APPENDIX C

National Science Foundation
Small Business Innovation Research Program

PROJECT SUMMARY

NSF AWARD NO.

NAME OF FIRM	Protein Solutions, Inc. (PSI)
ADDRESS	350 West 800 North, Suite 218, Salt Lake City, Utah 84103
PRINICPAL INVESTIGATOR (NAME AND TITLE)	R. Scheer, Research Scientist
TITLE OF PROJECT	Sensing Nicotine in Secondary Smoke Using Bioluminescence.
TOPIC TITLE	Bioengineering & Environmental Systems
TOPIC NUMBER AND SUBTOPIC LETTER	24C

This Phase I SBIR Project is to develop a nicotine sensor to facilitate behavior modification of active smokers.

Smoking continues to be a major health and environmental problem in this country. We propose to assess the feasibility of developing a novel biosensor utilizing bioluminescent bacteria. The biochemical pathways in the bacteria involved in bioluminescence are particularly sensitive to various toxins. Indeed, the inhibition of bioluminescence in certain bacteria is the basis of a widely used assay and is even found in the United States Pharmacopea, USP.

A unique strain of bioluminescent bacteria with a particularly strong sensitivity to nicotine will be cultured and maintained viable in a heart shaped plastic container containing adhesive on one side. The heart shaped container glows brightly and is very obvious in the dark. This plastic container is simply worn, like a self adhesive name tag, by the non-smoking spouse or room mate of a smoker. The nicotine and other toxic products in the secondary smoke permeate the heart shaped container and over a period of days to weeks cause the little glowing heart to progressively darken. Words are not necessary. The smoker knows that his/her actions has caused the death of the glowing bacteria.

The sensor will not be particularly quantitative or ultra sensitive. It is not designed for analytical or diagnostic purposes. It is simply designed for psychological effect. It is a behavior modification device.

We are convinced that its wide spread application would have a significant and profound effect in decreasing the incidence of smoking.

Potential Commercial Applications of the Research

A "behavior modification" nicotine sensor would have widespread application and would be distributed through many different outlets. It could be purchased in quantity and given away in large volumes. It is expected that it would be available in pharmacies and in all outlets where cigarettes and other tobacco products are distributed. We frankly anticipate a very large market.

KEY WORDS TO IDENTIFY RESEARCH OR TECHNOLOGY (8 MAXIMUM)

Nicotine, Smoking, Behavior Modification, Compliance, Bioluminescence.

CERTIFICATION PAGE

PPENDIX B (continued)

Certification for Principal Investigators and Co-Principal Investigators:

I certify to the best of my knowledge that:
(1) the statements herein (excluding scientific hypotheses and scientific opinions) are true and complete, and
(2) the text and graphics herein as well as any accompanying publications or other documents, unless otherwise indicated, are the original work of the signatories or individuals working under their supervision. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if an award is made as a result of this application.

I understand that the willful provision of false information or concealing a material fact in this proposal or any other communication submitted to NSF is a criminal offense (U.S. Code, Title 18, Section 1001).

Name (Typed)	Signature	Date
PI/PD R. Scheer	Robert J. Scheer	6/11/94
Co-PI/PD		
Co-PI/PD		
Co-PI/PD		

Certification for Authorized Company Representative

By signing and submitting this proposal, the individual applicant or the authorized official of the applicant institution is: (1) certifying that statements made herein are true and complete to the best of his/her knowledge; and (2) agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this application. Further, the applicant is hereby providing certifications regarding Federal debt status, debarment and suspension, drug-free workplace, and lobbying activities (see below), as set forth in Grant Proposal Guide (GPM), NSF 94-02. Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense (U.S. Code, Title 18, Section 1001).

Debt and Debarment Certification (If answer "yes" to either, please provide explanation.)

s the organization delinquent on any Federal debt? Yes ___ No__
s the organization or its principals presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department of agency? Yes ___ No__

Certification Regarding Lobbying

This certification is required for an award of a Federal contract, grant, or cooperative agreement exceeding $100, 000 and for an award of a Federal loan of a commitment providing for the United States to insure or guarantee a loan exceeding $150,000.

Certification for Contracts, Grants, Loans and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.
(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress or any employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.
(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

AUTHORIZED COMPANY REPRESENTATIVE	SIGNATURE	DATE
NAME/TITLE (TYPED) J.D. Andrade		6/11/94

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TABLE OF CONTENTS

D. IDENTIFICATION AND SIGNIFICANCE OF THE OPPORTUNITY

There is growing interest in the development and application of novel technologies which can decrease the costs of health care (10). A major contributor to health problems in this country is poor health practices. Among those activities which have the strongest correlation with poor long term health outcome is smoking (1).

There is general unanimity in the nation, with the possible exception of several tobacco company presidents, that there are considerable human and societal costs to continued widespread use of tobacco (1). Roughly 25% of the U.S. population still smokes. Tobacco use is the largest single preventable cause of death in the United States. It is responsible for over 85% of lung cancers and is the major factor in over 400,000 preventable deaths per year. That's larger than poor dietary or physical activity practices, alcohol consumption, fire arms, and motor vehicle industries.

The growing concern is the fact that the immediate family and other social acquaintances of smokers are also at risk. Environmental tobacco smoke (ETS) is also considered a health risk, although admittedly a much lower risk than direct smoking is. ETS is a complex mixture of thousands of compounds, many of which are carcinogenic or otherwise toxic. The most well known and specific of these is nicotine (2-4). There

have been measurements of the personal exposure to ambient nicotine inhaled by passive smoking in ETS environments in the range of 1-40 micrograms per hour. Daily exposure can be much higher. Nicotine is a very active and potent biochemical and is rapidly metabolized to a wide range of metabolites -- all of which can be detected in urine and other body fluids (6-8).

In spite of the major interest in inducing smokers to quite smoking or to smoke much less, and in minimizing environmental tobacco smoke, the health risks, and discomfort associated with smoke in the environment, there has been almost no application of behavioral modification or compliance enhancing technologies. In the 1992 National Science Foundation Workshop on Improved Delivery and Reduced Costs Of Health Care Through Engineering, it was noted that patient and public education and understanding of health risks is very important and that "technologies are needed to provide periodic or even constant reminders to encourage patients to resist temptation to reactivate bad habits or poor health practices." (10). Physicians and health care professionals rarely have the time to provide the extensive follow up and reminding which is critical in getting patients to stop smoking and to remain non-smokers (11).

Smokers fundamentally enjoy smoking. They not only enjoy the feel of the cigarette in their hands and in their mouth, but they enjoy and indeed become dependent on the biochemical and pharmacologic effects of smoking. They also enjoy the smell and ambiance of tobacco smoke. It is difficult for them to appreciate and to understand the concern which non smokers have with smoke in the environment.

Over the decades we have learned in education circles that one does not get kids or adults to learn by telling them that it's good for them or good for others. They don't learn from being told, and they don't even learn from reading. It is doing and experiencing that provides learning. That is the reason for the growing national interest in hands-on, interactive, process-based science and technology education. The same education strategies and theories hold for health related education. Patients or potential patients cannot be only told and even showing them is often ineffective (9-11). They must experience and learn for themselves. Their first heart attack generally makes learners and believers out of most of them.

Protein Solutions, Inc. is involved in the development of a range of products to enhance hands-on, discovery-based science and technical education. We use bioluminescence, light generated by living organisms, to generate interest, motivation, and enthusiasm in experiential learning. Our experience with these bioluminescence science education tools, with a wide range of audiences in a variety of environments, has convinced us that this same approach can be extremely effective for health related education and indeed for behavior modification.

Imagine a heart-shaped device, a self adhesive patch, which you would wear over your shirt pocket or on your lapel (Figure 1). The device contains a live, viable, non-toxic and harmless strain of marine bioluminescent bacteria. This heart-shaped patch would remain viable for weeks and even months as long as it is not exposed to toxic or other deleterious environments, such as very high or very low temperatures. The bacterial patch glows brightly in the dark as long as it is viable and healthy. Now imagine you live with a smoker. You sense and tolerate the secondary smoke which is ubiquitous in that environment. The bioluminescent bacteria in the patch are a special strain which is particularly sensitive to nicotine, and perhaps to other toxins in secondary tobacco smoke. The glowing heart patch goes black with time as the cumulative nicotine exposure increases and the bioluminescent pathway is shut down.

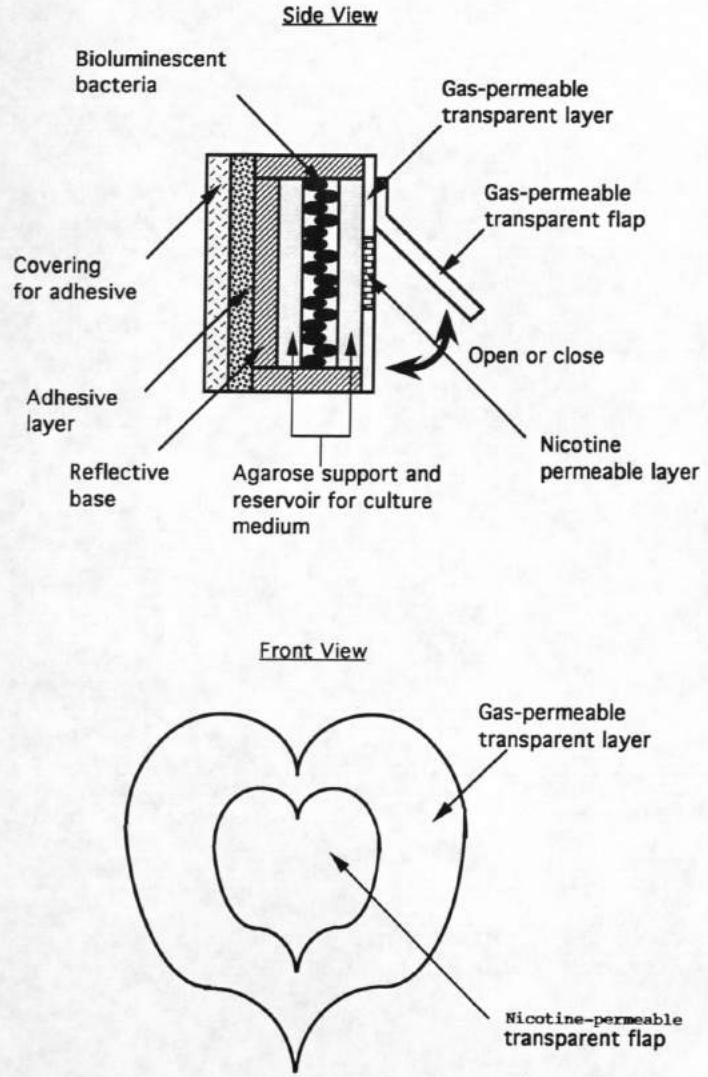

Fig. 1. Preliminary Design for a Nicotine-sensing Behavior Modification Device.

Imagine a social situation where the heart patches are passed out and worn. After the appropriate time, given appropriate levels of secondary smoke, the lights are turned off and the various patches examined. No words need be spoken. That is what we mean by direct experience and direct involvement.

PSI sees the possibility for a whole array of behavior modification sensors based on engineered strains of bioluminescent bacteria (12-26), dinoflagellates (39-42), and other bioluminescent organisms which can be readily maintained in sealed environments for indefinite periods (41,42). We have developed a set of technologies by which we can maintain bioluminescent dinoflagellates, marine phytoplankton, in sealed bag environments for indefinite periods. We have begun to work with bioluminescent bacteria for the past 6 months and have developed a collaboration with the Institute for Biophysics and its Bioluminescence Laboratory in Kraznoyarsk, Russia. This laboratory has over 2,000 strains of marine bioluminescent bacteria, several of which have very high sensitivities to nicotine. Dr. Valentina Kratasyuk has worked extensively on the sensitivity of bacterial bioluminescence to various chemical agents, including nicotine (see Consultants).

A recent National Science Foundation workshop on Cost Effective Health Care Technologies pointed out that technologies and devices for behavior modification and for patient compliance could have very major effects on decreasing the costs of health care in the United States (10). Psychology and behavior modification are very subtle sciences which can be greatly assisted and made more effective by the sensors we propose to develop.

E. BACKGROUND, RATIONALE, AND APPROACH

Bioluminescent marine bacteria are found in exceptionally high concentrations in the light organs of bioluminescent fish (27-31). Although the general anatomy and histology of some of these organs have been studied by marine biologists, there has been very little work on the means or mechanisms by which the bacteria can be maintained in such high concentrations. These bacterial light organs result in extremely bright bioluminescence. Anyone who has observed a flashlight fish exhibition in a marine aquarium can attest to the brightness of bacterial bioluminescence (27). Such brightness is not normally found in cultures of bioluminescent bacteria.

We propose a unique, novel, and highly effective means to dramatically detect and present the presence of environmental tobacco smoke and some of its major pharmacologic agents, including nicotine and carbon monoxide.

Toxic, or otherwise metabolically active chemicals and biochemicals can generally be assayed by their effect on sensitive living organisms (15). A wide range of bioassays for toxicity are well known and widely used in the medical device and health care products community for testing for safely purposes. The U.S. Food and Drug Administration requires a wide range of tests related to toxicity. It is becoming increasingly recognized that microorganisms, and bioluminescent bacteria in particular, are highly efficient, effective, and straightforward bioassay systems for toxicity. The so-called Microtox toxicity test system utilizing bioluminescent bacteria is now widely used in industry and in the health care products industry (17,18).

Nicotine is an active biochemical and is considered a toxin (33). Nicotine is a relatively simple alkaloid of low molecular weight. It is colorless and volatile, but also readily soluble in water, forming water soluble salts. It is considered one of the most toxic of all drugs, acting with a rapidity comparable to that of cyanide and markedly

stimulates the central nervous system. The drug is readily available from biochemical supply houses. It can be readily produced by simply lighting up a cigarette. It is also available in the nicotine patch for transdermal delivery, one of the most prescribed medications in the United States today. Nicotine patch therapy is effective in relieving the withdrawal symptoms associated with smoking cessation (34).

Nicotine is actually somewhat difficult to assay and generally samples require considerable processing before they can be assayed. Common methods include gas chromatography and radio immunoassay (5-8).

Nicotine's strong metabolic activities result in interference with the bioluminescence emission system of many strains of bioluminescent bacteria. This effect has been studied in a preliminary way by our Russian colleague and consultant Valentina Kratasyuk. Indeed, her laboratory is assessing the several thousand strains of bioluminescent bacteria which they maintain and study in order to select those strains which are most sensitive to nicotine in the most reproducible manner. Bioluminescent bacteria are classified into four genera and eleven species, all of which are represented in the Russian collection (21,22). They all have somewhat different properties, bioluminescence intensities, temperature requirements and nutrient requirements. Although the chemical processes involved in bioluminescence and the luciferase enzymes involved are expected to be very similar in all of these species, there are differences and there are subtleties which result in different bioluminescence characteristics under different conditions and, presumably, different susceptibilities to nicotine toxicity.

Bioluminescence bacteria are readily obtained from a variety of supply houses, culture collections, and can be easily isolated from marine sources (19-22). Indeed they are readily available from high school biology suppliers and are used in high school and undergraduate biology laboratory exercises. Most of the organisms are marine and require a sea water based culture medium. Various media and methods for culture are well known (19-22).

A very interesting feature of many marine bioluminescent bacteria is the phenomenon of auto induction, that is, these organisms do not bioluminesce until they produce a critical concentration of a chemical which then *induces* bioluminescence. The bacteria may be perfectly healthy and non bioluminescent at low concentration; greater numbers are required before the auto inducer reaches sufficient concentration to induce the culture to bioluminesce (22,23). The growth and luminescence of these bacteria is also sensitive to other components in the medium, including salt concentration (24), iron levels (25,26), and over all osmolarity (30).

There has been little concerted effort from an engineering perspective to try to design a culture for maximal light emission *in vitro*. This has been done successfully *in vivo*, however, through the symbiosis between bioluminescent bacteria and various bioluminescent fishes, including Flashlight and Pony fishes (27-31). Many bioluminescent fishes contain a highly dense and very bright culture of a symbiotic bioluminescent bacteria in their light organ. These light organs have been evolutionarily engineered to produce bright emission with even directional characteristics.

There has been little or no consideration of the structure and micro architecture of these light organisms from a bioengineering and biotechnology perspective. What we are proposing in this project is basically to construct and to apply *in vitro* a miniature light organ, containing bioluminescent bacteria -- essentially a biological flashlight which will glow or wink at you in the dark when healthy, but will progressively weaken and go black after exposure to environmental tobacco smoke and its nicotine.

It is interesting that fish light organs house the bacteria in specialized, highly structured, gland-like environments. The fishes have significant anatomical and physiological adaptations for maintaining the symbiotic bacteria and maximizing the luminescence while minimizing the growth of the bacteria.

The bacteria are present at high concentration and have little need to grow or multiply -- they are already present at high and potentially maximum density in light organs. Thus, unlike the growth of bacterial in *in vitro* culture, conditions are such that growth is minimized while luminescence is maximized. The growth rate of bacteria in a fish light organ is of the order of 20-30 times slower than in typical culture conditions. The fish may regulate growth through the regulation of osmolarity. Low osmolarity apparently increases luminescence and luciferase concentration but restricts growth (30). High osmolarity, comparable to that of sea water, stimulates growth but limits luminescence (30). This mechanism, although not fully proven, will be important in the design and engineering of an *in vitro* light organ with optimum characteristics.

The bacteria and/or the surfaces and micro architecture of the light organ may be optimized for bacterial immobilization and possibly even orientation. Vibrio harveyi is known to adhere to artificial fiber surfaces under a variety of conditions, including laboratory filters (32). These observations and these clues will likewise be used in this study to optimize our artificial light organ.

The sensor requires three very different technologies (Figure 1):

One, means to produce very high concentrations of bioluminescent bacteria in a very small volume;
Two, means to maintain the viability of such concentrated cultures for long periods of time, possibly through the use of a controlled nutrient delivery device, built into the sensor; and
Three, means to permit nicotine to be permeated through the device and delivered to the bacteria within. This is a difficult membrane permeability problem and will require sophisticated material and/or biochemical technologies.

The Figure shows a very preliminary design for a behavior modification device based on the inhibition of bacterial bioluminescence via nicotine in cigarette smoke. Referring to the side view in the figure and going from left to right we first have a simple adhesive covering which is removed to expose a pressure sensitive, skin compatible adhesive. The device is then pressed against the skin or against fabric where the adhesive layer binds it securely. This adhesive layer is analogous to that used in Band-Aids and other medical devices designed to make contact with skin.

The main structural component of the device is the reflective base. This may simply be a white, reflective film or it may actually be a metalized plastic film for mirror reflection purposes. In both cases, the purpose of the film is to redirect light emitted in the direction of the base towards the right, thereby enhancing the overall bioluminescence intensity. We may actually experiment with the reflecting elements commonly used in fish light organs, as well as more common metalized films.

Sitting on the reflective base is a relatively thick layer of transparent agarose gel whose liquid component is the bacterial culture medium. Bioluminescent bacteria are commonly grown on agar in an appropriate medium. We already produce luminescent thin films based on agarose containing luciferase and luciferin, we will experiment with the same agarose formulations containing the various bacterial culture media with the expectation of obtaining agarose/media films, which are optimally transparent. This

media reservoir will provide the nutrients, the buffering capacity, and other components required to produce a device which would have an appropriate shelf life (3-6 months) and an appropriate consumer usage period (1-2 weeks).

Sitting on and perhaps partially *in* the agarose film is the concentrated culture of bioluminescent bacteria. At this point in the design development we anticipate that the culture will be initially at maximum density, that is, we do not anticipate any need for significant growth in bacterial numbers during the lifetime of the device, analogous to the situation in fish photo organs. It is also possible that the bacterial concentration and the nutrient volume may be adjusted so as to permit growth to optimal and maximum density during the shelf life of the device, but that remains to be evaluated. The actual density of bacteria and the thickness of the bacterial culture layer will be studied and optimized with respect to overall bioluminescence intensity and lifetime of the device.

The bacterial film will then be coated with another layer of agarose containing culture medium. This layer will be relatively thin, however, as it must not significantly hinder oxygen and CO_2 transport from the front of the device. That layer will then be covered with a transparent, gas-permeable, and largely water-impermeable film, initially with low density polyethylene, which we now utilize for the growth of bioluminescent dinoflagellates in sealed bag cultures (41,42). Light emitted by the bacteria will be transmitted through the transparent agarose overlayer and the transparent gas-permeable outerlayer. Light emitted by the bacteria to the left will also be transmitted by the thicker, but still transparent, agarose sublayer and reflected. The gas-permeable transparent layer will not permit significant nicotine transport.

A major part of the project and a critical component of Phase II will be to optimize the characteristics of these outer membranes. For the initial Phase I device, however, we propose to use a gas permeable transparent flap or shutter. The wearer or operator of the device will keep this flap closed. It will be opened in situations in which secondary smoke and nicotine is expected. When open it will expose an underlying, central part of the film which is nicotine permeable. At this stage in the development, this part will also be permeable to water vapor, as well as other gases. This nicotine permeable layer also has to be transparent. It will likely be a microporous membrane with reasonable optical properties and good permeability to nicotine. The nicotine permeable region may be in the shape of a small central heart, as shown in the front view at the bottom of the figure. The nicotine impermeable region, the rest of the device, is a larger heart.

The purpose of the transparent flap is to permit the device to receive nicotine but to minimize dehydration due to water vapor loss when there is little or no nicotine in the immediate environment. Obviously this is a complication of the device which we hope to overcome during the Phase II research and development effort. This could be done by using an "intelligent" membrane which responds by increasing its permeability or porosity in the presence of nicotine (43). This is actually quite possible due to nicotine's alkaline nature. There are a variety of membranes whose characteristics can be made to change dramatically in the presence of slightly acid or slightly basic environments. We will demonstrate the feasibility of such a device during the Phase I effort by the manually operated flap approach.

In the presence of sufficient concentrations of nicotine, the central nicotine permeable region will become dimmer due to nicotine's interference with the bioluminescence pathway in the bacteria. The glowing heart will then show a diminished intensity or glow in its smaller internal heart; eventually the internal heart will go black, whereas the outer heart will remain bright, particularly at its edges. There will, of course,

be a gradient in nicotine concentration and in bioluminescence intensity in the interfacial region between the small and large heart.

We have not yet come up with an appropriate commercial name or trademark for this preliminary device. The term used in Neil Diamond's song based on the film E.T., "Heartlight," certainly comes to mind.

The fundamental point is that nicotine kills. It kills the bacteria, thereby eliminating their glow. It is doing the same thing, albeit at a much slower rate, to the smoker him/herself, and to those in his/her immediate environment.

We have no plan at this stage to develop a quantitative nicotine sensor, although that clearly is a distinct possibility and may be considered in future grant applications.

The purpose of the sensor proposed is to enhance the awareness of toxins in the environment and through such awareness to encourage modification of behavior which would lead to decreased concentrations of such toxins. The device will of course have to be tested in a variety of control environments, including kitchens. We certainly do expect that other volatile agents in the ambient environment will also tend to inhibit the bacterial bioluminescence, although it is certainly likely that the nicotine environment in and around smokers will be more active in inhibiting bioluminescence than other common agents.

F. PHASE I TECHNICAL OBJECTIVES

1) Comprehensive evaluation and assessment of the key species of bioluminescent bacteria and the various available strains with respect to their suitability for this application. Careful evaluation of the standard marine bacteria cell culture media (21,22) for this application. Selection of those species and strains with the appropriate brightness, robustness, and other properties.

2) An assessment of their sensitivity to nicotine, both in acute dosage and in chronic, or cumulative, dosage forms using volatile gas-phase nicotine. Based on acute and chronic nicotine sensitivity we will select up to 6 strains for further study.

3) Reverse engineering of fish light organs.

4) Incorporation of optimum bacterial culture media in an agarose/agar gel film.

5) Optimization of the agarose-based culture medium with respect to enhanced bioluminescence intensity and long term stability of the cultures.

6) Evaluation and assessment of appropriate gas-permeable transparent overlayers for the device.

7) Evaluation and assessment of appropriate nicotine-permeable films for the device.

8) Design and construction of the preliminary device for testing and evaluation purposes.

9) Assessment of stability, intensity, nicotine sensitivity and behavior of the device in a variety of end user environments.

G. PHASE I RESEARCH PLAN

Task One:

Our colleagues in Kraznoyarsk, Russia (see Personnel section) have already been screening strains in their bioluminescent bacteria culture collection for nicotine sensitivity in anticipation of the collaborative effort on this project. They have a wide experience with the use of bioluminescent bacteria for environmental toxin detection and assessment (see biosketch of Kratasyuk in Personnel section).

We will also continue to work closely with those investigators in the United States who have considerable experience in the use of bioluminescent bacteria for toxicity assay, in particular Dr. J. W. Hastings, who serves on PSI's Scientific Advisory Board (14,20). Based on the results of the Becvars from Texas, using their Lumitox assay (35), *Photobacterium leognothi* may be the most effective of the commonly available types for this application. However, we frankly expect that the screening of the extensive strain collection in Kraznoyarsk will lead to even more effective strains. The culture of these various strains will be conducted in a variety of standard media to obtain the appropriate base line conditions for task 5 below. In this task, the strains and media will be selected based on bioluminescence intensity and long term stability of the culture under high intensity, high brightness conditions.

Task Two:

The strain/media combination selected above will then be evaluated for nicotine sensitivity. Tobacco smoke will be produced in small, closed environments, using a controlled cigarette and smoking protocol, a measure of total tobacco smoke, and a semi-quantitative measure of nicotine concentration. This later will be done by LC/TLC procedure which we are developing for a different educational product, Cig Chem, a chemistry kit based on tobacco, combustion, and nicotine. The various cultures will be evaluated for their acute and chronic nicotine sensitivity, that is, both as a short term nicotine detector, as well as a long term nicotine dosimeter. Several culture combinations in each category will be selected.

Task Three:

We will continue our study and evaluation of the light organs in bioluminescent fish. This will not be done experimentally but rather will be done by discussion with marine biologists, anatomists, and histologists, who are expert in these areas, particularly Dr. James Morin and his co-workers at UCLA (29). We fully expect to stimulate considerable interest to reverse engineering fish photo organs, which would be relevant not only to this project, but to future projects aimed at developing living light sources.

Task Four:

In parallel with Tasks 1-3 above, we will be conducting a set of studies in developing bacterial culture media in a transparent gel environment. Normal media utilize agar and are constructed in such a way that they are largely translucent rather than transparent. We have had extensive experience with the use of agarose and with the ability to control the gelation of agarose via a combination of temperature and agarose molecular weight and other molecular characteristics. Low gelling temperature agaroses have been used by our group for some time to produce luminescent films for teaching and

analytical applications. We fully expect to be able to produce an agarose-base culture reservoir as required in the preliminary design of this device.

Task Five:

This is probably the most critical task in the overall project and will be a major part of the Phase I and the Phase II studies. Given what we have learned in Tasks 1-4 above, and particularly what is known from a reverse engineering study of photo organs, with respect to the chemical and environmental control of the bioluminescent bacterial population, we will begin to modulate and modify our culture material and indeed the design of the device to facilitate the long term stability of these bacterial cultures, coupled with very high bioluminescence intensity. Control and regulation of osmolarity and pH are the two immediate variables, as well as iron concentration (24,26,30,31). We fully expect that in order to develop an optimum device we may need to design a gradient in one or more of these parameters into the device itself, the gradient to help compensate for such factors as water loss with time and perhaps changes in Oxygen and CO_2 transport with time. Our experience with the development of gradient systems for other applications will be very valuable here (44).

Task Six:

Preliminary analysis by Mr. K. Foote of highly gas permeable polymer films for algae aquaculture applications (36) led to the selection of low density polyethylene for our initial studies (Table 1). Low density polyethylene has been used for the storage and propagation of plants in sealed environments (37).

Table 1: Best candidates for enclosing membrane material (from Ref. 36).*

Membrane Material	Gas Permeability mol*cm)/cm^2*sec*atm)*10^{-12}® CO2	O2	Rate of Water Vapor Transport (g*mil)/(100in^2*24hr) at 37.88 C
Silicone rubber (Silastic)	290	140	170
Low density polyethylene	5.49	1.02	1.3
Ethylene-vinyl acetate copolymer	12.2	1.71	2.5
Fluorinated ethylene propylene copolymer (FEP TEFLON)	3.40	1.52	0.4

* Data from *Modern Plastics Encyclopedia*, Vol. 64, 1988, pp 553-557.

Basically one desires maximum CO_2 and O_2 permeability with minimum water vapor transport. The polymer with the highest gas permeability is silicone rubber, but

that also has a very high rate of water vapor transport. Preliminary studies of cultures in silicone rubber bags showed a very rapid water loss, leading to increased salinity of the cultures to the point where cell death occurred. Silicone copolymers and silicones of varying cross-link density will be evaluated, however.

Low density polyethylene was selected because of the optimum combination of properties, because it is inexpensive, and because it is very easy to heat seal. Most of our experience to date has been with four mil thick polyethylene. We will examine a range of thicknesses and the trade off between thickness, mechanical properties, and gas transfer.

There are many types of low density polyethylene. We have not evaluated the various sources, various densities, crystallinities, and molecular weights. We also have not evaluated ethylene vinyl acetate copolymer, which is very promising and has a different ratio of CO_2 to O_2 permeability, which would be very interesting in terms of fundamental studies of photosynthesis and respiration ratios.

Probably the best material is FEP Teflon. CO_2 and O_2 permeabilities are comparable to polyethylene, with water vapor transport three times less. Unfortunately FEP Teflon is extremely expensive for this application, although it has been used, and is marketed as a cell-culture bag for small volume research applications (38).

We propose to perform limited studies with FEP Teflon and silicone rubber, and much more extensive studies with various grades and types of low density polyethylene and ethylene vinyl acetate copolymer.

Task Seven:

The nicotine permeable component will be selected from a careful study of polymer membrane materials, including those materials and components used in the cigarette industry, not only in cigarette production, but also for analytical purposes. Nicotine is sufficiently small and volatile that a variety of polymer membrane materials should be suitable. Admittedly we have little experience with this specific area, but we do have considerable experience in polymer materials and the permeability and diffusion characteristics of polymer systems.

We also have to deal with the transport of the nicotine through the agarose/media overlayer. These characteristics can be readily assessed by qualitative bioassay, using the bioluminescent bacteria themselves.

Task Eight:

In the last third of the Phase I project we will assemble overall devices for preliminary testing and evaluation. A major determinant will be bioluminescence intensity and the degree of darkness which is required to effectively see and utilize the device.

Task Nine:

The stability of the device and the efficacy of a chronic sensor for nicotine in environmental tobacco smoke will be critically evaluated. We certainly expect to provide a set of "Heart Light" devices in a very preliminary form to the reviewers of the Phase II application and with the Phase I final report.

H. COMMERCIAL POTENTIAL

This has been discussed in Section D.

I. PRINCIPLE INVESTIGATOR/KEY PERSONNEL

Rob Scheer, Ph.D., Principle Investigator on this grant does not have a strong background in marine biology or bacteriology. Rob is a materials engineer with considerable background in the properties of plastics and materials, including some recent modeling studies on the gas permeability of polymeric materials. His biosketch is attached.

He will be assisted by *Ms. Mara Lisonbee, a biology technician* with over two years experience in the culture of bioluminescent microorganisms and by Mr. Dong Min, a graduate student with considerable experience in the culture of bioluminescent bacteria.

In addition we have budgeted a half-time marine bacteriologist/microbiologist post-doc for the project.

Dr. Scheer will be assisted by *J.D. Andrade, Ph.D., President and Chief Scientific Officer* of Protein Solutions, who has been working on bioluminescence for the past 5 years. He has supervised PSI's work to date in the area of bioluminescent dinoflagellates. He has conducted courses for inservice elementary and high school teachers and has distributed *Pyrocystis lunula* cultures to at least 300 different teachers, friends, co-workers, and advisors for their input and critique. Joe has a strong interest in integrated science education. Dr. Andrade has been working on biomaterials and biotechnology problems for the past 25 years. He is an accomplished scientist with 5 books and over 100 peer-reviewed publication. Joe is also Director of the University of Utah's Center for Integrated Science Education (CISE). Although Joe is on the faculty of the University of Utah, he spends about 25% time with Protein Solutions, Inc., and will advise and assist on this project as needed. No funds are budgeted for his services.

A part-time undergraduate is also budgeted to assist in the project.

Advisors:

Dr. J.W. Hastings, Professor of Biology at Harvard University is one of the world's experts on dinoflagellate bioluminescence. Dr. Hastings serves on PSI's Scientific Advisory Board and will regularly provide input and guidance to the project.

Other experts with whom Dr. Andrade has already consulted over the last 2 years and whom have offered their advice include Dr. Barbara Prezelin, Department of Biological Sciences, University of California, Santa Barbara; Dr. L. Brand, University of Miami, School of Oceanography, Miami; Dr. James Morin, Professor of Biology, , Los Angeles; Dr. Robert Guillard, the National Phytoplankton Culture Facility, West Boothbay Harbor, Maine.

Robert J. Scheer
390 Wakara Way, Room 31, Salt Lake City, UT 84112
Ph. (801)585-3128
e-mail (rob.scheer@m.cc.utah.edu)

EDUCATION

Ph.D. in Materials Science and Engineering, September 1993, University of Utah, Salt Lake City, UT. Dissertation emphasis: Mechanical, interfacial, and surface study of composite materials.

B.S. in Mechanical Engineering, 1989, Duke University, Durham, NC. GPA 3.76. Emphasis: Fracture mechanics and failure analysis of polymeric materials.

UNIVERSITY HONORS

National Science Foundation Fellow, University of Utah Graduate Research Fellow, Dean's List Duke University, Academic All American

Duke University Magna Cum Laude, Scholastic Societies: Tau Beta Pi and Pi Tau Sigma.

EXPERIENCE

Principle Investigator

Protein Solutions, Inc. Salt Lake City, UT. 1994 - present. Directed research for the design and implementation of novel science education materials.

Research Assistant

University of Utah, Salt Lake City, UT. 1989 - 1994. Tested mechanical properties of polymers and composites, studied surfaces and interfaces, tested adhesive bonds on the microscopic scale, and developed stress analyses related to materials testing.

Instructor/Tutor

University of Utah and Salt Lake Community College, Salt Lake City, UT. 1991 - present. Planned, instructed, and graded for undergraduate physical science classes. Served as tutor and teaching assistant.

Engineering Technician

Sandia National Laboratory, Albuquerque, NM. Summer, 1988 and Summer, 1989. Designed engineering experiments for failure analysis of ceramic materials, and extensively researched current experimental techniques for determining material fracture toughness. Designed engineering experiments for strength testing of brittle materials, and performed CAD. Interacted with diverse engineering disciplines on a major research project.

AFFILIATIONS: American Society for Mechanical Engineers, ASM International, The Minerals, Metals, and Materials Society, American Physical Society, The Center for Biopolymers at Interfaces

PUBLICATIONS:

Scheer, R.J. and J.A. Nairn. "Variational Mechanics Analysis of Stresses and Failure Analysis in Microdrop Debond Specimens." *Composites Engineering*, Vol. 2, No. 8, pp. 641-654, 1992.

Scheer, R.J. Ph.D. Dissertation, "An Energy Based Analysis of Fiber-Matrix Adhesion." University of Utah, 1993.

Andrade, J.D. and R.J. Scheer. "Applying 'Intelligent' Materials for Materials Education: The Labless Lab™." *Proc., 2nd Annual Conference on Intelligent Materials*, Tech. Publ. Co., 1994, in press.

J. CONSULTANTS

Dr. V. Kratasyuk is a senior researcher with the Institute of Biophysics in Krasnoyarsk, Russia. She is an expert on the use of bioluminescent bacteria as biosensors and biomonitors of environmental toxins. She will spend ten days working in our labs and will supply lyophillized bacterial strains. She is already screening her bioluminscent bacteria collection for nicotine sensitivity. Her letter of collaboration (via E-mail) and resume are attached.

K. FACILITIES AND EQUIPMENT

The work on this project will be carried out by PSI, Inc., in its laboratories located at 350 West 800 North, Suite 218, Salt Lake City, Utah 84103. PSI is a member of the Center for Biopolymers at Interfaces at the University of Utah, one of the State's Centers of Excellence. PSI is also a key corporate participant in the University's Center for Integrated Science Education (CISE). PSI has a Technology Transfer agreement with the University of Utah Research Foundation. PSI's laboratories are equipped to perform the necessary biological, chemical, engineering, and evaluation studies. The laboratory space (1100 ft^2) includes a culture room and a general biology lab. Normal office and instrument room space is also present. Sophisticated equipment which may be required may be used by our team at the University of Utah

L. CURRENT AND PENDING SUPPORT

Current Support:

NSF Phase I SBIR, The Lableѕs Lab™ in Polymer Materials (ends 8/15/94) Phase II will be submitted by 9/15/94.

Pending Support:

1. Department of Energy SBIR, Luminescent Films based on Photoproteins (7/1/94 - 1/1/95) two man months.

2. NSF STTR, Direct Reading, Quantitative Biosensors for ATP-Dependent Processes &7/1/94 - 6/30/95) two man months.

3) NIH SBIR, Direct Reading, Quantitative Bioluminescent Biosensors, (10/1/94 - 4/1/95) on man month.

As PSI's sponsored R & D volume increases several Ph.D. level research personnel will be attracted and hired, although Dr. Scheer will serve as principle investigator and primary supervisor and scientist for these projects, the more routine scientific and engineering activities and tasks will be delegated to these new staff researchers. Dr. Scheer will retain a minimum of one man month of direct involvement with each of the projects for which he is serving as principle investigator.

PSI has invested $150,000 (provided primarily by its founders and major stock holders) in the initial studies and product development. PSI expects to continue funding the project from stock-derived funds. PSI is now discussing equity investments by a number of local investors and investment groups.

M. EQUIVALENT PROPOSALS TO OTHER FEDERAL AGENCIES None.

From: biolum@ibp.krasnoyarsk.su (Bioluminescence lab.)
Subject: letter from Dr.Kratasyuk

Press RETURN for more...

MAIL>

#1 13-DEC-1993 21:39:01.39 MAIL
To: JDANDRAD@CC.UTAH.EDU
Message-id: <ABfzM3jKJ6@ibp.krasnoyarsk.su>
X-Envelope-to: JDANDRAD
Organization: Institute of Biophysics
Return-Receipt-To: biolum@ibp.krasnoyarsk.su
X-Mailer: dMail (Demos Mail v 1.13a)
Lines: 56

Dear Josef Andrade,
It was a great pleasure for me to remember my visit to your laboratory. I want to express my deep gratitude to you and your colleques for my visit. We are going to do experiments with nicotins. And I will try to send you results as soon as possible. In Wasnington I met Michail Rybinski (Partne of NORTEK Scientific Group. 7205 Adrienne Glen Avenue Springfield, Virginia 22152-3540, USA, tel: 703-905-0957, fax: 703-644-4516, E-mail: p00383@psilink.com) and we discussed any possibilities to receive finuncial support of this work with nicotins. I discribed idea only in common and

Press RETURN for more...

MAIL>

#1 13-DEC-1993 21:39:01.39 MAIL
he was sure that he can help us. So if it is interesting you should call him and give a project, Michail will wait your call. May be it will be useful for us.
I send you also my resume. Sin

VALENTINA A. KRATASYUK

(Biographical Sketch)

Date of Birth: 1 December 1952

Place of Birth: Russia, Zlatoust, Chelyabinsk region,

Married, two daughters

EMPLOYMENT:

1991 - present Post-doctoral worker, Senior Researcher of Institute of Biophysics, Siberian Branch of Russian Academy of Sciences (IBP)

1989 - 1991 Senior Researcher of IBP

1986 - 1989 Researcher of IBP

1981 - 1986 Junior Researcher of IBP

1975 - 1981 Lab assistant, Junior Researcher of Institute of Physics named L.V. Kirensky, Siberian Branch of Russian Academy of Sciences

1970 - 1975 Student of Novosibirsk State University.

EDUCATION:

Senior researcher (Associate Professor), 1989, Institute of Biophysics of Siberian Branch of Russian Academy of Science, Krasnoyarsk 660036, Russia

Candidate of Biological Sciences, 1985, Institute of Biophysics of Siberian Branch of Russian Academy of Science, Krasnoyarsk 660036, Russia

Biologist (biochemistry) 1975 graduate of Novosibirsk State University.

HONORS:

1985 - Krasnoyarsk regions, Young Scientists Competition.

1984 - Siberian Branch of Russian Academy of Science, Young Scientists Competition.

1982 - National achievements exibition (Vistavka dostizheni narodnogo hozyastva), silver medal

1982 - IBP, Scientists Competition

1980 - IBP, Young Scientists Competition

1978 - IBP, Young Scientists Competition

1977 - Sigh of USSR's Inventor

DISSERTATION:

Kratasyuk V.A. 1985. Inhibitor's analysis of bacterial bioluminescent reactions. 173 pp.

PUBLICATIONS: total number 63

Publications cont/...

Kudryasheva N.S., Kratasyuk, V.A., Belobrov, P.I., Sherbinskaya, M.K., "Physical-Chemical Regularities of External Quenching of Bacterial Bioluminescence," from *Biological Luminescence*, Singapure: World Scientific, 1990, pp. 416-425.

Kratasyuk, V.A., "Principles of Luciferase Biotesting (Review)." from *Biological LuminescenceI*, Singapure: World Scientific, 1990, pp. 550-558.

Kratasyuk, V.A., Kim, N.B., "Luciferase Biosensors for the analysis of Aldehydes," from *Biological Luminescence*, Singapure: World Scientific, 1990, pp. 564-572

Kudryasheva, N.S., Kratasyuk, V.A., Belobrov, P.I., Shigorin, D.N., "Investigation of Biolumionescence Mechanism by Means of Molecular Quenchers," Prepr. N154B, Inst. of Biophysics, Krasnoyarsk, 1991 - 22s.

Kratasyuk, V.A., Egorova, O.I., Popova, L.Y., Orlova, N.Y., Lvova, L.S., "Bioluminescence Method Using for Determination of Corn Infected with Fungi," Prepr. N114B, Inst. of Biophysics, Krasnoyarsk, 1991 - 15s.

Bytev, V.A., Starushkin, A.I., Kitaev, y.N., Kratasyuk, V.A., Egorova, O.I., Orlova, N.Y., "Bioluminescent Experess-Lab for Determination of Corn Infected by Fungi" Abst. of All-Union Conf. on Clinics Diagnostics, 1992.

Kratasyuk, V.A., Kim, N.B., Abacumova, V.V., "Soluble and Immobilized Liciferase Using Aliphatic Aldehydes Analyses." Abstr. of All-Union Conf. on Fluorescence Methods, 1992.

Kudryasheva, N.S., Shalaeva, E.V., Zadorozhnaya, E.N., "Quanching by Quinones and Phenols - Wastewaters' Compounds," Prepr. N114B, Inst. of Biophysics, Krasnoyarsk, 1992 - 25s.

Kudryasheva, N.S., Shalaeva, E.V., Zadorozhnaya, E.N, Kratasyuk, V.A., "Bioluminescent Control of Quinones and Phenols in Cellulose Industry Wastewaters," Abstr. of Intern. Concress on Analytical Chemistry, London, 1992, Analytical Proceeding, 1992, Vol. 29, pp. 350.

Egorova, O.I., and Kratasyuk, V.A., "Luciferase Toxicity Testing in Ecology," abstract, 8th Annual Int. Symp. on Bioluminescence, Cambridge, U.K., Sept., 1994.

O. PRIOR PHASE II AWARDS

None.

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