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Toxicology Study No. S.0065662A
Protocol No. 0FMA-92-iv17-03-01 T,U,V,W

**MICROTOX® ACUTE TOXICITY TESTING OF NOVEL ENVIRONMENTALLY
SUSTAINABLE BINDERS FOR ENERGETIC FORMULATIONS**

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Microtox® Acute Toxicity Testing of Novel Environmentally Sustainable Binders for Energetic Formulations

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Study Completed

August 2019

Performing Laboratory

Army Public Health Center
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Aberdeen Proving Ground, MD 21010-5403

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Good Laboratory Practice Compliance Statement

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards, except for the following:

1. The test article characterization (purity) was conducted by the manufacturer and it is not known whether the testing was done in compliance with the above regulation.
2. Due to time constraints, the method of analysis for these compounds could not be validated by the Laboratory Sciences Directorate prior to the study start in compliance with study protocol and modification requirements. Because of this the dosing solutions used for all tests were verified after being frozen (at - 80 degrees Celsius) until the method could be validated by the LAB after the study was completed.

No deviations from the aforementioned regulation affected the quality or integrity of the study or the interpretation of the results.



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14 May 2020

Date

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COMMONLY USED TERMS

AERTA	Army Environmental Research and Technology Assessment
AFNOR	Association Française de Normalisation
APHC	U.S. Army Public Health Center
AR	Army Regulation
CASRN	Chemical Abstracts Service Registry Number
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
COGA	cyclooct-1-yn-3-glycolic acid
DA	Department of Army
DIN	Deutsches Institut für Normung
DMSO	dimethyl sulfoxide
DOD/DoD	Department of Defense
DoDI	Department of Defense Instruction
EC ₅₀	median effective concentration
ESOH	Environmental Safety and Occupational Health
GHS	Global Harmonization System
GLP	Good Laboratory Practice
HDI	hexamethylene diisocyanate
IBF-OH	p-(3-phenyl-1-isobenzofuranyl)phenol
IBF-Ome	1-phenyl-3-(p-{10-[3-phenyl-1-isobenzofuranyl]phenoxy]decyloxyphenyl)isobenzofuran
ISO	International Organization of Standardization
L	Liter
LAB	Laboratory Sciences Directorate
LC ₅₀	median lethal concentration
µL	microliters

mg	milligrams
mL	milliliters
mg/L	milligrams per liter
mg/ml	milligrams per milliliter
min	minutes
MRL	Minimum Risk Level
NVN	Nederlandse voornorm
OECD	Organization for Economic Co-operation and Development
PCL	PCL-(cyclooctyne)
ppm	parts per million
RDT&E	research, development, testing, and evaluation
SERDP	Strategic Environmental Research and Development Program
SOP	standing operating procedure
TDI	toluene diisocyanate
TOX	Toxicology Directorate
UNECU	United Nations Economic Commission for Europe
USACHPPM	U.S. Army Center for Health Promotion and Preventive Medicine

TOXICOLOGY STUDY NO. S.0065662A
MICROTOX ACUTE TOXICITY TESTING OF NOVEL ENVIRONMENTALLY SUSTAINABLE
BINDERS FOR ENERGETIC FORMULATIONS

1 Summary

1.1 Overview

The energetic and toxicological properties of cyclooct-1-yn-3-glycolic acid (COGA), 1-phenyl-3-(p-{10-[3-phenyl-1-isobenzofuranyl]phenoxy]decyloxyphenyl)isobenzofuran (IBF-Ome), p-(3-phenyl-1-isobenzofuranyl)phenol (IBF-OH), and PCL-(cyclooctyne) (PCL) have been assessed as potential replacements for isocyanate-based binder replacements in energetic formulations. This study evaluated the aquatic toxicity of COGA, IBF-Ome, IBF-OH, and PCL with the Microtox® Acute Toxicity Test System, a bioluminescent bacterial aquatic toxicity test. The data from this study are used to assist in making environment and health-based decisions regarding the design and selection of formulas and materials for further development of new munition compounds.

1.2 Purpose

The study provides environmental and occupational health information on new or replacement compounds for military use. This information is critical to the Research, Development, Testing, and Evaluation (RDT&E) of munition formulation alternatives. This study addresses, in part, the Environmental Safety and Occupational Health (ESOH) requirements outlined in Army Regulation (AR) 200-1 (DA 2007c); AR 40-5 (DA 2007a); and AR 70-1 (DA 2018); Department of Defense Instruction (DoDI) 4715.4 (DoDI 2018); and Army Environmental Research and Technology Assessment (AERTA) requirement PP-3-02-05 (AERTA 2018): *Compliant Ordnance Lifecycle for Readiness of the Transformation and Objective Forces*. This program is under the direction of the Department of Defense (DoD) Strategic Environmental Research and Development Program (SERDP).

Research, development, testing, training, and use of substances potentially less hazardous to human health and the environment is vital to the readiness of the U.S. military. Safeguarding the health of Soldiers, Civilians, and the environment requires an assessment of alternatives before fielded. Continuous assessments begun early in the RDT&E process can save significant time and effort during RDT&E, as well as over the life cycle of the items developed. Residues of pyrotechnics, propellants, explosives, and incendiaries found in soil, air, surface water, and groundwater samples, create environmental problems and interfere with training activities.

The DOD is identifying replacements for substances known to cause environmental and/or occupational risks to health. This toxicology study examined the aquatic toxicity of COGA, IBF-Ome, IBF-OH, and PCL using a bioluminescent bacterial toxicity assay and conducted the assay consistent with GLP standard regulations.

1.3 Conclusions

This study reports the aquatic toxicity for the new isocyanate-free binder replacements COGA, IBF-Ome, IBF-OH, and PCL-56 via the Microtox Acute Toxicity assay. Results show that IBF-Ome and PCL were not considered toxic; an EC₅₀ was not able to be determined at the maximal soluble concentration of 125 mg/L for both. The COGA and IBF-OH were slightly toxic (EC₅₀: 24.36 mg/L and 15.31 mg/L respectively). The IBF-Ome and PCL are not classified by the GHS Hazard Classes, while COGA and IBF-OH are Acute Category 3 (UNECE 2015).

1.4 Recommendations

The acute aquatic toxicity of COGA and IBF-OH are of slight concern, and further testing and evaluation should be continued to determine if environmental releases are likely following use of these test articles. The PCL and IBF-Ome were tested at their solubility limit in DMSO and did not cause toxicity, so are not able to be classified by GHS and are categorized as practically non-toxic by EPA hazard classes. Additional aquatic testing for these compounds may not be necessary. They also do not appear to be skin sensitizers or mutagens, although confirmatory testing is ongoing for both endpoints. Compared to the compounds that these are proposed to replace, COGA, IBF-Ome, IBF-OH, and PCL have lower toxicity concerns, although further *in vivo* testing may be necessary if development continues forward with these compounds.

2 References

See Appendix A for list of references

3 Authority

This technical report was conducted under the authority of Military Interdepartmental Purchase Request No. W74RDV90166292. The report addresses, in part, the ESOH requirements outlined in DoDI 4715.4, *Pollution Prevention* (DoDI 2018), AR 200-1, *Environmental Protection and Enhancement* (DA 2007b); AR 40-5, *Preventive Medicine* (DA 2007a); and AR 70-1, *Army Acquisition Policy* (DA 2018); and Army Environmental Research and Technology Assessment Requirement PP-3-02-05, *Compliant Ordnance Lifecycle for Readiness of the Transformation and Objective Forces* (AERTA 2018). The SERDP conducted it as part of an ongoing effort.

4 Background

Current regulations require the assessment of human health and environmental effects arising from exposure to substances in soil, surface water, and ground water. Applied after an item has been fielded, these assessments can reveal the existence of adverse environmental and human health effects that must be addressed, often at substantial cost. It is more efficient to begin the assessment of exposure, effects, and environmental transport of military-related compounds/substances early in the RDT&E process in order to avoid unnecessary costs, conserve physical resources, and sustain the health of those potentially exposed. A goal of this program is to investigate these new compounds with operational and/or ESOH issues.

Candidate compounds under development as new non-isocyanate binder replacements have been evaluated for acute aquatic toxicity.

National defense requires the development of unique energetic compounds to perform specialized mission requirements. These requirements also include the sustainable use of these materials in the environment, particularly during training operations. The use of isocyanate-containing binders is a concern due to their ability to cause dermal and respiratory sensitization, resulting in an asthma-like syndrome from HDI exposure, amongst other negative health effects (ATSDR, 1998 #1268). Additionally, some compounds under review are suspected of being carcinogens, such as toluene diisocyanate (TDI; ATSDR 2018).

The CDC's ATSDR has developed an intermediate inhalation minimum risk level (MRL) for HDI of 3×10^{-5} ppm based on nasal irritation and hyperplasia found in a rodent study (Mobay Corporation 1988). The chronic MRL is 1×10^{-5} ppm based on nasal cavity epithelial hyperplasia in female rats. For TDI, the MRL for acute inhalation is 1×10^{-5} ppm due to respiratory effects in humans (Vandenplas et al. 1999). A chronic duration inhalation MRL of 1×10^{-5} ppm was derived as a result of total decline in lung function in humans exposed in the course of their manufacturing jobs (Clark et al. 1998).

The SERDP is dedicated to finding replacements for isocyanate-containing energetic binders that will reduce or eliminate ESOH risks and decrease potential impacts on readiness and the costs associated with training (USACHPPM 2007). The energetic and toxicological properties of COGA, IBF-Ome, IBF-OH, and PCL are being evaluated as potential replacements for isocyanate-containing binders. Toxicity tests can be conducted *in vivo* and *in vitro*. *In vitro* methods have the advantage of being relatively inexpensive, high-throughput, and capable of addressing many mechanistic issues at the cellular and molecular levels. *In vitro* tests are ideally suitable and effective toxicity screening tools, especially when limited quantities of a compound are available. By identifying ESOH effects early in the acquisition process, unacceptable—or “regrettable”—replacement compounds can be identified.

The APHC TOX has been tasked with providing aquatic acute toxicity data for COGA, IBF-Ome, IBF-OH, and PCL to determine their potential to negatively affect the environment. The data from these studies will help in making recommendations for continued development and toxicity testing—resulting in appropriate exposure guidance.

Microtox is an acute toxicity testing system that uses a strain of naturally occurring bioluminescent bacteria, *Aliivibrio fischeri*—formerly *Vibrio fischeri* and still referred to as *V. fischeri* by the supplier of the reagents, Modern Water, Inc.—and will be referred to as *V. fischeri* in this report. The marine bacterial bioluminescence is tied directly to cellular respiration, which is fundamental to cellular metabolism and associated life processes. These non-pathogenic, marine, bioluminescent bacteria are sensitive to a broad range of toxicants resulting in a decreased rate of respiration and a corresponding decrease in the rate of luminescence. Reduction of the microorganism's light emission is proportional to the toxicity expressed as EC₅₀ (the midpoint of the effective concentration). This test has been shown to be an effective screening tool in assessing toxicity of varied chemical compounds compared with other bioassays. Comparisons of toxicity results using these methods for a variety of compounds found that *V. fischeri* were, in most cases, more sensitive than other aquatic

organisms (Dutka and Kwan 1981; McFeters et al. 1983; Riva et al. 2007). Thus, the results with Microtox tests are often useful screens in the assessment of relative toxicity to aquatic organisms. The bacterial bioluminescence aquatic toxicity test has been validated by the industrial, academic, and governmental testing communities; testing achieved official “Standards Status” in several countries including an ASTM® Standard (D-5660; withdrawn), ISO 11348-3 and Standard Method 8050 in the United States, AFNOR T90-320 in France, NVN 6516 (withdrawn) in the Netherlands, and DIN 38412 (Germany).

This report describes the toxic effect of COGA, IBF-Ome, IBF-OH, and PCL in the bacterial bioluminescent acute toxicity assay. Table 1 identifies the critical events and dates of this study.

Table 1. Critical Events

Critical Event	Date of Event
Non-Animal Use Protocol Approved	16 July 2019
Study Start Date	16 July 2019
Experimental Start Date	25 July 2019
Experimental Completion Date	6 August 2019
Study Completion Date	May 2020

5 Materials

5.1 Test Substance

NALAS Engineering, Centerbrook, Connecticut, completed synthesis of COGA (CASRN 917756-42-4), IBF-Ome (CASRN not Found), IBF-OH (CASRN not found), and PCL (CASRN not found). The molecular structure of the compound is shown in Figure 1.

The COGA was soluble at 200 mg/mL, IBF-Ome and PCL were soluble at 12.5 mg/mL, and IBF-OH was soluble at 20 mg/mL. Initial solubility was determined by solubility checks in the Ames assay (APHC 2016, 2017b). At the end of study, the final serial dilutions were frozen and held for analysis by the APHC Method Development Section Client Services Division for dose validation.

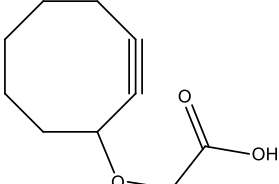
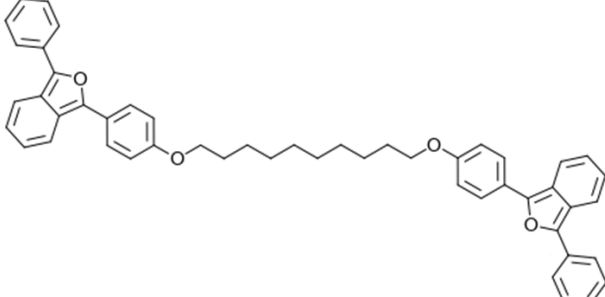
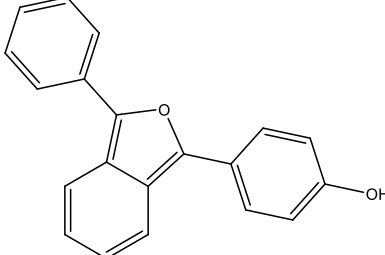
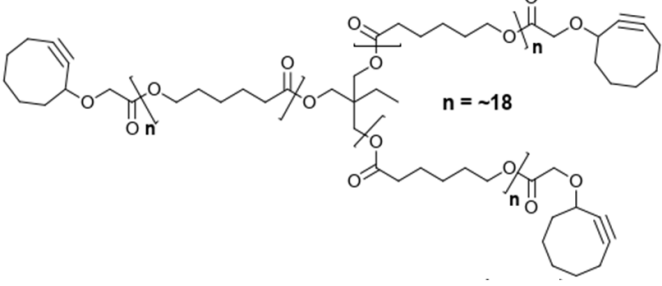
Cyclooct-1-yn-3-glycolic acid (COGA)	
Phenyl-3-(p-{10-[3-phenyl-1-isobenzofuranyl]phenoxy}decyloxy)phenylisobenzofuran	
p-(3-phenyl-1-isobenzofuranyl)phenol (IBF-OH)	
PCL-(Cyclooctyne)3 (PCL)	

Figure 1. Molecular Structures of Replacement Candidate Compounds

5.2 Test System

The Microtox Acute Toxicity Test reagent and associated media and solutions were obtained from Modern Water, Inc., New Castle, Delaware. The reagent is a freeze-dried preparation of a specially selected strain of the marine bacterium *V. fischeri* (also known as *A. fischeri*, formerly known as *Photobacterium phosphoreum*, NRRL number B-11177). Appendix D lists media, solution, and other necessary test materials with expiration dates and lot numbers. All reagents were stored according to manufacturer instructions as described in the TOX SOP 037 and study protocol (APHC 2017a, 2017c).

5.3 Positive Control

Phenol is the recommended standard or positive control for the test system. Phenol was purchased from Sigma-Aldrich, St. Louis, Missouri. Each vial of lyophilized *V. fischeri* was tested against the standard following reconstitution. Only vials with a calculated EC₅₀ of 13–26 mg/L at 5 minutes for phenol were qualified further use.

5.4 Quality Assurance

The APHC policy requires that all experiments and studies conducted by any element of the APHC TOX will be compliant with the applicable GLP standard guideline (APHC 2018). For this study, the test article dictates that the following GLP guideline applies (CFR 1989):

Code of Federal Regulations (CFR), Title 40: Protection of Environment, Part 792, Good Laboratory Practice Standards.

According to this policy and that these results may be used in regulatory decisions involving the EPA, these assays were conducted in compliance with GLP standards and followed the appropriate regulatory testing guidelines.

In compliance with the GLP requirements, the APHC Quality Systems Office audited critical phases of this study. A Quality Assurance Statement, provided in Appendix B provides the dates of these audits along with the audited phases and the dates that the results of the audits were reported to Management and the Study Director. Appendix C provides additional Quality Assurance/GLP-required archive information as well as the names of personnel contributing to the performance of this study.

6 Methods

6.1 Experimental Design

The experimental design and general procedures of this study were conducted under the APHC TOX SOP for the Microtox Acute Toxicity assay (APHC 2017a). The test kit is designed to determine the aquatic toxicity of a test material in compliance with the APHC TOX Type Protocol: “*Microtox Toxicity Testing System*” (APHC 2017c), and modifications. The modifications to the protocol are approved and signed by the Study Director. The electronic and hard copy versions of the protocol modifications are saved and archived with the protocol and the raw data.

6.2 Range Finding

In order to define an appropriate testing concentration range, a range-finding was conducted for each compound. For the 100x stock solution, compounds were dissolved in DMSO at their respective solubility limits: 200 mg/mL for COGA, 20 mg/mL for IBF-OH, 12.5 mg/mL for PCL and 12.5 mg/mL for IBF-Ome. Samples were serially diluted 1:2 in DMSO and further diluted 1:100 in diluent. Eight concentrations were tested in the range-finding. Reconstituted *V. fischeri* were added to each test concentration (10 µL), and samples were incubated and tested

for luminescence at 5, 15, and 30 minutes using the Microtox Model 500 Analyzer (Modern Water, Inc.). The EC₅₀ from the range-finding determined the final test concentration range.

6.3 Cytotoxicity Test

Following the range-finding, COGA and IBF-OH were tested in duplicate on three separate days. On each testing day, 25 mg/mL COGA and 20 mg/mL IBF-OH in DMSO were prepared as the stock solution for the main test. Eight serial 1:2 dilutions into DMSO were made, and then each of these were diluted 1:100 into the diluent for testing. Ten microliters reconstituted *A. fischeri* were added to each sample and luminescence measured at 5, 15, and 30 minutes as above. Results from the range-finding were confirmed for PCL and IBF-Ome following the same procedure.

6.4 Data Analysis

For each test, raw luminescence data were recorded at 5, 15, and 30 minutes by the Microtox analyzer. The EC₅₀ values at 5, 15, and 30 minutes were calculated by the MicrotoxOmni[®] software and further fitted to the Hill function using GraphPad PRISM[®] 5.04. All data (prints and files) were archived.

6.5 Criteria for a Valid Assay

The phenol-positive control must meet specified EC₅₀ criteria as stated in Section 5.3 for a test to be considered valid.

6.6 Aquatic Toxicity Hazard Categorization

Table 2 aligns the US EPA, OECD, and GHS categories for aquatic toxicity. We used the aquatic toxicity criteria of the EPA, the OECD and the GHS to categorize the potential aquatic toxicity of COGA, IBF-Ome, IBF-OH, and PCL.

Table 2. Ecotoxicity Assessment Scale

LC ₅₀ or EC ₅₀ Concentration Range (mg/L)	Hazard Categories (EPA 2017)	Hazard Classes (OECD 2001)	Acute Aquatic Toxicity (UNECE 2015)
< 0.01	Super Toxic	Acute Toxicity I (very toxic to aquatic life)	Acute Category 1
0.01 to 0.1	Extremely Toxic		
0.1 to 1	Highly Toxic		
1 to 10	Moderately Toxic	Acute Toxicity II (toxic to aquatic life)	Acute Category 2
10 to 100	Slightly Toxic	Acute Toxicity III (harmful to aquatic life)	Acute Category 3

LC ₅₀ or EC ₅₀ Concentration Range (mg/L)	Hazard Categories (EPA 2017)	Hazard Classes (OECD 2001)	Acute Aquatic Toxicity (UNECE 2015)
100 to 1000	Practically Nontoxic	—	—
> 1000	Relatively Harmless	—	—

7 Results

7.1 Microtox Acute Toxicity and Risk Assessment

Microbial toxicity has been used to estimate aquatic (fish) toxicity, and *A. fischeri* responses are considered the most sensitive (Dutka and Kwan 1981; McFeters et al. 1983; Riva et al. 2007). The toxicity of COGA, IBF-Ome, IBF-OH, and PCL to a species of marine bacteria, *A. fischeri*, was measured using the Microtox acute toxicity test system at 5, 15, and 30 minutes. For each test compound, three independent experiments were performed in duplicate. Table 3 presents the toxicity data (EC₅₀ and the 95% Confidence Interval) and risk assessment. Appendices E-H present the data for Microtox analyses of all four compounds. This evaluation suggests COGA and IBF-OH are “Slightly Toxic” with low concern to aquatic life, and that IBF-Ome and PCL are “Practically Nontoxic” (Table 3).

Table 3. Microtox Toxicity and Risk Assessment

Compound	Microtox EC ₅₀ (mg/L) ^a [95 percent CI]			Hazard Categories (US EPA 2017)	Hazard Classes (OECD 2001)	Acute Aquatic Toxicity GHS (UNECE 2015)
	5 min	15 min	30 min			
COGA	23.59 [21.91- 25.39]	24.36 [22.79- 26.05]	24.99 [23.55- 26.52]	Slightly Toxic	Acute Toxicity III (harmful to aquatic life)	Acute Cat. 3
IBF-Ome	>125	>125	>125	Practically Nontoxic	—	—
IBF-OH	11.63 [3.73- 36.24]	15.31 [9.136- 25.65]	14.53 [8.823- 23.91]	Slightly Toxic	Acute Toxicity III (harmful to aquatic life)	Acute Cat. 3
PCL	>125	>125	>125	Practically Nontoxic	—	—

Note:

^aThe value of EC₅₀ at 15 min is used for the risk assessment.

8 Conclusions

This study reports the aquatic toxicity for the new isocyanate-free binder replacements COGA, IBF-Ome, IBF-OH, and PCL-56 via the Microtox Acute Toxicity assay. Results show that IBF-Ome and PCL were not considered toxic; an EC₅₀ was not able to be determined at the maximal soluble concentration of 125 mg/L for both. The COGA and IBF-OH were slightly toxic (EC₅₀: 24.36 mg/L and 15.31 mg/L respectively). The IBF-Ome and PCL are not classified by the GHS Hazard Classes, while COGA and IBF-OH are Acute Category 3 (UNECE 2015). These replacement compounds are equivalent to or less toxic than TDI and HDI, which are predicted to be Acute Category 2 or 3 respectively for aquatic toxicity (ECHA 2020a; 2020b).

9 Recommendations

The acute aquatic toxicity of COGA and IBF-OH are of slight concern, and further testing and evaluation should be continued to determine if environmental releases are likely following use of these test articles. The PCL and IBF-Ome were tested at their solubility limit in DMSO and did not cause toxicity, so are not able to be classified by GHS and are categorized as practically non-toxic by EPA hazard classes. Additional aquatic testing for these compounds may not be necessary. They also do not appear to be skin sensitizers or mutagens, although confirmatory testing is ongoing for both endpoints. Compared to the compounds that these are proposed to replace, COGA, IBF-Ome, IBF-OH, and PCL have lower toxicity concerns, although further *in vivo* testing may be necessary if development continues forward with these compounds.

10 Point of Contact

Dr. Emily N. Reinke, the Study Director, is the point of contact for this project. She may be reached at DSN 584-3980 or commercial 410-436-3980.

Toxicology Study No. S.0065662A, August 2019

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APPENDIX A

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Appendix B

**QUALITY ASSURANCE STATEMENT
MICROTOX ASSAY**

For: Toxicology Study No. S.0065662A, Protocol No. 0FMA-92-iv17-03-01 T,U,V,W, Microtox® Acute Toxicity Testing Of Novel Environmentally Sustainable Binders For Energetic Formulations the following critical phases were inspected/audited by the Quality Systems and Regulatory Compliance Office (QSARC):

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Type Protocol Good Laboratory Practice Standard Review	03/01/2017	03/01/2017
Test Article Specific Protocol Modification Reviews	07/16/2019	07/16/2019

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Analytical Chemistry Support – QA review of Dosing Solution Concentration Verification	12/06/2016	12/06/2016
Microtox - Reagent and Test System Storage and Labeling requirements	05/02/2018	05/05/2018
Microtox - Data Processing and Raw Data Documentation Procedures	05/02/2018	05/05/2018
Microtox - Compliance with GLP requirements for Test Facility SOPs	05/02/2018	05/05/2018
Microtox - Calibration Verification of Equipment - Balance and Pipettes	05/02/2018	05/05/2018
Microtox Test Study Endpoint Criteria Compliance	08/06/2019	08/06/2019
Study Raw Data Good Laboratory Practice Standard Review	04/20/2020	04/20/2020
Final Study Good Laboratory Practice Standard Report Review	04/20/2020	04/20/2020

Note 1: All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Note 2: This report has been audited by the Quality Assurance Unit (QSARC), and is considered to be an accurate account of the data generated and of the procedures followed

Note 3: In addition to the study specific critical phase inspections listed here, general facility and process based inspections not specifically related to this study are done monthly and are also listed here in accordance with QA Standard Operating Procedure.

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Date: 2020.04.21 14:28:55 -04'00'

Michael P. Kefauver
GLP Quality Assurance Specialist, QSARC

04/21/2020

Date

APPENDIX C

ARCHIVES AND STUDY PERSONNEL

C-1 Archives

All raw data, documentation, records, protocols, contributing scientist reports, and a copy of the final report generated as a result of this study will be archived in the storage facilities of the TOX Directorate, APHC, for a minimum of five (5) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

Records on the test system will be archived by the TOX Directorate for a minimum of five (5) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

The present study used the Toxicology Study No. S.0065662A, Protocol No. 0FMA-92-iv17-03-01 T,U,V,W.

The protocol, raw data, summary data, and the final report pertaining to this study will be physically maintained within Building E-2100, APHC. These data may be scanned to a computer disk. Scanned study files will be stored electronically with the study data in the archive.

Archived SOPs can be found in the Master Control database at APHC. Maintenance and calibration logbooks may be found in Room 1026, Building E-2100, APHC, Aberdeen Proving Ground, MD, 21010.

Archivist: Martha Thompson

C-2. Personnel

Management: Mark Johnson, Ph.D., D.A.B.T., Director, Toxicology; Michael J. Quinn, Ph.D., Division Chief, Health Effects Division (HEF)

Study Director: Emily N. Reinke, Ph.D., Biologist, HEF

Technical staff: Taryn Brown, ORISE Fellow

Quality Assurance: Michael P. Kefauver, Chemist, Quality Systems Office

APPENDIX D

MICROTOX TEST REAGENTS

Table D-1. Microtox Test Reagents

Microtox Reagents	Source	Lot #	Date Expiration
Modern Water Microtox Diluent	Modern Water	18F4135A	07/2021
Modern Water Microtox Acute Reagent	Modern Water	17H4227	09/2019
Dimethyl sulfoxide	Sigma	RNBG8238	06/2020
Phenol	Sigma-Aldrich	BCBW8224	
Modern Water Microtox Reconstitution Solution	Modern Water	18C4048	03/2021

APPENDIX E

COGA MICROTOX TEST DATA TABLES AND CALCULATIONS

Table E-1. Test concentrations - COGA

Nominal Concentration (mg/mL; 100x test concentration)	Corrected Working Concentration ^a (mg/mL; 100x test concentration)		
	Test 1	Test 2	Test 3
0.195			
0.391			
0.781			
1.56			
3.12			
6.25			
12.5			
25			

Note:

^aCorrected Working Concentrations were unavailable at the time of this report. Concentration verification had not been performed by the APHC Method Development Section.

Table E-2. COGA EC₅₀ at 5, 15, and 30 Minutes

COGA EC ₅₀ (mg/L; 95% CI) ^a		
5 minute	15 minute	30 minute
23.59 [21.91-25.39]	24.36 [22.79-26.05]	24.99 [23.55-26.52]

Note:

^aEC₅₀ values calculated for the marine bacteria, *A. fischeri*

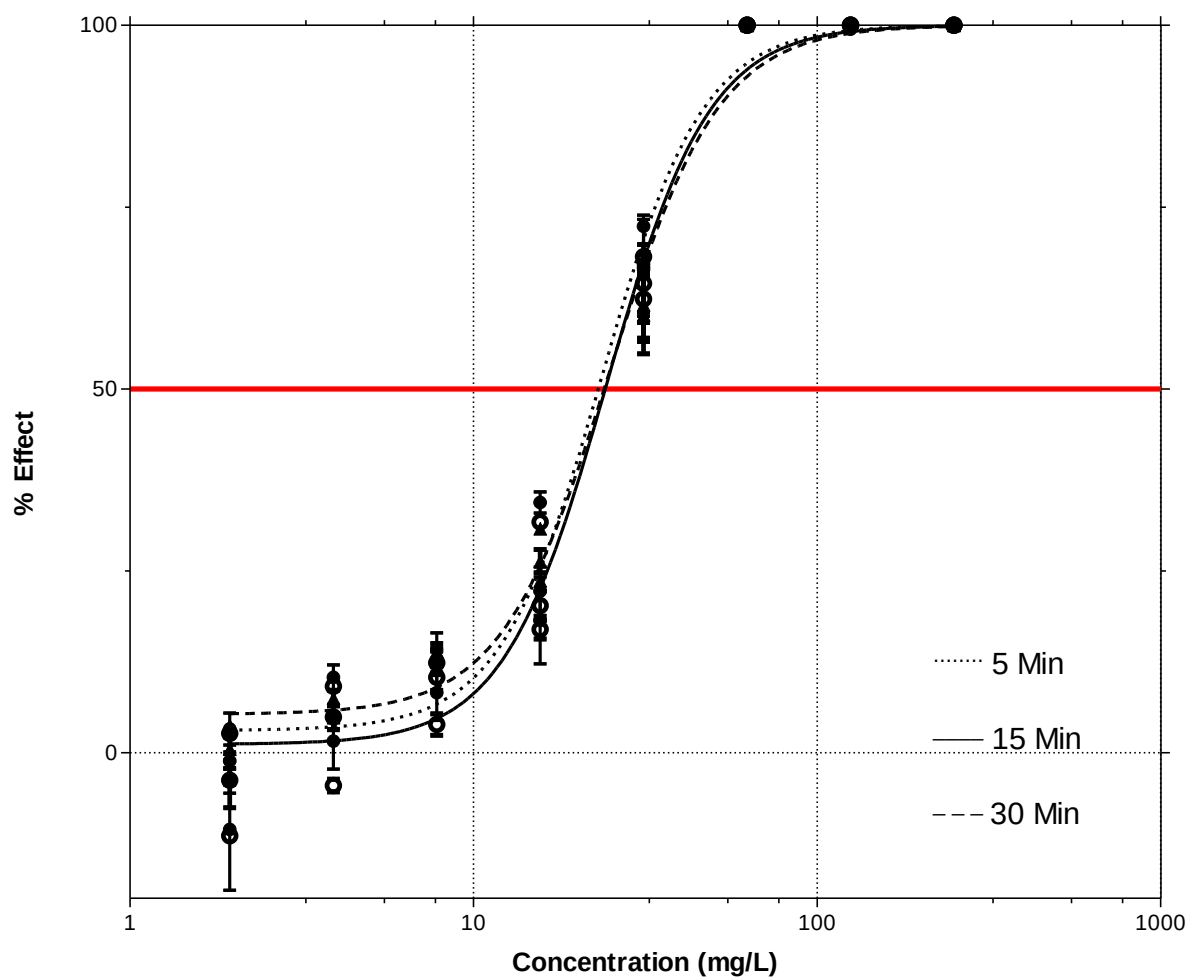


Figure E-1 – Microtox toxicity of COGA: Red line represents median effect.

APPENDIX F

IBF-Ome MICROTOX TEST DATA TABLES AND CALCULATIONS

Table F-1. Test concentrations – IBF-Ome

Nominal Concentration (mg/mL; 100x test concentration)	Corrected Working Concentration ^a (mg/mL; 100x test concentration)		
	Test 1	Test 2	Test 3
0.0977			
0.195			
0.391			
0.781			
1.56			
3.13			
6.25			
12.5			

Note:

^aCorrected Working Concentrations were unavailable at the time of this report. Concentration verification had not been performed by the APHC Method Development Section.

Table F-2. IBF-Ome EC₅₀ at 5, 15, and 30 Minutes

IBF-Ome EC ₅₀ (mg/L; 95% CI) ^a		
5 minute	15 minute	30 minute
>125	>125	>125

Note:

^aEC₅₀ values calculated for the marine bacteria, *A. fischeri*

APPENDIX G

IBF-OH MICROTOX TEST DATA TABLES AND CALCULATIONS

Table G-1. Test Concentrations – IBF-OH

Nominal Concentration (mg/mL; 100x test concentration)	Corrected Working Concentration ^a (mg/mL; 100x test concentration)		
	Test 1	Test 2	Test 3
0.156			
0.313			
0.625			
1.25			
2.5			
5			
10			
20			

Note:

^aCorrected Working Concentrations were unavailable at the time of this report. Concentration verification had not been performed by the APHC Method Development Section.

Table G-2. IBF-OH EC₅₀ at 5, 15, and 30 Minutes

IBF-OH EC ₅₀ (mg/L; 95% CI) ^a		
5 minute	15 minute	30 minute
11.63 [3.73-36.24]	15.31 [9.136-25.65]	14.53 [8.823-23.91]

Note:

^aEC₅₀ values calculated for the marine bacteria, *A. fischeri*

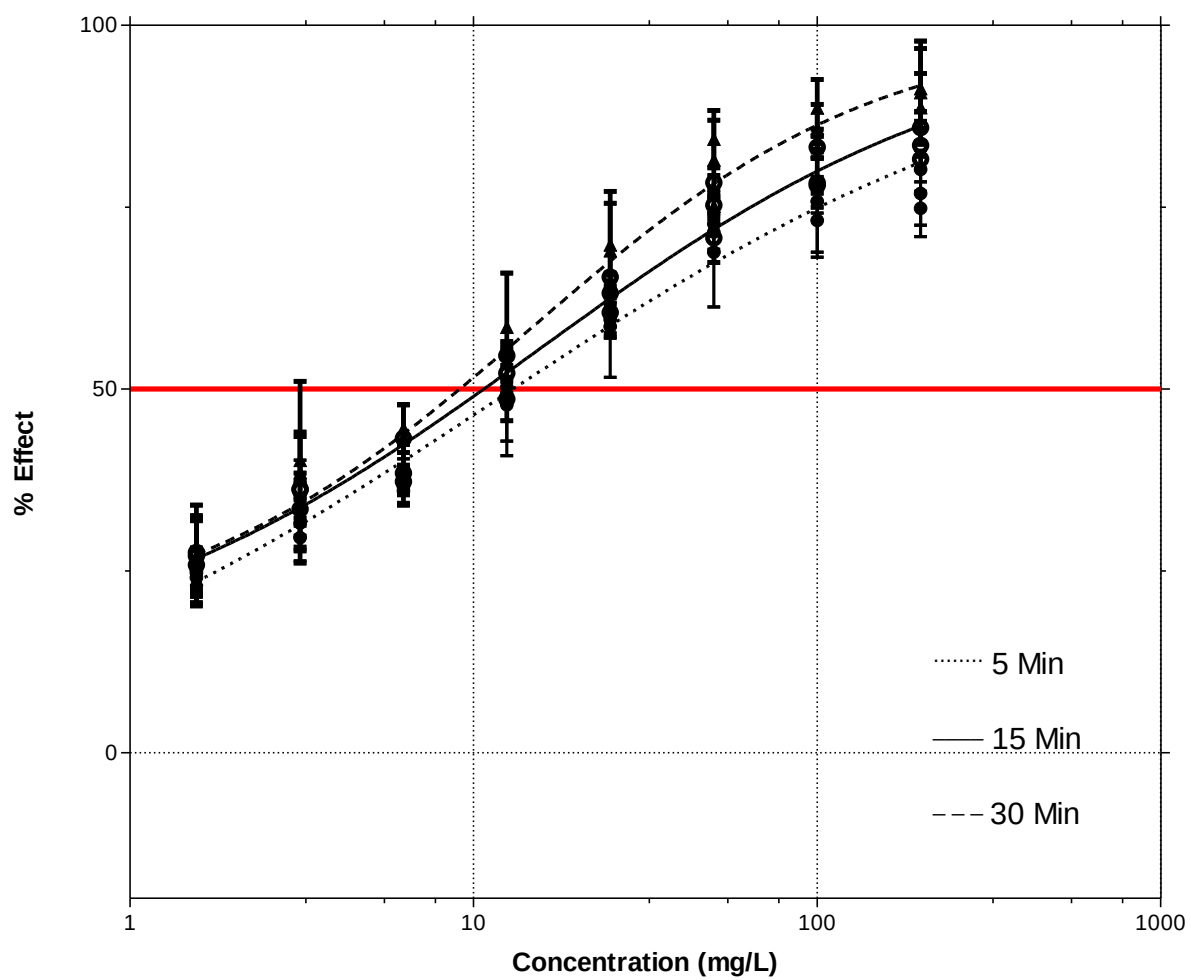


Figure G-1 – Microtox Toxicity of OBF-OH - Red line represents median effect

APPENDIX H

PCL MICROTOX TEST DATA TABLES AND CALCULATIONS

Table H-1. Test Concentrations – PCL

Nominal Concentration (mg/mL; 100x test concentration)	Corrected Working Concentration ^a (mg/mL; 100x test concentration)		
	Test 1	Test 2	Test 3
0.0977			
0.195			
0.391			
0.781			
1.56			
3.13			
6.25			
12.5			

Note:

^aWorking concentration measured by the APHC Method Development Section, final corrected concentrations were calculated from lowest measured working concentration.

Table H-2. PCL EC₅₀ at 5, 15, and 30 Minutes

PCL EC ₅₀ (mg/L; 95% CI) ^a		
5 minute	15 minute	30 minute
>125	>125	>125

Note:

^aEC₅₀ values calculated for the marine bacteria, *A. fischeri*