Unattended Remote Electrobiotic Toxic Agent Sensor

JPL Task Plan No. 80-6204 Rev A

A research and development project for the

Dr. Alan Rudolph
Activity Detection Technologies
BAA 01-05
3701 N. Fairfax Drive
Arlington, VA 22203-1714

Quarterly Report
May 31, 2002

Contact:
Dr. Robert C. Stirbl
Senior Engineering Staff /Concept Development
Space Experiments Systems & Technology Section
Jet Propulsion Laboratory/Caltech
4800 Oak Grove Drive
Pasadena, CA 91109
Voice: (818) 354-5436 mobile (818) 635-6793
Robert.C.Stirbl@jpl.nasa.gov
# Table Of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Executive Summary</td>
<td>3</td>
</tr>
<tr>
<td>B. Technical Progress</td>
<td>4</td>
</tr>
<tr>
<td>C. Milestone Report</td>
<td>17</td>
</tr>
<tr>
<td>D. Future Issues and Technology Transition</td>
<td>18</td>
</tr>
<tr>
<td>E. Appendix: Program Background</td>
<td>19</td>
</tr>
</tbody>
</table>
A. EXECUTIVE SUMMARY

FUNDS: Total Received: $908.2K = FY ‘01 $248.2K + FY ‘02 $615.2K Obligated as of 02/28/02: $752.2K Funds Exhaustion: 90-122 days

Program Deliverables:

- Identify behavioral metrics response characteristics to specific chemicals
  Adapt multi-node computer model of *C. elegans* from lab to APS (9/01 – 5/02)
  Demo agent dose-rate correlation w/ lab motion detection software (2/01 – 9/02)
  Produce *C. elegans* genetically eng./screened for specific hypersensitivity (2/02 – 09/02)
  Test *C. elegans* response to a panel of agents (10/02 – 2/03) Report results (2/03)

- Investigate *C. elegans* for hypersensitivity to specific agent
  Design (genetically engineer/screen) *C. elegans* for hypersensitivity to one (or more) of a panel of selected specific agents (6/02 - 9/02) Report results (9/02)

- Demonstration of *C. elegans* imaging readout with APS electronic biocoupling
  Investigate biocoupling by shadow time differencing (9/01 – 11/02)
  Establish readout patterns from APS CMOS chip (10/01 – 6/02)
  Design, fab prototype biocoupling interface electronics test chip (6/02 – 01/03)
  Demonstrate *C. elegans* imaging readout (1/03 – 02/03) Report results (02/03)

Progress For This 3rd Quarterly Report Period:

1) **Demonstrated reproducible, sigmoidal behavioral metrics, dose-response curves for *C. elegans* movement in the presence of toxic agent simulants, aldicarb and arsenite.** The EC50 for aldicarb is 0.8mM while the EC50 for arsenite is 8.2mM for the parameter mean positive velocity. (5/02) **On-Schedule**

2) **Identified three candidate hypersensitive mutant *C. elegans* strains tested for movement in response to increasing doses of the toxic agent simulants aldicarb and arsenite.** The cat-4 mutants, with weak cuticles, are 2.7-fold more sensitivity to aldicarb (EC50 = 0.3 mM) and 3.6-fold more sensitive to arsenite (EC50 = 2.3 mM) than are wild-type *C. elegans*. The strain NL130, carrying engineered deletions of *pgp-1* and *pgp-2*, encoding P-glycoprotein-type transporters, is 2.0-fold more sensitive to aldicarb (EC50 = 0.4 mM) and 2.6-fold more sensitive to arsenite (EC50 = 3.2 mM) than wild-type *C. elegans*. Another strain NL152, genetically engineered to lack *pgp-1*, *pgp-3* and *mrp-1* (a toxin transporter), also showed increased sensitivity to aldicarb and arsenite but is somewhat movement impaired in the absence of toxic agents. Construction of triply mutant strains and use of these strains in direct screens for hypersensitivity is scheduled next as well as tests of additional candidate mutations developed in Caltech’s lab as well as in other labs as they become available. (5/02) **Ahead of Schedule**

3) **Initiated two test screens to determine the optimal method for identifying of *C. elegans* mutants hypersensitive to toxic agent simulants (i.e. arsenite) now that robust, repeatable dose-response curves have been achieved.** The screen tests, carried out in 12-well microtiter dish format, are for reduced viability or reduced movement in the presence of low (2.5-5 mM) levels of NaOAs. These tests also allow us to determine the best method of handling and analyzing large numbers of worms. Following the tests, we will readily scale up to intense screening. (5/02) **On-Schedule**

4) **Demonstrated simpler JPL analysis algorithm, applied to multiple (~10) worm dose response curve videos provided by Caltech (at 1 Hz rate), is sufficient to**
**discern a factor of 2 change in dose.** Grew, video taped & analyzed motion metrics of NSWC supplied mosquito larvae. *(5/02) On-Schedule*

5) **Constructed a portable breadboard design for toxic agent panel field-testing.**

Completed breadboard of portable laptop motion processor with APS sensor test chamber & wireless controller IC design for toxin field testing of large numbers of *C. elegans* and possibly other candidate sentinel organisms in a deployable type environment. Designed/fabricated new PDMS microfluidic *C. elegans* chambers and demonstrated contact APS imaging of *C. elegans* through the chamber’s 5 um bottom wall. *(5/02) Ahead of Schedule*

**B. Technical Progress**

**Dosage Response of *C. elegans* to toxins aldicarb and arsenite**

We have extended and refined our analysis of *C. elegans* movement in the presence of increasing concentrations of aldicarb (2-methyl-2-(methylthio) propionaldehyde O-methylcarbamoyloxime) and sodium-arsenite (NaAsO₂). We increased the exposure time of wild-type *C. elegans* to aldicarb from 12 to 30 minutes. We also tested a wider range of aldicarb concentrations to further test the limits of nematode response to this agent. Using new conditions of concentration and time of exposure, we have tested 7 individual wild-type nematodes for each concentration of aldicarb. We have determined that the concentration reducing wild-type mean forward velocity by 50% (EC₅₀) is 0.8mM aldicarb (Figure 1). We have also increased the concentrations of arsenite tested and have increased the number of worms analyzed from 5 to 16 for concentrations below 40 mM. At arsenite levels of 40-80 mM, many nematodes die so we have tested fewer animals (n = 6) at those levels. We have determined an EC₅₀ of 8.2mM NaAsO₂ for wild-type *C. elegans* (Figure 2).

**Candidate mutations**

We have tested three candidate hypersensitive mutant *C. elegans* strains for movement in response to increasing doses of aldicarb and arsenite (NaAsO₂). The candidate mutant *cat-4* is thought to have a weak cuticle as it is hypersensitive to several disparate agents such as the neurotransmitter serotonin and the detergent SDS. We have shown that *cat-4* mutants are 2.7-fold more sensitive to aldicarb (EC₅₀ = 0.3mM vs. 0.8mM; Figure 1) and 3.6-fold more sensitive to arsenite (EC₅₀ = 2.3mM vs. 8.2mM; Figure 2) than wild-type *C. elegans*. The strain NL130 was genetically engineered to delete the genes for two P-glycoproteins encoded by *pgp-1* and *pgp-2* (Broeks, A. et al., EMBO Jour. vol. 14 no.9 pp.1858-1866, 1995). P-glycoproteins are membrane transporters in certain cells that protect the cell against environmental toxins by moving such agents out of the cell. The *C. elegans* mutant lacking these two P-glycoproteins is hypersensitive to colchicine and chloroquinone. We have shown that NL130 is 2.0-fold more sensitive to aldicarb (EC₅₀ = 0.4mM vs. 0.8mM; Figure 3) and 2.6-fold more sensitive to arsenite (EC₅₀ = 3.2mM vs. 8.2mM; Figure 4) than wild-type *C. elegans*. The strain NL152 was genetically engineered to lack the gene for *mrp-1* as well as those for *pgp-1* and *pgp-3* (Broeks, A. et al., EMBO Jour. vol. 15 no.22 pp.6132-6143, 1996). The *mrp-1* gene encodes a C.
*elegans* homolog of the mammalian multidrug resistance-associated protein (MRP), another transporter that protects cells from toxins. NL152 also showed increased sensitivity to aldicarb (EC50 = 0.15mM vs. 0.8mM; Figure 5) and arsenite (EC50 = 7.8mM vs. 8.2mM; Figure 6). However, NL152 is also somewhat movement impaired in the absence of toxic agents. We are currently constructing a triply mutant strain lacking cat-4, pgp-1 and pgp-3 to test whether it has additive sensitivity.

**Test Screens**

We have initiated two test screens (reconstructions) to determine the optimal method for identifying mutants hypersensitive to arsenite. Arsenite was chosen over aldicarb for screens because screens for mutants hypersensitive to aldicarb have been conducted in other laboratories (reviewed by Mendel, J., Neuron. 1999 Oct;24(2):287-8.) and have yielded several mutant strains that will be tested in our candidate mutant assays. Both test screens use cat-4 mutant *C. elegans* to mimic new mutants hypersensitive to arsenite. If we are able to identify cat-4 mutants, which are moderately hypersensitive to arsenite, in our test screens, we should easily be able to identify our target mutants, which are robustly hypersensitive to arsenite, when we perform a full-scale mutant screen.

The first test screen will detect hypersensitive mutants with either reduced viability or reduced movement in the presence of low (2.5-5 mM) levels of NaAsO2. Adult worms are placed on numbered standard 12-well culture plates in the absence of NaAsO2 and allowed to lay eggs for a given amount of time. Most wells are seeded with wild-type hermaphrodites but a few are seeded with cat-4/+ test mutants. These mimic presumptive heterozygous mutants obtained following mutagenesis of a population of *C. elegans*. The hermaphrodites are then transferred to cognate plates containing NaAsO2, allowed to lay eggs for the same amount of time, and removed. When the eggs have matured to adults, NaAsO2 + and – wells of the same number are compared for the number of live individuals and their movement. Movement will be scored either by eye, or by video taping followed by statistical image analysis of movement of the population. One fourth of the offspring of the test mutant heterozygotes should be cat-4 homozygotes and show increased sensitivity to NaAsO2. In a full-scale screen, comparing movement and viability both in the presence and absence of NaAsO2 will allow us to identify and eliminate from further analysis mutants that have reduced viability or movement due to mutations in genes not related to NaAsO2 sensitivity. The population on the NaAsO2-plates will also serve as a source for further generations so that the mutants can be isolated as a homozygous population and analyzed further for dose response and hypersensitivity to other agents.

The second test screen will detect mutants hypersensitive to NaAsO2 with respect to movement. Wild-type and cat-4/+ heterozygotes will be placed individually on numbered standard 12-well culture plates and allowed to lay eggs for a limited time in order to synchronize the population of their offspring. This is necessary since movement is most easily assayed in adults. When the offspring of these hermaphrodites reach adulthood, the population in each well will be divided, with one half being placed in a well containing NaAsO2 and the other in a well without arsenite. After allowing time for
the NaAsO₂ to affect movement (3 hours as in our dosage response assays), the population in each pair of wells will be compared. One fourth of the population from a cat-4/+ hermaphrodite should show reduced movement on NaAsO₂ compared to wild-type worms.

These test screens are necessary to verify that researchers will be able to recognize hypersensitive mutants that will occur as a small proportion of the large number of C. elegans examined during a full-scale mutant screen. These tests also allow us to determine the best method of handling and analyzing large numbers of worms.

These screens will be a major focus over the next few months.

**Figure 1. cat-4 mutant hypersensitivity to aldicarb.**

![Graph showing mean positive velocity for aldicarb response]

**Figure 2. cat-4 mutant hypersensitivity to arsenite.**

![Graph showing mean positive velocity for arsenite response]
Figure 3. NL130 mutant strain hypersensitivity to aldicarb.

Figure 4. NL130 strain hypersensitivity to arsenite.
Figure 5. NL152 strain hypersensitivity to aldicarb.

Figure 6. NL152 strain hypersensitivity to arsenite.
Refined JPL Algorithms for Toxin Agent Sensing via Multiple C. elegans

The prospects for using C elegans to monitor for environmental hazards is based on the assumption that observable behavioral changes can be reliably detected when the worms are exposed to such toxins. In experiments where known doses of toxic agents were introduced to the nematodes in a controlled environment, simple motion evidence indicates that both Aldicarb and Arsenite, both toxic agent simulants, significantly impacts worm motion in a recognizable and repeatable manner.

Single Worm Studies

Single worm motion evidence is based on tracking of genetically identical N2 strain of wild C. elegans. These worms were observed over a 5-minute time span in controlled conditions at Caltech. The individuals were plated in solutions of toxins prior to observations (30 minutes for Aldicarb, 3 hours for Arsenite). A tracking system followed the individuals while they were moving on agar. At least 6 worms were evaluated for each of the doses, reported in the Figures 7 and 8 test summaries.

Figure 7. Motion vs. milli-Molar concentrations of Aldicarb for 6 wild type nematodes. Plots show the average motion of the worms at the relevant dose. The one-sigma error bars show the deviation in individual worm motion averages.

Figures 7 & 8, linear in mM concentration, indicate that over the time period measured; individual worms show variation in motion. Approximately 20 microns/second differentiate the means of the individual worms while the deviation in speed was of the
same magnitude for the Aldicarb-treated worms. Nonetheless, the curves are quite clear. They show that a population of worms will have mean speeds that are different under controlled conditions. While it is unlikely that the standard deviation of the worm motions will be significantly different than those shown in the figures, a sufficiently large sample of worm motions could be used in discrimination of those dose levels.

The Arsenite worms trials show a similar impact. In this case, the minimum difference between the mean speeds is approximately 10 microns/sec while the mean deviation is nearly 30 microns/sec. Examining a single worm in either a Aldicarb or Arsenite environment is thus insufficient to accurately determine dose. In fact, for a field system, the results would be even more suspect due to other variants (such as temperature and humidity for instance) likely to be present and totally unaccounted for in the above curves.

**Multiple Worm Data**

Examining multiple worms on a field of agar, we show similar behavioral changes in nematode population behavior in the presence of toxins (see Table 1). In this case, 12 worms are allowed to roam while a fixed camera records their position every second. The frame to frame speed (in terms of pixels) is calculated for each worm. No attempt is made to track individual worms over time (just frame to frame) so the resultant statistics are actually comprised of the speeds of the individual, observable worms, not the individual
worm’s average speeds over the full 5 minutes observation period. The data shows that the average frame-to-frame motion of the worms is quite comparable with the average speeds of the individuals (a pixel being about 10 microns).

Table 1

<table>
<thead>
<tr>
<th>Dose (Arsenite mM)</th>
<th>Speed (pixels per second)</th>
<th>Deviation (pixels per second)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.36</td>
<td>2.49</td>
</tr>
<tr>
<td>1.25</td>
<td>3.24</td>
<td>2.14</td>
</tr>
<tr>
<td>2.5</td>
<td>1.89</td>
<td>1.78</td>
</tr>
<tr>
<td>5.0</td>
<td>1.70</td>
<td>1.85</td>
</tr>
<tr>
<td>10.0</td>
<td>0.851</td>
<td>0.90</td>
</tr>
</tbody>
</table>

This suggests that it is sufficient to capture the results of the individual worm data using a large number of nematodes. How then to separate out the various dose responses with such a large variance in motion? This of course is the key question. What we need to do is to assume that there is populations mean and variance captured by the individual mean speeds. That is, the data of the first experiment (if enough data were gathered) would show what the actual mean speeds of a population of worms would be dosed at the particular level of toxin. If we were to then follow a sufficiently large number of worms, we could employ a sample means test to get at the likelihood of the underlying dose. For these tests, the number of worms captured by the tests reduces the deviation about the mean sample by the square root of the number of worms. For instance, the mean speed of 25 worms dosed in some solution of Aldicarb would have a deviation of only 4 microns/sec from the population mean at whatever dose level they were exposed to (20 microns/sec divided by square root of 25). A simple Bayesian test could then be employed to determine the most likely dose (weighted by the cost function of having made an incorrect estimate, if so desired).

**Developing a Fieldable Toxin System Design**

Having reduced the number of measurements made on the worms from approximately 15 frames per second down to one, the computational effort needed to extract relevant measurements from nematode behavior is now well within the capability of almost all modern processing systems (including very low power units). The major difficulties are to account for the shown variance in mean speed data for any given dose level and to understand how other variations might impact a field system (worm age, temperature, humidity, etc.). Currently we have little understanding of any of the other variants impacting a field system. However, the shown variance of the test data can easily be accommodated provided that we can sample enough worms to conduct a reliable mean test.

The field system proposed here attempts to address both sources of variance. For the natural variation of nematode speed, the system will need to provide measurements from a large number of worms. As indicated in the prior section, tracking of multiple worms will allow us to employ a sample mean comparison. This reduces the deviation about the
mean by the square root of the sample number. For Aldicarb, tracking of at least 9 worms would provide a 3 sigma separation between the doses tested providing a fairly accurate estimate of the dose levels in lab conditions. For Arsenite, the resolution provided by tracking 9 worms would allow us to distinguish every other dose level tested.

To account for field variants, we propose outfitting the sensor with 3 APS chips or 3 channel sectors on one chip. One chip or sector would have no dose in the channel to establish baseline motion measurements for no toxin, one chip or sector would provide a target dose level (allowing us to estimate a dose response curve), and the final chip or sector would apply concentrated air samples to the other chip to provide an environmental dose. On each chip, a large number of wild or modified worms would be released. Their motion would be estimated at the 1 Hz update rate. Computationally, this approach is within current reach, either with the commercial 486 processor board running at reduced speeds or with a designed FPGA for power efficiency.

Figure 9 shows the component parts of our current breadboard system for monitoring *C. elegans* (or other microscopic bio-systems) for toxins. This system will be outfitted with micro-fluidic devices so that we can gather sufficient statistics on how the overall population of certain *C. elegans* strains responds to toxins. The device now works off a laptop computer. The system gathers an image each second, segments out the location of the individual worms and measures their frame-to-frame motion. Over a 5-minute interval, mean motion statistics are gathered. A number of such systems can be rapidly made and used for collecting of data. Figure 10 shows the breadboard system connected to the portable laptop computer. Figure 11 are example outputs of the program implemented to run on the PC/breadboard system.

![Figure 9](image.png)

Figure 9. Shows the breadboard system for planned *C. elegans* field data collection effort. From the top left corner in a clockwise direction shows an unpopulated APS interface board, breadboard top cover with illumination diffuser, white LED light source, and the breadboard unit with cables connecting to laptop (PCMCIA card required).
Figure 10. Breadboard with laptop processing package. This setup can be used to gather statistics on the motion of biological systems. Toxins can be introduced into the breadboard system.

Figure 11. Processing of multiple nematodes. The first picture is the original frame (in this case from the microscopic setup at Caltech). The second frame is after the application of pre-processing steps prior to thresholding. The final frame shows the centers of the worms which are then matched to worm locations in the subsequent frame to generate motion measurements (this is done at 1 frame per second).

**Microfluidic chambers on CMOS chips.**

Ultimately we will need a method of introducing nematodes for observation on the APS chips. Our first priority plan is to use soft microchambers based on microfluidic devices of S. Quake and Axel Scherer, et. al. developed under DARPA’s BioFlips support. Towards this end, we have micro-fabricated (with Mark Adams and Stephen Quake at Caltech) silicone chambers to hold nematodes for observation on the APS chips. These rectangular chambers have intake and exhaust ports and are gas permeable. We have fabricated several chambers to test for optimization of the chamber height dimension for analyzing worm movement and easy of inputting of nematodes. Chambers with 800, 250, 200 and 150 micron in height were tested. Those chamber heights shown in microscope photographs Figures 13-16 allowed facile introduction of nematodes but do not fully constrain the z-axis movement. Narrower chamber heights will also be
fabricated and tested to explore which optimal behavioral metrics will be achievable in the field portable toxic agent sensor.

The newly constructed breadboard was also used to display *C. elegans* in the microfluidics via contact imaging through the chamber’s 5 um thick bottom placed on top of the APS array and illumination with the breadboard’s packaged white light LED.

Figure 12 –Micrographs of nematodes in custom molded PDMS microfluidics 7 mm x 7 mm chambers (a) 800um chamber height with a 5 micron bottom wall thickness photographed at 25X magnification; (b) 250um chambers height with a 5 micron bottom wall thickness photographed a 12X magnification.

Figure 13. Nematodes in 200 micron high 7 mm x7 mm microfluidics chamber with a 5 micron bottom wall thickness photographed at 6x microscope magnification.

Figure 14. Nematodes in 200 micron high 7 mm x 7 mm microfluidics with a 5 micron bottom wall thickness photographed at 50x microscope magnification.
Figure 15. Nematodes in a 150 micron high, 7 mm x 7 mm microfluidic chamber with a 5 micron bottom wall thickness photographed at 6x microscope magnification.

Figure 16. Nematodes in a 150 micron high, 7 mm x 7 mm microfluidic chamber with a 5 micron bottom wall thickness photographed at 50x microscope magnification.
**NSWC Collaboration:** Mosquito Larvae tests using the *C elegans* automated behavioral system

Based on discussion with Dr. Rayms-Keller on using the JPL/Caltech portable automated motion sensing system for *C. elegans* on mosquito larvae toxic agent test challenges as well as JPL bio and chem agent *C. elegans* test trials at Dahlgren, initial tests were performed on mosquito larvae (eggs sent to Caltech/JPL by Navel Surface Warfare Center [NSWC]) to determine if algorithms suitable for tracking the nematodes could be modified to take measurements of mosquito larvae. Figure 17 provides example still image of a single mosquito larva from the video tape collects made at Caltech. Figures 18-20 show the data output from slightly modified JPL algorithm currently running on the *C elegans* breadboard behavioral test system. The mosquito larvae data may be analyzed further to determine data variation for these zero-dose organisms. Future work could compare the mosquito larvae dose responses with that of the *C. elegans*.

![Figure 17. Mosquito larva. Potential organism who’s movements can be analyzed by the same algorithms currently running on the breadboard APS system.](image1)

![Figure 18. Motion of mosquito larva. The graph plots the total distance moved (in terms of pixels) between each frame (15 FPS).](image2)
Figure 19. Shows the orientation of the bounding box’s principal axis of the mosquito larva. Both 0 and 180 represent the organism parallel with an image row.

Figure 20. Shows the ratio of the largest to smallest dimension of the bounding box on the mosquito larva.
C. Milestone Report on Program Deliverables:
  Identify behavioral metrics response characteristics to specific chemicals
  Adapt multi-node computer model of C. elegans from lab to APS (9/01 – 5/02) MET
  Demo agent dose-rate correlation w/ lab motion detection software (2/01 – 9/02) MET
  Produce C. elegans genetically eng./screened for specific hypersensitivity (2/02 –09/02) OnGoing
  Test C. elegans response to a panel of agents (10/02 – 2/03) Report results (2/03) Planned

Investigate C. elegans for hypersensitivity to specific agent
  Design (genetically engineer/screen) C. elegans for hypersensitivity to one (or more) of a panel of selected specific agents (6/02 - 9/02) Report results (9/02) OnGoing

Demonstration of C. elegans imaging readout with APS electronic biocoupling
  Investigate biocoupling by shadow time differencing (9/01  – 11/02) MET
  Establish readout patterns from APS CMOS chip (10/01 – 6/02) MET
  Design, fab prototype biocoupling interface electronics test chip (6/02 – 01/03) OnGoing
  Demonstrate C. elegans imaging readout (1/03 – 02/03) Report results (02/03) OnGoing

Progress For This 3rd Quarterly Report Period:

FUNDS: Total Received: $908.2K = FY ‘01 $248.2K + FY ‘02 $615.K Obligated 02/28/02 $752.2K: Funds Exhaustion 90-122 days

1) Demonstrated reproducible, sigmoidal behavioral metrics, dose-response curves for C. elegans movement in the presence of toxic agent simulants, aldicarb and arsenite. The EC50 for aldicarb is 0.8mM while the EC50 for arsenite is 8.2mM for the parameter mean positive velocity. (5/02) On-Schedule

2) Identified three candidate hypersensitive mutant C. elegans strains tested for movement in response to increasing doses of the toxic agent simulants aldicarb and arsenite. The cat-4 mutants, with weak cuticles, are 2.7-fold more sensitivity to aldicarb (EC50 = 0.3 mM) and 3.6-fold more sensitive to arsenite (EC50 = 2.3 mM) than are wild-type C. elegans. The strain NL130, carrying engineered deletions of pgp-1 and pgp-2, encoding P-glycoprotein-type transporters, is 2.0-fold more sensitive to aldicarb (EC50 = 0.4 mM) and 2.6-fold more sensitive to arsenite (EC50 = 3.2 mM) than wild-type C. elegans. Another strain NL152, genetically engineered to lack pgp-1, pgp-3 and mrp-1 (a toxin transporter), also showed increased sensitivity to aldicarb and arsenite but is somewhat movement impaired in the absence of toxic agents. Construction of triply mutant strains and use of these strains in direct screens for hypersensitivity is scheduled next as well as tests of additional candidate mutations developed in Caltech’s lab as well as in other labs as they become available. (5/02) Ahead of Schedule

3) Initiated two test screens to determine the optimal method for identifying of C. elegans mutants hypersensitive to toxic agent simulants (i.e. arsenite) now that robust, repeatable dose-response curves has been achieved. The screen tests, carried out in 12-well microtiter dish format, are for reduced viability or reduced movement in the presence of low (2.5-5 mM) levels of NaOAs. These tests also allow us to determine the best method of handling and analyzing large numbers of worms. Following the tests, we will readily scale up to intense screening. (5/02) On-Schedule

4) Demonstrated simpler JPL analysis algorithm, applied to multiple (~10) worm dose response curve videos provided by Caltech (at 1 Hz rate), is sufficient to
discern a factor of 2 change in dose. Grew, video taped & analyzed motion metrics of NSWC supplied mosquito larvae. (5/02) On-Schedule

5) Constructed a portable breadboard design for toxic agent panel field-testing.
Completed breadboard of portable laptop motion processor with APS sensor test chamber & wireless controller IC design for toxin field testing of large numbers of *C. elegans* and possibly other candidate sentinel organisms in a deployable type environment. Designed/fabricated new PDMS microfluidic *C. elegans* chambers and demonstrated contact APS imaging of *C. elegans* through the chamber’s 5 um bottom wall. (5/02) Ahead of Schedule

D. Future Issues and Technology Transitions

One of the foci of next quarter’s effort will be to increase sensitivity, starting with some of the hypersensitive screens to develop better nematode strains, as well other genetically engineering approaches such as transgenes that alter synaptic transmission. Another focus will be engineering the microfluidic chambers and their use on the APS chips to reproduce Caltech’s results in a field-deployable system design. At the same time that Caltech’s laboratory assays are at full steam, we want to be able to test engineered strains on as near to a fieldable device as possible.

Contacts: R. Stirbl and P. Sternberg spoke with Dr. Alfredo Rayms-Keller, the Biotechnology Group Leader (Code B51), at the Chemical Biological Systems Technology Division of the Naval Surface Warfare Center, Dahlgren Division. In telephone discussions and emails after the conference, Dr. Rayms-Keller again invited JPL/Caltech to work together with their DARPA-funded mosquito larvae toxin-sensing group to test toxic agent challenges with our portable automated motion sensing system for *C. elegans* as well as about JPL/Caltech *C. elegans* bio and chem agent test trials at Dahlgren. Discussions covered working together on surety testing of real agents, as well as providing expert technical assistance and procedural review of simulant sensitivity protocols. His colleague Ryan Mackie sent mosquito eggs to P. Sternberg’s lab that were subsequently hatched and taped for a zero-dose behavioral baseline. Both the Caltech and JPL automated toxin metric sensing system were tried on the mosquito larvae motion tapes. The JPL processing algorithm was able to produce several zero toxic baseline parameters (see Section B text) from the Caltech mosquito larvae videos tapes of NSWC provided eggs. We plan further discussions with Dr. Rayms-Keller.

JPL is continuing to work with Caltech President Fund support on incorporating an evaporated multilayer interference filter on top of a special purpose APS to permit on-chip laser-induced fluorescence studies that could have applicability to several DARPA TBB/AD/ADT efforts (i.e. Dr. Yosishacham-Diamond, Director of Nano Science and Technology Projects at Tel-Aviv University, Dr. Greenbaum’s algae fluorescent sensing efforts at Oakridge National Labs, use of the APS-based fluorescent sensing platform, Dr. Danny Dhanasekran from Temple University, doing work with modifying yeasts olfactory signal transduction systems, as well as Phil McFadden and Frank Chaplen of the University of Oregon to explore its use in their work on living cell chromatophore sensors for toxic agent sensing). These efforts could benefit from using the high
performance CMOS imager system JPL/Caltech are developing to capture a better fluorescent protein signal than is possible with commercially available products for monitoring bacteria, yeast, and human cells.

Caltech’s Mark Adams is now making good progress with a totally integrated JPL-based APS CMOS array contact microfluidic fluorescent diagnostic system under DARPA BioFlips support to Axel Scherer and Steve Quake; (DARPA Program Manager: Robert Campbell).

Another potential area of collaboration that was discussed at the DARPA PI conference and in recent phone discussions is working with Dr. Wikswo from Vanderbuilt University, who is assessing changes in metabolic function in cells and other model systems with the development of a MicroPhysiometer as an indicator to activity detection in nanoliter microfluidic channels that could be provided by Caltech.

Based on the suggestion of Dr. Rudolph and, a review is continuing into the applicability of motion feature metrics and statistical tools employed in the motion metric studies applied in the study of other biological entities. We’ve also had discussions with Dr. James Cupello who plans to visit JPL this summer to discuss how we might utilize design of experiment (DOE) techniques.
E. Appendix: Program Background

**GOAL:** The Jet Propulsion Laboratory (JPL), and Caltech (CIT), with the support of DARPA, will conduct a research and development program to produce a prototype of a revolutionary, quantitative, ultra-sensitive sensor capable of toxic-agent detection and classification. This low-cost, long-life, wireless detector will be designed to be packagable in a highly integrated, miniaturized enclosure ruggedized for high-impact deployment.

This advanced detector concept integrates a newly developed, automated quantitative metric of the response of the sub-millimeter nematode worm *C. elegans* to known external stimuli that Caltech has already demonstrated for altered gene function. The essence of this task is to transfer these demonstrated metrics into on-chip image processing for autonomous readout and to produce new signature metrics for agents of specific Department of Defense (DOD) interest by genetically engineering *C. elegans*. The proposed effort will employ JPL techniques to insert this proven metric for analyzing *C. elegans* movements onto a miniaturized sensing platform using an inexpensive, ultra-low power, “smart” complementary metal oxide semiconductor (CMOS) imager chip as a contact on-chip microscope that replaces the macro-scale, large laboratory microscope and processing computer. This biotechno-symbiosis sensor comprising a biological entity will respond predictably to a specific toxic environment and will act, in effect, as a quantitative, wireless “canary-on-a-chip” activity detector. For this proposed effort *C. elegans* nematodes will be engineered or selected for their improved agent sensitivity. They will then be plated in an appropriate nutrient medium onto the electronic biocoupling/processing detector array system that senses and reports any quantized reaction to that specific agent. Biocoupling is accomplished by the processing of lensless contact images of this selected bioentity’s motion. When movements characteristic of a given toxic signature are detected via the on-chip processing, the processor autonomously takes action to transmit the signal. Activity detection of signatures from unknown nerve agents could also induce a response resulting in a transmitted signal.

**BENEFIT:** Unlike conventional methods, this approach does not rely upon classical large-scale sensible parameters coupled with a separate external computer-based algorithm to deduce the presence of toxic agents. Toxicity is determined directly by a sensed metric of nematode movements in response to the agent(s) to which they were bred to react.

This innovation, as discussed, is anticipated to lead to the demonstration of a compact, highly sensitive, wireless detection system capable of functioning covertly in an unattended environment. Because this sensor uses a sub-millimeter biological entity as its first line of sensitivity and capitalizes upon the widely available and power-efficient CMOS-compatible integrated microimager electronics, its reliability is very high and it’s cost very low.

**TECHNICAL APPROACH:** The essence of this effort (see schedule in figures 1 & 2) is the following: 1) quantification of dose response to agent simulants in a laboratory set-up at Caltech, 2) proof-of-principle investigation and demonstration of on-chip *C. elegans* imaging with off-chip processing for autonomous readout, followed by 3) a transfer of these demonstrated agent dose software metrics into chip-level hardware to produce a new signature metric agent-activity assay of specific DOD interest via 4) genetically engineering the *C. elegans* for increased sensitivity and agent specificity, and 5) conducting actual agent tests at government field test facilities (i.e., Dalhgren, Dugway, USAMRIID, Battelle Labs, etc.). The effort employs Caltech expertise in adapting the existing metrics for on-chip worms, and JPL techniques to insert this proven metric software for analyzing *C. elegans* movements onto a miniaturized sensing platform using an inexpensive, ultra-low power, “smart” CMOS imager chip as a contact microscope with on-chip and hybridized processor interframe feature detection that replaces the macro-scale, large laboratory microscope and processing computer. This biotechno-symbiosis sensor comprising a biological entity will respond predictably to a specific toxic environment and will act, in effect, as a quantitative, wireless “canary-on-a-chip” activity detector. For this proposed effort, a strain of mutated or genetically engineered bioentity, *C. elegans* nematodes, will be selected for their improved agent sensitivity.

**Key Milestones**
The milestones and anticipated deliverables from this task are the following:

**Phase I:**

- Demonstration of *C. elegans* imaging readout with APS electronic biocoupling
- Report of agent-simulation dose-rate correlation with laboratory automated motion software
- Demonstration of a genetically engineered *C. elegans* hypersensitivity to one (or more) of a panel of selected agents.
- Phase I interim report

**Phase II:**

- Demonstration of software-to-hardware transfer of motion-metrics functions
- Demonstration of a prototype of a complete electrobioptic toxic-agent sensor
- Design of an integrated sensor system to include the motion-metrics ASIC, the APS biocoupler, and a wireless transmitter
- Final report

*Caenorhabditis elegans* with desired properties can either be engineered using transgenic and recombinant DNA technology, or identified by screening mutagenized populations of worms for genetic variants with the desired properties. Caltech has carried out hundreds of such genetic screens in other projects.

To extend the range of response or alter the sensitivity to agents, *C. elegans* can be partially “humanized” by constructing transgenic strains expressing human genes in addition to, or instead of, nematode genes. Another potential issue is the sensitivity of *C. elegans* to a particular compound. Significantly higher concentrations of agents are typically needed to exert effects on *C. elegans* than on mammalian tissue culture cells. However, some strains with altered cuticle (e.g., *cat-4*) generally lower doses are needed, indicating that the cuticle might be engineered to allow higher sensitivity. Moreover, the ability to genetically alter *C. elegans* should allow hypersensitivity to specific agents to be programmed, overriding any problems in permeability. Another approach to the permeability problem is to engineer sensory neurons that ultimately control motility through olfactory, mechanosensory, and chemosensory modes to respond to the agent of interest. Specific promoters for such cells exist, and the metrics developed by Caltech are expected to be sufficient as readout of these neurons.

In Phase I as we are calibrating the imaged readout of the *C. elegans* on an APS chip, it will be necessary to compare the responses measured by the APS electronic biocoupler with those obtained under the microscope. This specific task will involve the comparison with metrics measured on Petri plates in bacterial lawns with respect to known genetic alterations affecting muscle contractile force generation; general neural transmission; and specific neural transmitters. These metrics include amplitude of sinusoidal wave of muscle contraction, rate of wave propagation along the worm's body, and frequency of muscle contraction. We will adapt demonstrated quantifiable metrics of the *C. elegans* movements to respond to specific agent simulants of DoD interest. The existing multi-node *C. elegans* computer model will be modified to reflect the new response signatures, and responses of known genetic alterations will be quantified. This task will help define the requirements for genetically engineered *C. elegans* that are hypersensitive to specific agents. *C. elegans* responsive to a specific panel of toxic agents of strategic DoD interest will be designed during this phase. In addition Phase I efforts will produce *C. elegans* designed for specific agent hypersensitivity by well-understood genetic engineering techniques. The *C. elegans* response to a panel of agents will be tested to determine characteristic response signatures, and the movement metrics quantifying the new signatures will be refined. This task will involve the demonstration of a specialized *C. elegans* made hypersensitively responsive to a specific agent or agent class via screening techniques and genetic engineering, where these engineering efforts seek to make the nematodes sensitive to agents not typically affecting them (e.g., “humanizing” the nematode by insertion of mammalian genes into its genome).
Phase II of this program will take place over the following eighteen months during which we will produce *C. elegans* designed for a broader or expanded class of agent hypersensitivity by well-understood genetic engineering techniques. The *C. elegans* response to an extended panel of agents will be tested to determine characteristic response signatures, and the movement metrics quantifying the new signatures will be refined. This task will involve the demonstration of a specialized *C. elegans* made hypersensitively responsive to both new specific and broader agent classes via screening techniques and genetic engineering, as performed in Phase I. Other genetic engineering efforts will control permeability of nematodes to exogenous agents. Screening efforts will proceed in parallel to include “irrational” or serendipitously harvested “design” principles, in addition to the genetic engineering approach. In the meantime, the circuit design of the FPGA-development breadboard biocoupling electronics will be integrated into a test chip and characterized for different bias conditions and temperatures.

A second task in Phase II will also involve integrating the motion-metric analytic functions into hardware. As with the biocoupler, the motion-metric electronics will first be configured as a FPGA-development breadboard for ease of component changes to facilitate optimization. The software functions will then be modeled into their respective hardware elements, integrated with the APS biocoupler, and tested first for functionality and then with the new *C. elegans* and agent simulant panels, as well as real agent panel tests at government field test facilities (i.e., Dugway, USAMRIID, Battelle Labs, etc.). The ultimate goal is to design and fabricate a miniaturized, agent-activity relevant, application specific integrated circuit (ASIC) embodying the operations of the motion-metric FPGA-development breadboard hardware and software.

The final task in Phase II is to design and produce the integrated system architecture to include the new motion-metrics ASIC and the APS biocoupler with a wireless communication system. An associated laboratory setup for a full-system array will be characterized to demonstrate the capabilities of integrating both proven and new design approaches. The final product of this effort is expected to be a prototype of a highly-integrated, ultra-low power, Electrobiotic Toxic-Agent, Dose Response Sensitive, Activity Sensor that is anticipated to substantially improve sensitivity for such applications as advanced mission support, treaty compliance, and stand-off situational awareness.

**Meeting Activity Detection Technology (ADT) Program Goals**

This work is directly in line with DARPA’s ADT programmatic goals of using biological systems for the detection of chemical, biological, and other toxins of interest to DOD needs. Although this effort primarily pertains to the Assay Development and the Chip Interface Engineering and Design areas of research identified in BAA 01 05, throughout the course of developing an electrobiotic sensor we will address, to some degree, each of the remaining four research areas. For example, the “shelf-life” of the *C. elegans* is an obvious concern, which falls under Longevity and Stability; how we extract useful information from the sensor relates to Computational Design, Statistical Analysis and Data Mining Tools; Sample Collection and Preparation must be addressed; and System Modeling and Integration, specifically the integration, must be dealt with to assure proper interconnection among the various critical components. The electrobiotic sensor brings in the full-system viewpoint of a biocoupled means for the detection and classification of toxins.
Figure A1. (below) A two-phase program enables discretely identifiable development stages.

Figure A2. (below) Phase I 18 month program – Detailed from Fig. A1

3rd Qtr Progress: On or Ahead of Schedule, Dose Response Now Reproducible, Hypersensitivity Screens Underway, and a Preliminary Breadboard Design for a Field Portable Toxic Agent Sensing System was Built