and lasts only a fraction of a second [2] but individual cells, depending on the species, will flash repeatedly with repeated stimulation. These dinoflagellates will be used in NIGHT COLONY to produce light when agitated or mechanically stimulated.

Approach

We propose to develop integrated science educational toys using bioluminescence as the "discovery" and observation vehicles. NIGHT COLONY will consist of a transparent container of "sea pets", an owner's manual, food packets, a sampling pipet, and a NIGHT SLIDE for observation (Appendix A).

The technology involved in the development of NIGHT COLONY requires R&D efforts in the culture, growth and handling of the bioluminescent microorganisms in supplemented sea water The technology further includes means to stimulate the bioluminescence by physical and chemical means. One major advantage over chemiluminescent products* is the ability to control the light emission, essentially to induce light on command. Growth of the organisms to the high densities needed for NIGHT COLONY requires optimization of the culture conditions and of the sea water composition.

* The Lite Stiks sold by American Cyanamid are based on chemiluminescence and do not involve living organisms.

PSI is currently working on the development of culture techniques for production of the microorganisms. PSI has substantial experience and knowledge as to the temperature, salinity and nutrient requirements, including trace metals and vitamin supplements, of the Pyrocystis noctiluca, Pyrocystis lunula and Gonyaulax polyedra bioluminescent organisms

In addition, we propose to design the appropriate materials and configuration for NIGHT COLONY which would have the greatest visual and tactile appeal to generate interest in children, their parents, and their teachers. Currently several configurations are under consideration, including a flat rotatable disk design and a gas permeable polymer molded into the shape of an animal to be sold as a NIGHT PET* .

The requirements for shipping and storage of the NIGHT COLONY will be optimized. For example, questions such as how much carbon dioxide must be stored in the NIGHT COLONY to maintain an adequate shelf life of the organisms will be addressed.

PSI is uniquely positioned to enter the educational bioluminescence toy market. We have had extensive interaction with experts in the field, including those knowledgeable about dinoflagellates and other bioluminescent organisms[10]. J.D. Andrade, President of PSI, has been involved in the field of bioluminescence for over 3 years. One of our staff has attended a workshop on marine phytoplankton culture and techniques at the Provasoli Guillard Center for the Culture of Marine Phytoplankton in Maine.

In addition, we have been actively working with educators and administrators from the Salt Lake City and Davis County school districts to introduce bioluminescence into an experimental science curriculum[8]. We are also working with the Children's Museum of Utah to develop an interactive, hands-on bioluminescence exhibit[9]. We sincerely believe that the development of this technology will result in an expanding, successful and profitable business venture which will stimulate children's fascination with science and educational toys.

Anticipated Results

The proposed R&D project has been planned and organized to test the feasibility of developing and maintaining a viable dinoflagellate culture for use as a science educational toy. We anticipate successful demonstration of feasibility by growing and fabricating containers and cultures of these microorganisms. We propose work leading to:

1. High density cultures of single and mixed populations of dinoflagellates which may be maintained viable for up to six months with little care or attention. We expect to identify specific feeding and CO2 requirements for their viability and to design an optimum product configuration.

2. A polymeric membrane capable of providing the necessary gas requirements for these organisms which may be fashioned into a shape attractive to children. The gas needs of the dinoflagellates will be a function of temperature and of the light requirements and must be determined. Several gas permeable polymers are commercially available which may satisfy these requirements. Our staff has

* Since the organisms are plants and utilize photosynthesis to maintain their life and function, access to atmospheric carbon dioxide (CO_2) is necessary

extensive experience with gas transfer membranes and in the design of products with the needed gas transfer requirements.

3. An instruction manual which will be interesting, and informative to children, ages 10 to 14, which will help stimulate the child's interest to learn. A specific aim of this manual will be to encourage personal discovery and experimentation processes.

After feasibility is demonstrated by this Phase I project, we will immediately move into Phase II to further develop and optimize NIGHT COLONY and to move into production and commercial sales. We anticipate no difficulty in obtaining funding for the follow-on Phase II (optimization and test marketing) and Phase III (full commercialization) of the project.

Several novelty products are currently on the market which generate light through chemiluminescence, such as "light stiks" (see footnote on page 5) . However, these do not address the educational market or attempt to stimulate scientific interest. These products lack the flexibility, versatility, efficiency and control available with bioluminescence. To our knowledge, no other product is available or under development which can compete with NIGHT LIFE TOYS.

PROGRAM OBJECTIVES

The proposed R&D project is aimed at accomplishing the following specific objectives:

A. Develop methods to produce high densities of different dinoflagellates and their mixtures for optimum bioluminescence.

B. Develop the process to produce cultures which have high stability and which guarantees a product life of three to six months.

C. Optimize the proprietary culture medium with intent to patent the resulting formulation.

D. Develop an optimum container/membrane for appropriate CO2 requirements and consider patent protection.

E. Establish shipping and storage requirements to maintain a viable culture of the dinoflagellates.

F. Develop the teaching and educational materials and product accessories to enhance customer interest and science education.

PROGRAM PLAN

The program objectives will be reached by the performance of 15 specific tasks (keyed to Objectives A-F above):

Task A-1. Selection of dinoflagellates with sufficient bioluminescence- There are several known species of bioluminescent dinoflagellates. PSI has identified and studied three varieties, Pyrocystis noctiluca, Pyrocystis lunula, and Gonyaulax polyedra. With

further exploration of the literature and consultation with experts in the field, it may be that other species are more suitable for this application. These will be identified and final selections made based on availability, bioluminescent intensity and duration, ease of culture, ease of transport, and long term viability.

Task A-2. Design, fabrication and installation of incubators and lighting systems- Dinoflagellates maintain normal circadian rhythms and are sensitive to fluctuations in temperature and light. Following consultation and based on our current experience, incubators and lighting systems will be designed, built and installed in our laboratory. Different organisms respond differently to light/temperature cycles so it may be that several incubators will be needed to maintain the different species. PSI has investigated the possibility of acquiring floral or restaurant chillers which may prove appropriate for our needs. These techniques are needed to generate the high densities and volumes of organisms for use in the NIGHT COLONY product.

Task A-3. Development of culture/transfer techniques- Although there are several references on culturing dinoflagellates, our experience demonstrates that certain "tricks of the trade" must be developed for successful manipulation of these micro-organisms. We recently participated in a workshop sponsored by the Provasoli Guillard Center for Culture of Marine Phytoplankton in which the important criteria for successful maintenance of phytoplankton were discussed and practiced. Techniques developed so far will be optimized and recorded for each of the selected dinoflagellates.

Task A-4. Identification of division rates- Each species of dinoflagellates has a specific life cycle and division rate. Obviously it will be important to select those organisms which will provide the maximum division rates for culture while minimizing any fouling of the media from cell death or metabolites. The culture media has an influence on the rate of division and will be explored in efforts on Task C-1 below.

Task A-5. Maximization of cell densities for maximum bioluminescent intensity- Currently, PSI has developed sufficient expertise to maintain moderate density cultures of the Pyrocystis strains. Experiments will be conducted to maximize the cell densities of individual species by altering light cycles, temperature and availability of CO2. These experiments will again be explored simultaneously with efforts to optimize the artificial sea water (Task C-1).

Task B-1. Influence of temperature/ light on long term stability- Using the optimum cell densities determined above for each species selected, experiments will be conducted to determine the influence of varying light cycles and temperatures for the purpose of long term stability and product life issues. These factors are critical in design of the product itself as well as in the development of packaging and shipping containers to maintain a viable, dense culture of dinoflagellates.

Task B-2. Media rejuvenation/fouling experiments- Requirements for long term maintenance will necessarily address the issue of rejuvenation and fouling. Questions which must be answered include: How long can the culture be left unattended (assuming adequate CO2) without the depletion of nutrients? Do the organisms degrade their own environment with metabolites or decay products? Answers to these types of questions will be critical not only to culturing these organisms by PSI but also for the consumer. These issues will be addressed in the maintenance instructions packaged with the product (Appendix A).

Task B-3. Mixed cultures- Experiments will be conducted to determine if mixed cultures, i.e. more than one species present in a single culture medium, will provide a more favorable product, either in terms of intensity or duration of light, or longer term stability of the culture. Since the different species of dinoflagellate respond differently to stimulation, it may be that a NIGHT COLONY consisting of a mixture of organisms will prove more optimal. Each of the selected organisms will be cultured in combination with the others and observations made as to light intensity, duration, long-term viability of the culture and growth rates. The dinoflagellates are plants and should therefore coexist without problem.

Task C-1. Optimization of artificial sea water and supplements- Several commercially available sea waters are under investigation for use in the NIGHT COLONY. Examples of these include Guillard F/2 Supplement® and Instant Ocean®. However, it has been found that the dinoflagellates require additional supplements such as manganese chloride, ferrous chloride, biotin and B12 vitamin. In addition to the commercial sea waters, PSI intends to explore the use of several different natural occurring sea waters, including dilutions of water from the Great Salt Lake. We will do extensive testing to develop formulations for optimization of growth, and bioluminescence emission. Additives to increase the viscosity of the medium will be explored for purposes of forming a homogeneous suspension of the organisms. An example of a material which may be considered is polyethylene glycol, a non-toxic, inert polymer. The optimum media will be proprietary and possibly patentable.

Task C-2. Optimization of container configuration/materials- It has been our experience that the dinoflagellates appear to reproduce more quickly in larger gallon jugs rather than small flasks. This will be explored with each organism. Choice of materials may also influence the reproduction rates. For example, do various plastics influence the growth differently than glass?

Task D-1. Determination of CO2 requirements- Dinoflagellates use CO2 and expire O2 as part of their photosynthetic processes. The rate per cell will determine the surface area and permeability of the materials used in the culture. Experiments will be performed to determine gas exchange requirements and limits. For example, is it possible to pressurize NIGHT COLONY with CO2 for shipping and storage without hurting the organisms? The CO_2, HCO_3, pH equilibria will be carefully studied. The respiration of the organisms will also be studied, including the feasibility of a totally sealed system (an Ecosphere or Biosphere)[4].

Task D-2. Identification of appropriate gas transfer membranes-When CO2 requirements are established for particular cell densities, various materials will be evaluated for permeation rates of CO2, O2, and water.. Examples of materials which may be evaluated include siloxane and urethane polymers. The manufacturability of these polymers in the desired configuration will be evaluated.

Task D-3. Design and fabrication of container- Following selection of the appropriate material, ultimate designs and configurations for NIGHT COLONY will be developed. Some of this work may be subcontracted to a local design engineering firm. Rotatable disks and animal shapes have been considered thus far. If a rotatable disk shape is used, the addition of neutral buoyancy particles will be explored which will stimulate the dinoflagellates to luminesce when the disk is rotated.

Task E-1. Stress testing of cultures for limits of viability- Experiments will be conducted to determine limits of viability which will be critical in shipping and shelf life.

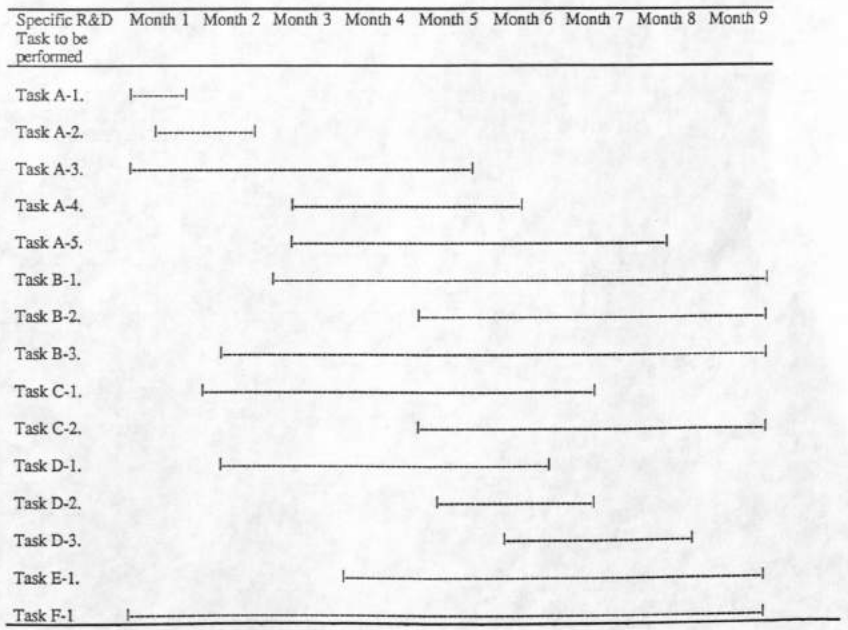
Parameters which will be tested include temperature, partial and total darkness, and CO2 storage capacity.

Task F-1 We will continue the development of supporting teaching and educational materials which will be available as product accessories for inquisitive customers and for teachers and schools.

It is emphasized that the over-all objectives of the tasks described above, including testing to be carried out, is to establish feasibility of growing dinoflagellates and designing and building a NIGHT COLONY product. The proposed project will be followed by Phase II and Phase III studies, which are outside the scope of this proposed Phase I effort.

The proposed schedule and time requirements for carrying out each of the identified tasks are summarized in Table 1.

TABLE I. PROPOSED SCHEDULE FOR R&D WORK



POTENTIAL PROBLEMS

No serious or insurmountable difficulties are anticipated. Areas of potential concern are listed below with appropriate discussion and possible solutions.

1. Algal contamination of culture- It may be possible, if careful handling and transfer techniques are not used, that other organisms such as algae may contaminate the culture media. It may be necessary, if this is determined to be a problem to suppress their growth by composition adjustment of the culture media. However, it is likely that the unique nutritional requirements of dinoflagellates will themselves act as inhibitors. The media used for reproduction and maintenance of dinoflagellates are inhibitory to many other microorganisms.

2. Safety testing- Because of the high safety expected of toys and related products, safety and toxicity testing of the media and of the organisms will be carried out. Nelson Laboratories, located in Salt Lake City, is a contract laboratory for toxicity testing and will be used to test product safety. No allergic or toxic indications have been reported for these dinoflagellates.

3. Cycle Maintenance- Dinoflagellates respond to light and dark cycles. The cultures and products will have their light-dark cycles adjusted to synchronize with the end user's needs and expectations.

FACILITIES

Most of the work on this project will be carried out by PSI, Inc. in its laboratories located at Northgate Business Center, 825 No. 300 West, Suite 145 in Salt Lake City and affiliated laboratories at the University of Utah. PSI is a member of the Center of Biopolymers at Interfaces at the University of Utah, one of the State's Centers of Excellence (Appendix D). PSI will be a key corporate participant in the University's proposed Center for Integrated Science Education (CISE). PSI has a Technology Transfer agreement with the University of Utah Research Foundation (Appendix D). Our laboratories are equipped to perform the necessary biological, chemical, engineering, and evaluation studies. Sophisticated equipment which may be required may be used by our team on a time-sharing cooperative basis at the University of Utah.

CONSULTANTS AND SUBCONTRACTS

PSI anticipates utilizing other bioluminescence and marine biology experts for consultation, in particular, Professor W.G. Hastings, Department of Biology, Harvard University. Dr. Hastings is internationally recognized for his work on bacteria and dinoflagellate (photoalgae) bioluminescence. He and his co workers have worked out the basic chemistry of dinoflagellate bioluminescence. He has applied these systems for the study of biological circadian rhythms, and is published widely on the subject.

Dr. Trish Stoddart, Professor of Educational Studies at the University of Utah, and Rene Stofflett, Ph.D. candidate in Educational Studies, will advise and assist in the development and design of the product for the elementary school age group.

PSI will involve an outside design engineering individual/firm in the development of the final configuration of NIGHT COLONY on a subcontract basis.

PSI expects to contract the design and production of packaging and shipping containers to the Utah Paper Box Co., Salt Lake City.

KEY PERSONNEL

Suzanne Winters, Ph.D., Vice President, Product Development, will serve as Project Manager. Dr. Winters has worked in technology development for the past 4 years with CardioPulmonics, Inc. as Director of Membrane Technology, where she was responsible for materials development of a gas transfer membrane. She recently joined Protein Solutions, Inc. where she is Vice President for Product Development and is working on bioluminescence-based technologies. She holds a Bachelors Degree in Zoology and and Ph.D. in Pharmaceutics from the University of Utah (Appendix C).

Joseph D. Andrade, Ph.D. Chairman, Department of Bioengineering, University of Utah, and President of Protein Solutions, Inc., has been studying bioluminescence for over 3 years, primarily for science motivation and education. He has taught high school general science,chemistry, and biology and has assisted in elementary school science instruction on a regular basis for a 3 year period. He has a strong interest in integrated science education. Dr. Andrade has been working on biomaterials and biotechnology problems for the past 25 years (Appendix C).

CURRENT AND PENDING SUPPORT & FOLLOW ON FUNDING

PSI has no current outside support. However,a proposal is in preparation for submission to the National Science Foundation SBIR program. This proposal will be submitted in June. If funded, the Phase 1 NSF application would be initiated in early 1992. Phase 2 Federal SBIR funds would be requested at the appropriate time.

PSI also expects to submit Federal SBIR grants to NASA and the Environmental Protection Agency for continued R&D in areas related to NIGHT LIFE educational materials.

In addition to the anticipated Federal SBIR funds, PSI has already invested $50,000 (provided by its founders and major stock holders) in the initial studies and product development. PSI expects to continue funding the project at the same level. Thus this is really a $100,000 project, of which $50,000 in State SBIP support is requested. PSI is now discussing major equity investments by a number of local investors and investment groups.

PRE-EXISTING RELEVANT PATENTS AND INVENTIONS

PSI has already developed several proprietary culture media formulations, used with existing commercial artificial sea waters. PSI expects to patent a unique technology, now under development, which will permit the product to be shipped and maintained with minimal damage to the organisms. This technology should have market potential in aquaculture and biotechnology. PSI would then license the technology for suitable royalties to one or more companies involved in aquaculture and/or biotechnology.

FINANCIAL/LEGAL INFORMATION

PSI's lawyer is Mr. Jay Sheen with Moyle and Draper, Salt Lake (phone (801) 521-0250). PSI's accounting firm is Peterson, Silar, and Stevenson, P.C., Salt Lake City (phone (801) 328-2727).

MARKET ASSESSMENT

The initial market for PSI's bioluminescence products are children, their parents and their teachers. With the recognition that our educational system must increase the emphasis on science and mathematics, a dramatically increased demand for products which have a significant educational component will be seen during the 90's[1,3].

Although PSI has not had the resources to do a complete market analysis, it is clear that the market is large. For example there have been over ten million Ant Farms sold by its original inventor and developer, Milton Levine. There at least two other manufacturers of ant farms who have a significant fraction of the market. The estimated sales volume for these ant farms and accessories is $25 million.

Market assessments performed by Toy Manufacturers of America estimates sales of Activity Toys, which include educational toys, for 1989 to be $1.4 billion. The segment of this market specifically addressing educational and scientific toys was $45 million in 1989[5].

A key target for PSI's marketing emphasis will be museum and science center gift shops. According to a recent ASTC Science Center Survey[6], museum stores represent a substantial source of revenue in some institutions. Numerous articles are appearing in journals across the nation from flight magazines to Business Week describing museum shopping and the "chic" emporiums for unique gifts[7]. Smithsonian Museum Shops stock merchandise to sell with the philosophy that *every* item must reflect each museum's collection and special exhibits and be educational and salable.

PSI has been in contact with The Children's Museum of Utah and has agreed to work with them to erect a display dedicated to bioluminescence. The museum has indicated a strong interest for the exhibit as well as items for the gift shop (Appendix D). A joint proposal to NSF's Informal Science Education Program will be submitted in August, 1991.

PSI is now obtaining information as to the total volume and subcategories of sales in museum gift shops nationally. Additionally, PSI is investigating the total budget and budget subcategories for science-related supplies and equipment in elementary, junior high and high schools throughout the nation. The National Science Teachers Association wields tremendous buying power and hosts shows and exhibitions for manufacturers of science-related products. Other groups to which ads and mailings can be specifically targeted include the School Science and Mathematics Association, The National Education Association, and related groups.

PSI's competitors would be manufacturers of scientific educational toys. However, no other bioluminescent toys or science kits are currently on the market.

It is expected that PSI"s bioluminescence products will retail in the price range from roughly $15 to $100.

In summary, the educational toy market is already very large and is likely to be expanding in the next decade, based on the recognition of need for a renewed emphasis in science education. The ability to produce bright light in various patterns and under various degrees of stimulation will attract potential buyers to PSI's bioluminescent products. That attraction and novelty, coupled with the educational potential of the product, should guarantee a strong and loyal clientele.

MARKETING PLAN

It is PSI's intention to develop and manufacture NIGHT COLONY in Salt Lake City as the first of a line of educational toys based on bioluminescence. NIGHT COLONY falls into PSI's division of liquid products. Follow-on products in the dry category involve technology consisting of a powder containing the protein and the chemicals required for efficient bioluminescence, derived from a marine crustacean. When the powder is wet with water, the enzyme is reactivated and a brilliant bioluminescence results (see Appendix B). This dry technology lends itself to the development of products such as paper, inks and paint. The market for these types of toys was over $200 million in 1989. A NIGHT FARM, a bioluminescent earthworm farm, analogous to the ant farm, is under consideration, as well as a more sophisticated teaching toy, NIGHT LAB, a complete laboratory with accessories for more in-depth exploration of the phenomenon of bioluminescence.

Initial test marketing of all of these products will be in local specialty shops such as Gregory's Toys and Adventures. Managers of these businesses have expressed significant and enthusiastic interest in these products (Appendix D) Direct marketing via the publications of the National Science Teachers Association and related groups will be used at a later date.

The second market segment on which PSI will concentrate is the museum shops[6,7]. As mentioned previously, The Children's Museum of Utah has shown interest in a bioluminescence exhibit with bioluminescent products in their gift shop (Appendix D).

PSI intends to have its first product, NIGHT COLONY, on the market by mid 1992, with extensive marketing and distribution planned to follow. The second product, NIGHT FARM, should be launched by mid 1993.

PRODUCT PROTECTION

These products will incorporate a wide range of proprietary innovations and components, including the design of the container, the selection of highly bioluminescent strains, the particular nutrient compositions, and the development of various chemical enhancers for bioluminescence. The particular formulations, designs, and innovations are all patentable and proprietary. PSI expects to have a significant technological lead over any competition which may attempt to copy its creative products. That technological lead will be protected by patents and by trade secrets.

UTAH OPERATIONS

Anticipated growth of the new business resulting from the development of NIGHT LIFE TOYS is summarized as follows:

ANTICIPATED BUSINESS GROWTH	1991	1992	1993	1994	1995
Utah employees:					
Scientists	1	2	3	5	10
Technicians	1	3	7	12	25
Sales and Admin	1	1	4	6	15
Payroll: (annual) in $1,000	50	200	500	1,500	2,000
Sales Forecast: (annual) in $1,000	0	300	800	2,000	5,000

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5. *Toy Industry Fact Book: 1990-91*, Toy Manufacturers of America, 1991.
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8. State Office of Education; Bruce Griffin and LaMar Allred: Davis School District; David Steele and LaMont Jensen: Salt Lake School District; Ken Powell.
9. Richard Morris, The Children's Museum of Utah.
10. D. Anderson, Woods Hole, Mass; D. Brand, Univ. of Miami; B. Prezelin, Univ. of California Santa Barbara; R. Guillard, Boothbay Harbor, Maine.

BUDGET JUSTIFICATION

Personnel: S. Winters is employed by Protein Solutions, Inc. She will be working on this project at least half time. Four person months are budgeted for the nine month duration of the project. An additional full-time technician will be working closely under Dr. Winter's supervision. He/she is fully budgeted.

Permanent Equipment: Incubators are required in order to have a controlled, reliable environment for the extensive culture studies proposed. It is far cheaper to control the environment in an incubator than to contract for highly sophisticated research space with expensive environmental controls. Partial support of a computer is also budgeted for computerized design, data analysis, and record keeping.

The consultants category is for advice and services of experts in dinoflagellate culture. This includes Dr. Woody Hastings, Department of Biology, Harvard University, and a number of other individuals with whom we have been in contact[10]. Their compensation will be limited to the Utah SBIP maximum rate of $320 per day. Their travel is budgeted separately. We anticipate sharing the travel expenses of these consultants with the appropriate components of the University of Utah, as they are all highly respected biologists and biotechnologists and would probably give lectures on campus as well.

$4,000 is budgeted for engineering and design services for prototype product development and for consultants.

We have also budgeted $5,000 for membership in the University of Utah Center for Biopolymers at Interfaces. This membership provides us with a unique range of facilities and expertise at very minimal cost.

Travel funds are also requested to permit the project director to attend at least one meeting of the National Science Teachers Association and of the Association of Science and Technology Centers. Both of these groups have industrial exhibitions, and this travel is essential in developing an appropriate marketing analysis and marketing plan. Travel is also budgeted for the consultants.

Indirect Costs are 16% of total costs: The indirect costs are primarily for the support of an office manager, for accounting and legal services, and for space rental.

APPENDICES

A. NIGHT-COLONY Owners Manual -- Draft

B. Dry Product Demonstration

C. VITAS

D. Letters

E. Business Plan (available on request -- not included due to page limitation).

DRAFT

NIGHT-COLONY
Owner's Manual

Introduction

Congratulations! You are now the proud owner of NIGHT-COLONY -- a kit containing bioluminescent NIGHT-LIFE living cells. These sea pets will "talk" to you by giving off blue light in the dark. They have to work during the day -- to make their food -- and so they only "play" at night. They normally live at the surface of the ocean, where they absorb carbon dioxide from the air. They also absorb sunlight to get energy. They make their own food -- by photosynthesis.

You have two different NIGHT-LIFE creatures -- they are both a little hard to see (they're only about the size of the dot on the letter i!). But you can see them by the light they give off.

The round, flat one could be called "Fire-Cell" (its scientific name is pyrocystis noctiluca). The other, the thin one, is called "Night Moon", because it has a moon shape (its real name is pyrocystis lunula).

Set Up

Your NIGHT-LIFE is already in its NIGHT COLONY home -- a circular container with a cap on the top and full of seawater. The circular house is already mounted in a rectangular holder. Take the holder out of the cardboard packing and set it on a table or desk -- put it near a window where it can get bright light -- or put it where there is a strong room light or desk lamp. As these small creatures multiply and grow they will entertain and educate you with their light displays.

Although you will be able to see a little light from your Night Colony right away, your night creatures are tired from being cooped up and being shipped, and they will need a week or so to regain their strength, so be patient with them.

Your sea critters don't like very hot or very cold temperatures -- so be sure to keep them in a room where the temperature stays between 10 to 20°C (50-70°F), otherwise they get very unhappy and may even die!

Your NIGHT COLONY is shipped full of seawater -- that protects the critters from shaking and turbulence during shipping. We have also carbonated the water to give them the carbon dioxide they need.

Very slowly unscrew the top part of the cap -- as you start to unscrew it, you'll notice carbon dioxide bubbles forming -- just as when you open a carbonated soft drink. Open the cap slowly, but do not take it off! This will let the sea critters "breath" fresh carbon dioxide from the air. The cap has two parts -- the upper part is used to permit air to move in and out of your NIGHT COLONY.

The lower part of the cap can be completely unscrewed -- do that now. Pour about 1/3 of the solution out into a drain. Now screw the cap back. Be sure the top part of the cap is loose to let air in. By pouring out some of the water you've made more room for NIGHT COLONY and

for the air it needs. Now just leave it alone -- in the light -- so it can get better and healthy.

At night, around dinner time, cover it with the black cardboard cover in your NIGHT COLONY box. When you get up in the morning, take the black cover off, so your critters can absorb light. Remember, if their room doesn't get bright light from the window, then turn on the room light for them.

They use light, carbon dioxide, and minerals in the seawater to make their food and to multiply. Your sea critters are algae -- plants -- and use photosynthesis to live. They are really very important because they help produce an environment on planet Earth which allows all of its other life forms to live and multiply. When you breathe, you take in oxygen and produce carbon dioxide as a waste product. Your NIGHT LIFE uses carbon dioxide to make its food and actually produces oxygen, which goes up into the atmosphere, and which you breathe. So in a way, your NIGHT LIFE creatures are responsible for the oxygen which you need to breathe. So you can see they are really pretty helpful and useful guys. Take good care of them.

Don't try to talk with them or experiment with them during the day -- they aren't interested -- they're too busy working and making food (anyway, that's when you're in school and doing your homework!). They don't like to light up during the day so even if you turn off all the lights and put them in a dark room, they won't produce any light for you. They prefer to light up at night. So the only time you can really play with them is at night.

Around dinner time, put them to "bed" by putting the black cover over them. Then, when you're finished with your homework, just before bed, when it's dark outside, and with the room lights off -- and it's REALLY DARK -- carefully remove the black cover -- and you'll "see" your NIGHT LIFE! Tap gently on their house and they'll talk to you by giving off light! Don't get them too tired all at once. Cover them back up -- and you can both go to bed! (Remember to uncover them in the morning!).

Care and Feeding:

Your NIGHT-COLONY needs very little care. It needs:

- light (during the day).
- darkness (keep it covered at night except when you're looking at it).
- moderate temperatures (10-20°C, 50-70°F).
- gentle conditions -- don't shake them or stimulate them too much. If they get too tired, they might die.

As your sea critters live and multiply, they use up the nutrients in the seawater -- also, they might multiply too much and overpopulate their home. So -- every 3 weeks you'll need to thin out the population and provide fresh seawater. Here's what you need to do:

Find the Food Packets in your NIGHT COLONY box. Put 8 ounces (250 ml.) of distilled water in a cup -- you'll need to use a measuring cup to do this -- Mom or Dad can help you. You can get distilled water in any supermarket or at the same store where you bought NIGHT COLONY. DO

NOT USE purified water or any other type -- use only water which says "DISTILLED"! Now add the powder in one packet of NIGHT COLONY FOOD to the cup of distilled water and stir Put the Food Solution next to your NIGHT COLONY for a few hours -- this lets it adjust to the temperature in the room. Then pour out about half the water in your NIGHT COLONY (open the cap and unscrew it completely, then gently pour out -- into a drain or other container -- about half of the NIGHT COLONY water). Now pour back into your NIGHT COLONY the fresh Food Solution you made. Do this gently and slowly.

Now put the cap back on tightly. Be sure the upper part of the cap is slightly open to let air in. There -- that's it. You've now "fed" your NIGHT COLONY with fresh sea water. Remember, you'll need to do this every 3 weeks.

There's a coupon in your NIGHT COLONY box to order more Food Solution when you need it (10 packs of food are in the box -- that's good for 30 weeks -- over 6 months). You can also get more food at the store where you bought NIGHT COLONY.

Experiments -- Playing with NIGHT COLONY*

You are a scientific pioneer! Your NIGHT COLONY can be used for scientific studies and observations. And you can contribute to learning more about these important organisms. The light given off by your night critters is BIOLUMINESCENCE -- a word which just means light produced by living organisms.

Your bioluminescent creatures are part of a large family known as dinoflagellates (nope -- they're not related to dinosaurs! Dinosaurs are very BIG, dinoflagellates are very small). Dinoflagellates and their cousins, the diatoms, are very important to life on Earth. They live on the surface of the oceans, where they take in carbon dioxide and give off oxygen (remember -- they're plants!). They are a major producer of oxygen -- the oxygen you need to breath!

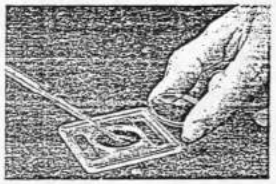
Want to see what they look like? Get a magnifying glass -- the best one to use magnifies about 10 times. You can get one from the same store where you bought NIGHT COLONY -- or use the order coupon attached to this sheet.

A few of your dinos will be attached on the wall of their plastic container -- right where the sea water meets the air. You've already seen that those guys can light up very brightly. So find some and try to look at them with the magnifying glass. You'll see them, but you can't see very much detail. That's because your dinos are very small.

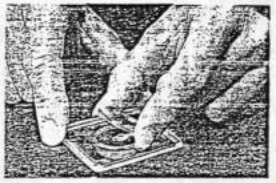
* Protein Solutions, Inc. is now developing NIGHT-LAB, a complete set of science kits and instructions for science projects and studies involving bioluminescence.

Want to see more? You'll need a microscope or a slide projector. In your NIGHT COLONY kit is a 35 mm NIGHT SLIDE, designed to hold some water. It looks like this:

1. The Deep-Well Slide is easy to use. All you do is remove the cover.



2. add the specimen to be projected, and



3. replace the cover. The slide is ready to project from a standard 35-mm slide projector. When using liquid specimens overfill the well to eliminate trapped air bubbles.

With your fingernail, a coin, or a paper-clip, remove the little cap in the center of NIGHT-SLIDE. Now take the PIPET, packed in your NIGHT COLONY box. Practice using the pipet with a little tap water. Learn how to pick up and release 1/2 ml. of water (see the markings on the pipet and the instructions on it).

Now empty out the tap water in the pipet. Then unscrew the cap on the top of your NIGHT-COLONY, and carefully remove 1/2 ml of the water with the pipet. Screw the cap back on your NIGHT COLONY. Release the liquid in the pipet into the center of your NIGHT-SLIDE. The little NIGHT SLIDE container should now be completely filled. Now gently put the NIGHT-SLIDE cap back on, press it down to seal it. With a tissue or small piece of toilet paper, clean up any loose liquid and be sure the outside of your NIGHT-SLIDE is completely dry. If it leaks, then seal the cap more carefully. If you can't seal it, ask someone to help you. If it still doesn't seal, you'll need a new NIGHT-SLIDE.

With your filled and sealed NIGHT-SLIDE, you are now ready to observe your Dinos --(finally!)

Use a microscope or a slide projector. Use your NIGHT-SLIDE as a specimen or sample for the microscope. Focus and presto! -- you'll see your sea critters! If you have any trouble, check the instructions with the microscope.

To use a slide projector, first set it up (be sure one of your parents or your teacher is there to help). Again, check that your filled NIGHT-SLIDE is dry and sealed -- otherwise you might damage the slide projector! Gently put the NIGHT-SLIDE in the projector, turn on the projector light, and focus. There they are! Depending on the lens in your projector and how far away is the screen, your dinos should be about 1 cm or about 1/2 inch in diameter on the screen. Now you can really see them!

See the big round one -- the pyrocystis noctiluca -- he looks like a big sphere. And the moon-shaped one -- the pyrocystis lunula. You may also see some of the cells dividing. Turn off the projector! If you leave it on too long, you'll cook your dinos -- and you might even

melt the NIGHT-SLIDE. Sorry, you probably can't do this in the dark and see the individual cells bioluminesce.

When you're finished, open up and wash your NIGHT-SLIDE in tap water, dry, and put away for next time -- and put away the microscope or slide projector.

Your NIGHT COLONY can rotate -- it also has some coral on the bottom, which moves through the water when you rotate it. At night, while watching NIGHT COLONY in action, rotate it and see what happens. The waves produced by the rotation motion, and the movement of the sand, cause your NIGHT LIFE to bioluminesce. You already know they light up when you tap their house - or even if you jump on the floor. Why don't you experiment and see what tapping force you need to get light?

Lots more experiments are described in the book, Observing Bioluminescent Dinoflagellates, which you can order using the order form attached to this sheet.

Trouble Shooting

1. Your NIGHT COLONY kit arrived with little or no water in it. Oops -- it leaked out! Someone may have cracked or broken the container during shipment. Fill out the coupon below and mail the whole box -- with everything in it -- back to us. We'll send you a new NIGHT COLONY.

2. You can't open the top cap to let air in. Look at the drawing and picture below carefully -- and follow the instructions. If you still can't make it work, get an adult to help you. If you still can't have your parent or teacher call us at 1-800-XXX-YYYY and we'll work it out.

3. Check their temperature. There's a little liquid crystal temperature detector on the left front of your NIGHT COLONY -- if it reads too high, you are in trouble! (Actually, you're not, but your dinos are). They don't like high temperature. Move them to a room or place where the temperature is better (see Set Up instructions), then do what it says below (Trouble Shoot #4).

4. Everything seems okay, but there's no light. Maybe your dinos are just too tired from all their travels. Leave them alone for about 2 weeks -- be sure they get plenty of light during the day. They may just need some time alone to rest and get better.

5. The container looks polluted and yucky. You've grown some other creatures. There are all kinds of algae and they can grow, too. We sell a cleaning kit you may want to buy and try -- or we'll replace the whole NIGHT COLONY for you for 1/2 price if you mail in your polluted NIGHT COLONY. So we can study it -- just order NIGHT COLONY replacement on the Order Form.

-Appendix B-

NIGHT PAINT:*
An Introduction to PSI's Dry Technology

Japanese soldiers during WWII were issued a handful of nearly transparent particles about 1 to 2 millimeters in diameter. These were dried out crustaceans of the species cypridina hilgendorfi, a small crustacean which was plentiful at that time in the Sea of Japan. This bioluminescent crustacean, when stimulated or agitated in the water, secretes chemicals through two or more sets of glands which, when they mix in the nearby water, produce a bright metallic blue light. These creatures are bioluminescent. They not only occur in the Sea of Japan, but throughout the Caribbean and in many other regions of the world.

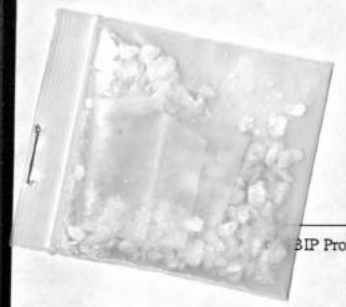
These particular bioluminescent organisms have the unique property that when they are dried out and ground up into a fine powder, bright light is emitted when they are simply wet. The water reactivates the enzyme and the biochemical process which produces bioluminescence.

When the sailors needed a little bit of light, perhaps to read a map by without attracting attention, they would simply take some of the powder and wet it in the palm of their hand.

A packet of this pre-ground crustacean is attached below. It is the brown, fishy smelling powder in the inner packet. The large blue material in the outer pocket is simply a drying agent. Take the inner packet with you into a dark room, a bathroom or closet with the door closed and the lights out will be adequate. Wait a few seconds for your eyes to adapt to the dark, then wet your finger with a little tap water and touch your finger to the powder in the packet. You should see a bright blue fluorescent looking glow. This is *not* fluorescence or phosphorescence, but *bioluminescence*, chemical light whose ingredients where produced by a living organism.

Continue to play. As you touch the powder with your wet finger, look at your finger, touch the powder to your hand, to your wrist, to your forehead. If you have youngsters nearby, they will be delighted, excited, and very curious. That is what PSI's bioluminescent educational products are all about: motivation, curiosity, and interest.

PSI expects to develop a variety of bioluminescent-based powder, ink, and paint systems for children's toys and novelties and for the educational products market.



*SBIP Proposal -- for information only.

Joseph Andrade
VITA Summary

Joe Andrade, Chairman of the University of Utah Department of Bioengineering, was born in Hayward, California on July 13, 1941. He received a B.Sc. degree in Materials Science and Engineering from San Jose State University in 1965 and a Ph.D. on Metallurgy and Materials Science from the University of Denver in 1969.

From 1969 to the present he has been on the faculty of the University of Utah. Joe served as Dean of the College of Engineering from 1983 to 1987. He has over 150 publications and has edited 5 books in the area of materials in medicine, biocompatability, and surface chemistry. He is Professor of Bioengineering, Materials Science and Engineering, and Pharmaceutics. During his 22 years at the University of Utah he has directed a large interdisciplinary research program averaging roughly $300,000/year in external funds. His 40 graduate students have positions in academia, industry, and government.

In 1981, together with Sung Wan Kim and a group of former students and coworkers, he founded Biomaterials International (Albion Instruments), a manufacturer of medical anesthesia monitoring equipment. Albion Instruments has annual sales (1989) of about $3,000,000 and employs 55 people in a site at the Salt Lake International Center. The firm is now Ohmeda-Salt Lake.

In January 1988, together with Peter Gerity and Jim McRea, Joe founded Protein Solutions, Inc. (PSI), a biotechnology firm focused on applying proteins to a wide range on consumer products and bioluminescent products for the toy and education markets.

Joe holds 3 patents with 4 applications pending. In 1983 he co-founded the Center for Biopolymers at Interfaces, a University/State/Industry consortium which now has 24 corporate members and sensors. The Center is directed by Dr. Karin Caldwell of the University of Utah. He is now forming the Center for Integrated Science Education (CISE), a proposed new State Center of Excellence.

Joe Andrade has a long and strong track record of working with industry. He has lectured at -- and consulted for -- numerous biomedical and health care companies and has been awarded many industrial fellowships and research contracts.

He has worked closely with the State of Utah Department of Community and Economic Development, with the Board of Regents, and with the Utah Legislature in fostering University-Industry interactions and economic development.

He has an international reputation in the medical device/biomaterials research and industrial communities.

GREGORY'S
TOYS & ADVENTURES

March 15, 1991

Joseph D. Andrade, President
Protein Solutions, Inc.
6009 Highland Drive
Salt Lake City, Utah 84121

Dear Joe:

I was pleased to learn that Protein Solutions, Inc. is proceeding with its plans to develop a range of children's discovery products based on bioluminescence. I have reviewed your description of the proposed NIGHT-COLONY product and feel that it would be very popular with our customers.

As you know, Gregory's Toys and Adventures provides toys and related products for a broad audience. We also have a very significant educational component in our stores. As I am sure you are aware, the science area is one that continues to draw more interest from consumers as well as educators. It is a real growth category. Our clientele prefer products which are safe, sound, educational, and entertaining. Your proposed NIGHT-COLONY product meets all of these requirements.

We look forward to participating in the initial test marketing of NIGHT-COLONY, which I understand may be available as early as late in 1991, hopefully in time for the pre-Christmas season.

We will be happy to display and to feature your product in our local and out of state stores. We look forward to your continued progress.

Sincerely,

Greg A. Gohlinghorst
President, Gregory's Toys & Adventures

CORPORATE HEADQUARTERS
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