RDT&E BUDGET ITEM JUSTIFICATION SHEET (R-2 Exhibit)							DATE February 2006
APPROPRIATION/BUDGET ACTIVITY RDT&E, Defense-wide BA2 Applied Research			R-1 ITEM NOMENCLATURE Biological Warfare Defense PE 0602383E				
COST (In Millions)	FY 2005	FY2006	FY2007	FY 2008	FY 2009	FY 2010	FY 2011
Total Program Element (PE) Cost	155.360	148.108	112.242	110.695	110.618	110.914	110.414
Biological Warfare Defense Program BW-01	155.360	148.108	112.242	110.695	110.618	110.914	110.414

(U) Mission Description:

(U) DARPA's Biological Warfare Defense project is budgeted in the Applied Research Budget Activity because its focus is on the underlying technologies associated with pathogen detection, prevention, treatment and remediation. This project funds programs supporting revolutionary new approaches to biological warfare (BW) defense and is synergistic with efforts of other government organizations.

(U) Efforts to counter the BW threat include developing barriers to block entry of pathogens into the human body, countermeasures to stop pathophysiologic consequences of biological or chemical attack, host immune response enhancers, medical diagnostics for the most virulent pathogens and their molecular mechanisms, biological and chemically-specific sensors, advanced decontamination and neutralization techniques, and integrated defensive systems, including detection of chemical and biological agents in sealed containers at entry points of facilities. This program also includes a unique set of BW sensors that will greatly improve sensitivity while decreasing response time. Program development strategies include collaborations with pharmaceutical, biotechnology, government, and academic centers of excellence.

(U) Program Accomplishments/Planned Programs:

	FY 2005	FY 2006	FY 2007
Unconventional Therapeutics	38.380	37.202	35.000

(U) This thrust is developing unique and unconventional approaches to ensure that soldiers are protected against a wide variety of naturally occurring, indigenous or engineered threats. Past successes in this effort have come from developing therapeutics that are designed to work against broad classes of pathogens. This has led to several significant transitions, a separate thrust in Anthrax countermeasures, and most recently

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a new program at DTRA that directly capitalizes on previous DARPA investments. Work in this area has also uncovered new approaches to therapeutics that, rather than attacking specific pathogens, enhance human innate immune mechanisms against broad classes of pathogens. Not only will these approaches be more effective against known pathogens, they also promise to offer substantial protection against unknown pathogens including engineered pathogens and emerging pathogens from third-world environments. An emphasis is on the discovery and development of technologies that will allow a rapid response (within weeks) to unanticipated threats, whether they are naturally encountered emerging diseases or agents from intentional attack. In this regard, this thrust addresses the development of in vitro systems that directly mimic the human immune response and can be used for rapid development and screening of human vaccines. An additional focus is the development of entirely new technologies that will allow the rapid, cost-effective manufacture of complex therapeutic proteins such as monoclonal antibodies and vaccine antigens.

(U) Program Plans:

- Demonstrated and transitioned to DoD a phage-based therapy and assay for anthrax.
- Demonstrated the ability of CpG to enhance the effectiveness of the Anthrax Vaccine Absorbed (AVA) anthrax vaccine by 8-fold.
- Demonstrated a new antimicrobial target for CIPRO-resistant anthrax that is common to all CIPRO-resistant organisms.
- Demonstrated siRNA as a new platform against highly virulent influenza and other respiratory viruses.
- Demonstrated inhibitors of apoptosis as potential countermeasures to anthrax and other pathogens.
- Developed in vitro fabrication of three-dimensional tissue constructs, bioscaffolds and bioreactors.
- Demonstrated that precursor immune cells can be expanded and differentiated into reactive T cells in the artificial immune system.
- Demonstrated generation of functional immune structures by dendritic cells in the artificial immune system.
- Demonstrated antibody class switching in the artificial immune system.
- Develop and demonstrate an integrated in-vitro immune system that will emulate the human immune response in order to provide a means of evaluating new BW vaccines and therapeutics.
- Demonstrate the ability to predict known vaccine immunogenicity in humans solely by testing in the artificial immune system.
- Develop a scalable, stable, *in vitro* system for the production of the cellular components of human blood to reduce military logistics burden and improve survival during CBRN attack.
- Develop and validate new in vitro systems to predict toxicology of vaccines and immune modifiers.
- Develop a technical framework for the synthesis of millions of doses of a protein therapeutic within 12 weeks.
- Develop new approaches for rapid, high-yield synthesis of therapeutic proteins in bacteria, fungi, and yeast.

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- Develop new methods for purification of therapeutic proteins from high-yield fermenters.
- Develop new approaches for assuring correct folding and mammalian post-translational modification of proteins by bacteria and fungi.

	FY 2005	FY 2006	FY 2007
External Protection	11.000	15.500	16.542

(U) This program is developing and demonstrating a variety of external protection technologies to protect soldiers from the hazards of chemical, biological and radiological attack and other hazards such as large unstable weapons stores. The program includes the autonomous detection and self-cleaning of surfaces contaminated by an attack, and the safe neutralization of hazardous materials.

(U) Program Plans:
- Developed new approaches for self-decontaminating surfaces that will be self-cleaning and be able to deactivate spores.
- Developed and demonstrated new approaches for widespread external decontamination.
- Design, develop and demonstrate systems to detect contaminated surfaces down to the human toxicity levels, and to remove the contamination to below those levels.
- Develop and demonstrate active coatings that can be applied to buildings to provide protection against chem-bio attacks.
- Develop and demonstrate a microbial based demilitarization of such hazardous materials as explosives stockpiles.

	FY 2005	FY 2006	FY 2007
Advanced Diagnostics	6.000	7.854	8.000

(U) In the early stages, many illnesses caused by biological warfare (BW) agents have flu-like symptoms and are indistinguishable from non-BW related diseases. Early diagnosis is key to providing effective therapy. The advanced diagnostics program will develop the capability to detect the presence of infection by biological threat agents, differentiate them from other pathogens (including those of non-BW origin), and

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identify the pathogen even in the absence of recognizable clinical signs and symptoms (i.e., while the pathogen numbers are still low). Novel approaches including the use of breath and advanced mathematical analysis will be examined.

(U) Program Plans:

- Demonstrated new rapid microfluidic diagnostic technique for separation of pathogens and immune cells.
- Demonstrated the detection of volatiles specific for explosives in the breath of explosive handlers.
- Develop hyperspectral approaches for presymptomatic diagnosis of exposure to pathogens or other medical issues (including naturally occurring disease) that affect soldier health and performance.
- Validate the presence of explosive volatiles in breath in the presence of a number of confounder variables.
- Adapt biosensors for breath-based diagnostics.
- Evaluate and demonstrate multiplexed pathogen detection in microliter samples.
- Develop new mathematical and diagnostic approaches to interpret biosignature data from individuals to determine if there will be a change in physiological status from health to disease and vice versa. Use these data to identify the kind of disease and need for treatment.

	FY 2005	FY 2006	FY 2007
Sensors	46.480	48.000	35.000

(U) The Sensors program goal is to develop a unique set of BW sensors that will greatly improve sensitivity and response time to bacteria, viruses and/or toxins.

(U) The overall goal of DARPA's Handheld Isothermal Silver Standard Sensor (HISSS) program is to develop a sensor that is capable of detecting the entire biological warfare threat spectrum (bacteria, DNA viruses, RNA viruses and protein toxins) with the same "silver standard" specificity as current laboratory techniques, but in a fast, reliable, handheld unit. Today, this standard is achieved for DNA and RNA threats using polymerase chain reaction, which is slow because of the associated temperature cycling. For proteins, the standard is met using Enzyme Linked Immunosorbent Assay (ELISA), which requires skilled laboratory technicians to complete. The equipment required for these tests is bulky and difficult to use under field conditions. Under HISSS, DARPA will develop fundamentally new ways to exploit previously developed identification

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mechanisms (DNA and RNA primers, protein antibodies) in an integrated, isothermal system that will allow a single, handheld sensor to detect the full range of BW threats.

(U) Program Plans:
- Developed isothermal assays for DNA, RNA and protein toxins and demonstrated a false-alarm rate equivalent to the current laboratory technology.
- Developed a microfluidics testbed for assay optimization and system integration.
- Demonstrated that HISSS isothermal assays have a false alarm rate that is better than the current laboratory technology.
- Develop stabilized reagents for fieldability.
- Design and build a prototype HISSS device.
- Characterize HISSS prototype in laboratory and operational environments.

(U) Triangulation Identification for Genetic Evaluation of Biological Risk (TIGER). Most nucleic acid-based sensors search for an exact sequence match to some unique part of each pathogen. This requires a unique set of primers and probes for every target pathogen; it also means that the sensor can only determine whether that specific (portion of the) target pathogen is present. DARPA is developing a new kind of DNA-based sensor that searches out the universal parts of the genetic code and looks for species-specific variation between these regions. This TIGER sensor will enable a universal sensor for all pathogens and also holds the promise of detecting the presence of never-before-seen (bio-engineered) agents.

(U) Program Plans:
- Develop capability to perform phylogenetic classification of unknown or genetically modified organisms.
- Optimize system to perform automated calibration to quantify number of organisms present in samples and allow multiplexing of primers to reduce costs.
- Transition and deploy fieldable prototype to support operational bio-protection efforts at USAMRIID and NHRC San Diego and forensic analysis at National Bioforensic Analysis Center (NBFAC).

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(U) Spectral Sensing of Bio-Aerosols (SSBA). Active probing of bioaerosols with electromagnetic (EM) energy holds the promise of extremely fast, and potentially long-range, detection and identification of bio agents. Only a small portion of the EM spectrum is exploited in today's trigger sensors (e.g., optically based particle sizers, sometimes enhanced with fluorescence measurements). However, anecdotal evidence suggests that other portions of the spectrum may offer substantial improvement in trigger sensors, as well as potentially agent-specific discrimination capability. Various types of spectra in the visible, infrared, and additional UV wavelengths are being measured in laboratory or early prototype systems. Additional spectral information such as UV fluorescence lifetime and single particle mass spectroscopy is also being evaluated. DARPA is investing in this approach, beginning with cross-spectrum data collection and performance models, followed by prototype sensor development. An aerosol testbed has been developed to provide calibrated exposures of threat agent simulants.

(U) Program Plans:

- Completed bioaerosol testbed and standardized data-collection protocols to allow the new sensor technologies to be challenged with both threat agent simulants and typical interferents such as diesel smoke, pollen and natural fibers.
- Investigated spectral response of chemicals unique to BW agents (e.g., picolinic acid in anthrax spores).
- Collected data, and developed performance model, for concepts that exploit a wide part of the electromagnetic (EM) spectrum (e.g., Raman scattering, terahertz spectroscopy, laser-induced breakdown spectroscopy, coherent Raman anti-Stokes spectroscopy, IR/photoacoustics, etc.).
- For sensors that can characterize and separate single particles, evaluated use of mass spectrometry for particle identification.
- Downselected to most promising concepts; design, build, and test prototype sensor.
- Characterize prototype behavior in operational environments.

(U) Rapid Proteomics to Detect Bioengineered Threats. The goal of this program is to develop and employ rapid proteomic approaches for the detection and identification of bioengineered microorganisms. These organisms potentially can be designed to have the lethal effects of dangerous pathogens by incorporating the genetic sequences that encode toxins into otherwise benign, naturally occurring organisms. The general concept for this type of bioengineering is well known and used extensively industrially to produce such products as human insulin. Clearly such biotechnology could be subverted to produce materials dangerous or deadly to humans while evading conventional detection. A novel approach to detecting such organisms would target the active, functional components required to engineer the organisms. For instance specific types of antibiotic resistance are nearly universal properties of engineered organisms. A method will be developed that uses rapid protein detection

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coupled with bioinformatics to target the proteins associated with these antibiotic resistance genes. Thus this universal pathway to bioengineered organisms would be detected. This approach would close a critical gap in the detection of advanced biological threats to the military.

(U) Program Plans:
- Develop target sets of protein signatures indicative of genetic manipulation.
- Develop and test proteomic tools to detect target protein signatures.
- Integrate proteomic tools into detection systems for genetically manipulated organisms.
- Conduct laboratory and field tests of detection system.
- Transition systems to Joint Program Executive Office - Chem Bio Defense (JPEO-CBD).

(U) Remote Radiation Detection via Intercepted Ultraviolet Scintillation (RRaDIUS). The RRaDIUS program will develop coatings containing crystals that scintillate ultraviolet (UV) photons upon interaction with ionizing radiation. RRaDIUS will also develop deployment and detection systems that will allow for surreptitious coating application and detection of UV photons at a distance. A notional approach includes the firing of a "paintball" that will coat a vertical surface. Resulting UV photons emitted from the coating will be intercepted remotely to determine the radiation level at the coated surface. RRaDIUS will leverage coating technologies developed in the Wide Area Radionuclide Detection (WARD) portion of the Radiation Decontamination (RD) Program. RRaDIUS will also develop new technologies for the collection and amplification of signal from small area (square centimeter range) coatings at a distance.

(U) Program Plans:
- Evaluate RD Program/WARD-developed technologies to identify a UV scintillant that is activated via ionizing radiation and emits UV photons near the solar blind region.
- Incorporate the scintillant into a ballistic-delivered coating or paint; match with existing ballistic paint delivery systems.
- Develop a remote detection, UV camera capable of identifying and quantifying the UV scintillation photons at a distance of at least 100 meters, and sustaining an indirect detection capability of a minimum of 1 microGray (100 microrads) per hour.

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	FY 2005	FY 2006	FY 2007
Immune Buildings	21.000	12.500	0.000

(U) DARPA is developing technologies for integrated defensive systems to be employed in military buildings to protect and respond to the emerging threat of aerosolized Chemical, Biological and Radiological (CBR) releases. The approach is to modify and augment the infrastructure of buildings to allow them to sense and defeat an attack by bio or chem agents in real-time and to find and remove hazardous radiation left behind by a "dirty bomb." The program has three goals: to protect the human inhabitants from the effects of the agents; to restore the building to function quickly after the attack; and to preserve forensic evidence for treatment of victims, if necessary, and for attribution. For CB releases, the DARPA focus is on the challenging problem of protection from internal releases of agent, where active and timely control of airflow is required to prevent a building's HVAC system from spreading the agent throughout the building. To enable such building-protection systems, DARPA is developing component technologies such as optimized filtration systems, advanced neutralization techniques, active building coatings, and remediation techniques appropriate to biological, chemical, and radiological decontamination. In addition, DARPA is investigating the systems-level issues of integrating and optimizing such active systems, including the integration and adaptation of sensors, as well as the simulation of threat events and emergency responses. Several new chemical and biological sensors have been identified for development to address problems that are unique to the building application. Self-assembling nano-structures for building sealants will be investigated to quickly and inexpensively coat building exteriors and completely seal the building, thereby making effective the defensive strategy of sheltering in place. These efforts have used full-scale test facilities to determine the effectiveness of protection components and the optimal architectures for protection. These systems are being transitioned to a full-scale demonstration of a complete building protection system at a military installation and will also leave behind a software tool for the design and optimization of building-protection systems for other military facilities.

(U) Program Plans:

- Developed high-payoff component technologies in the areas of filtration, neutralization, and decontamination; and matured sensors for active CWA/BWA defense applications.
- Continue development of neutralization and building sealant technologies and reduced-false-alarm CW and BW sensors.
- Transitioned rapid-viability testing methods to USAMRIID and Department of Homeland Security (DHS); and decontamination techniques to Joint Program Executive Office–Chem Bio Defense (JPEO-CBD), DHS, and Environmental Protection Agency (EPA).
- Demonstrated performance of component technologies in full-scale prototypes.

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- Optimized active protection system concepts and demonstrated performance in full-scale tests.
- Integrated existing models, and developed new models when required, into a software toolkit that enables performance predictions for protective architectures for diverse building types.
- Selected a site for full-scale demonstration in an operational military building.
- Characterize the demonstration site facility and develop a prototype active protection system optimized for that site.
- Validate toolkit predictions in full-scale test beds and at demonstration site.
- Extend the software toolkit to provide cost analysis of protective system and further validate with performance and cost data from the demonstration site.
- Develop Stimulated Hyper-Accelerated Radionuclide Kinetics (SHARK) technologies to hyper-accelerate description of radioactive contamination within building materials and to rapidly mobilize the contamination of outer building surfaces for more efficient removal.
- Install complete IB protective system in an active military facility at Ft. Leonard Wood, MO.
- Transition IB systems to the US Army Chemical School and US Army Corps of Engineers.

	FY 2005	FY 2006	FY 2007
Chem Bio Defense (CBD) Portal Security	5.000	0.000	0.000

(U) There is an enormous payoff in preventing the release of biological warfare agents (BWAs) and chemical warfare agents (CWAs), rather than trying to minimize the damage they cause once released. For this reason, DARPA has invested in technologies and systems to prevent such materials from entering buildings, either in packages or mail, concealed in normal maintenance materials such as wax or paint or as an item hand-carried by a visitor. A variety of energy sources and sensors have been evaluated for their ability to penetrate package and container materials and obtain signatures for anomaly or hazard detection/identification. Novel destruction methods for BWAs were also evaluated. This program is transitioning to the OSD Chemical/Biological Defense Program, PE 0603384BP, Project TT3.

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(U) Program Plans:
- Evaluated non-intrusive technologies for destruction of biological agents (e.g., ultrasound, variable frequency microwave and new techniques for X-Ray and gamma irradiation) and/or for the detection of chemical agents (e.g., associated particle neutron elemental analysis, tera-hertz spectroscopy, dielectric spectroscopy, and swept frequency acoustic interferometry).
- Selected the most promising approaches, and used laboratory instrumentation to evaluate collateral damage and false alarms.
- Developed performance models, carried out system trades, and developed required prototypes/components.

	FY 2005	FY 2006	FY 2007
Threat Agent Cloud Tactical Intercept Countermeasure (TACTIC)	8.500	12.500	10.000

(U) The TACTIC Program will develop and demonstrate the capability to (1) rapidly detect, discriminate and identify an airborne chemical warfare agent/biological warfare agent (CWA/BWA) battlefield threat at stand-off distances, and (2) use countermeasures to neutralize and/or precipitate the threat before it reaches the targeted troops. This program will investigate identification methodologies including: bead-based assays for biological molecules, fluorescent assays for chemicals, retro-reflector assays for chemical and biological agents; all of which can be interrogated with stand-off optical detectors. To accomplish the removal of the threat, technologies that mimic the seeding of rain clouds will be developed for particulate bio-agents, and technologies that polymerize chemical agent vapor will be investigated. Upon successful demonstration of the identification and removal technologies, a system will be developed to demonstrate the removal of chemical and biological simulant clouds from the battlefield.

(U) Program Plans:
- Investigate technologies for CWA/BWA standoff assays that rapidly (within one minute) identify agents.
- Investigate technologies to remove the agent cloud so as to eliminate the threat to unprotected war-fighters.
- Develop models of identification and removal technologies. Carry out systems trades between competing identification and removal technologies.
- Integrate optimal identification and removal components into a prototype system.
- Test prototype system in scaled aerosol test chambers.

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- Demonstrate system in full-scale field trials.
- Transition to Joint Program Executive Office - Chem Bio Defense (JPEO-CBD).

	FY 2005	FY 2006	FY 2007
Mission-Adaptable Chemical Sensors (MACS)	5.500	10.652	7.700

(U) At present, chemical sensors are unable to combine sensitivity (parts-per-trillion) and selectivity (unambiguous identification of molecular species) with low false alarm rate. This effort will develop a sensor, based upon rotational spectroscopy of gases that will have superior capability in all categories; it will achieve the highest possible sensitivity (parts-per-trillion) for unambiguous detection of all chemical species. A preliminary blind test showed complete and unambiguous identification with a sampling time of one second and a false alarm probability below 0.001%. At present, the program has investigated the nature of the atmospheric background "clutter" at the parts per billion (ppb) level and below to enable the identification of target signatures at highest sensitivity. The program will focus on reduction of size and simplicity of function to achieve portability and simultaneous detection of a large number (hundreds) of species. The capabilities will far surpass all other current sensors.

(U) Program Plans:

- Design and build a table top form factor, high-sensitivity sensor system and demonstrate its performance in a high-clutter atmospheric background.
- Demonstrate fractionation and related improvements to the system for simultaneous identification of multiple species in seconds.
- Refine table top form factor design and build a fully portable, high-sensitivity sensor system.
- Demonstrate the fully portable, high-sensitivity sensor system in a high-clutter atmospheric background.

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	FY 2005	FY 2006	FY 2007
Center for Water Security	1.000	0.000	0.000

(U) Program Plans:
- Established the Center at the University Wisconsin-Milwaukee through engaging essential technical personnel, acquiring state-of-the-art instrumentation dedicated to researching new and highly effective methods of water quality sensing.
- Continued to develop the use of the new methodologies through partnerships with public and private sector agencies to address water security issues related to civilian and military needs.

	FY 2005	FY 2006	FY 2007
Asymmetrical Products for BWD	2.000	1.300	0.000

(U) Program Plans:
- Continue to develop a technical approach to induce mucosal immunity against BioWarfare (BW) pathogens. Modeled and synthesized a cytokine-based family of compounds that stimulates mucosal immunity.
- Identify likely cytokine molecules and their combinations that result in resistance to pathogens.

	FY 2005	FY 2006	FY 2007
Center for Tropical Disease Research and Training	2.800	0.000	0.000

(U) Program Plans:
- Continued to examine *Leishmania* parasites to identify both *Leishmania* and sand fly molecules that may be useful in developing a protective vaccine against leishmaniasis, a serious disease affecting soldiers returning home from Iraq.

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	FY 2005	FY 2006	FY 2007
New Approaches to Weaponized Infections Organisms	1.000	0.000	0.000

(U) Program Plans:
- Evaluated potential new targets for antibiotics based on enzymes.

	FY 2005	FY 2006	FY 2007
Noninvasive Biomodulation	2.600	2.100	0.000

(U) Program Plans:
- Demonstrated new non-invasive approaches to biomodulation.

	FY 2005	FY 2006	FY 2007
Antimicrobial Research Program	2.100	0.000	0.000

(U) Program Plans:
- Developed new approaches for antimicrobial compounds.

	FY 2005	FY 2006	FY 2007
Bioscience Center for Informatics	1.000	0.000	0.000

(U) Program Plans:
- Developed new mathematical concepts to attack large biological data sets.

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	FY 2005	FY 2006	FY 2007
Chemically Programmable Immunity	1.000	0.000	0.000

(U) Program Plans:
- Demonstrated the use of novel strategies to usurp the natural immune system to fight and remove pathogens.

	FY 2005	FY 2006	FY 2007
Specific Gas Detector	0.000	0.500	0.000

(U) Program Plans:
- Develop new approaches for the detection of specific gases.

(U) **Program Change Summary:** *(In Millions)*	**FY 2005**	**FY 2006**	**FY 2007**
Previous President's Budget	159.567	145.354	144.050
Current Budget	155.360	148.108	112.242
Total Adjustments	-4.207	2.754	-31.808
Congressional program reductions	-0.123	-2.146	
Congressional increases	0.000	4.900	
Reprogrammings	0.000		
SBIR/STTR transfer	-4.084		

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(U) Change Summary Explanation:

FY 2005	Decrease reflects the DOE transfer directed by P.L. 108-447 and the SBIR/STTR transfer.
FY 2006	Increase reflects four congressional adds for biological defense enhancements, gas detection and noninvasive biomodulation offset by undistributed reductions for Section 8125 and the 1% reduction for Section 3801: Government-wide rescission.
FY 2007	Decrease reflects the completion and transfer of the Immune Building program, completion of the TIGER and SSBA sensor systems, and repricing of several countermeasure, diagnostic, and sensor programs.

(U) Other Program Funding Summary Cost:

- Not Applicable.

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