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This technical report details efforts of our first month of research to develop biosensors for detection of toxic compounds. Progress to date in the following areas is reported: 1) detection of carcinogens using cloned lux genes in various strains of E. coli, 2) stabilization and immobilization of E. coli biosensors by lyophilization, and 3) development and fabrication of a portable photodiode light detection system.

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PRINCIPAL INVESTIGATOR: Dr. Reinhardt A. Rosson

CONTRACTOR: BioPhotonics, Inc.

CONTRACT TITLE: Bioluminescence for Detection of Trace Compounds

START DATE: 1 September 1989

RESEARCH OBJECTIVE: To demonstrate the feasibility of bioluminescent testing for detection of toxic compounds and to develop an inexpensive photodiode based light detection system for field measurement of low level bioluminescence,

WORK IN PROGRESS: This initial report is the result of several weeks of active research since the signing of the contract at the end of September 1989. Work which has already been initiated, as well as work soon to be initiated, is briefly described in this report. Our first progress report, due 1 January 1990, will detail the results of our efforts more fully.

1. **Detection of carcinogens using cloned *lux* genes in *E. coli*.** We are attempting to optimize our system for low levels of endogenous bioluminescence. As has been previously demonstrated, when genes from *P. leiognathi* PL721 (plasmid pSD721) were cloned and expressed in *E. coli*, some strains did not express the cloned genes while other strains did. One strain, *E. coli* B18, had very low background luminescence and was highly sensitive to the addition of carcinogens. To optimize this system, in addition to working with *E. coli* B18[pSD721], we are testing other strains of *E. coli* for levels of endogenous expression of luminescence when transformed with pSD721. We hope to find strains with even lower background luminescence which are still highly sensitive to the presence of carcinogens. We are using the electroporation technique to rapidly and efficiently transform a variety of *E. coli* strains with pSD721, and screening these for rapid response to the presence of carcinogen. We will settle on one biosensor for further testing of the sensors response to a wide range of carcinogens as well as for studies of compounds that may interfere with our biosensor's response to carcinogens.

2. **Stabilization and Immobilization of *E. coli* biosensors.** We are testing lyophilization as a means of stabilizing our biosensors at known numbers and metabolic state so that the potential maximum luminescence in response to the presence of toxins can be standardized. We are also initiating shelf-life studies of these lyophilized biosensors; these studies will continue

for the duration of this six-month feasibility study. Studies of various other means of immobilization and stabilization of biosensors will begin in the next few weeks.

3. Development of the light detection system. We have settled on an initial design for our light detection system. The system will utilize an array of photodiodes, each with solid state amplification. We are in the process of fabricating initial prototypes with a variety of photodiodes for testing the level of sensitivity. The system is being initially developed with a Metrabyte® A/D card in an IBM PC for automated data acquisition. In the near future a dedicated data acquisition system will be completed to allow the entire light detection system to be portable.

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