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8252 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5403

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Toxicology Directorate

**Tiered *In Vitro* Toxicity Testing of the Novel Environmentally Sustainable
Binders for Energetic Formulations**

Prepared by Drs. Emily N. Reinke and Valerie H. Adams, Health Effects Division

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4800 Mark Center Drive
Suite 17D03
Alexandria, VA 22350-3605

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Authors

Emily N. Reinke, Ph.D., DABT
Valerie H. Adams, Ph.D., DABT

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Toxicology Directorate
Health Effects Division
MCHB-PH-HEF
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Good Laboratory Practice Compliance Statement

The studies described in this report were 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 (LAB) prior to the study start in compliance with study protocol and modification requirements. At the time this report was finalized, LAB had not achieved the capability to provide concentration verification for COGA, PCL, IBF-OH, IBF-Ome.

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

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Emily N. Reinke, Ph.D., D.A.B.T.
Study Director
Health Effects Division

Date

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Date: 2022.05.19 14:06:23 -04'00'

Valerie H. Adams, Ph.D., D.A.B.T.
Study Director
Health Effects Division

Date

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TOXICOLOGY STUDY NO. S.0058638-18
TIERED IN VITRO TOXICITY TESTING OF THE 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), PCL-(Polycaprolactone-cyclooctyne trimer) 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 in several *in vitro* test systems, including the Ames mutagenicity assay, the Microtox® Acute Toxicity Test System, and skin sensitization via the human cell line activation test (h-CLAT) and the direct peptide reactivity assay (DPRA). Data from the h-CLAT were also used to predict a Hazard Category (United Nations Economic Commission for Europe (UNECE) 2015) for acute oral toxicity. 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 purpose of this study is to provide toxicology data to support environmental and occupational health hazards on COGA, IBF-OH, IBF-Ome, and PCL as a new or replacement energetic compound 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 Department of the Army (DA) Regulation (AR) 200-1 (DA, 2007b), AR 40-5 (DA, 2020), and AR 70-1 (DA, 2018), Department of Defense (DoD) Directive (DoDD) 4715.23 (DoD 2018), and Army Environmental Research and Technology Assessment Requirement (AERTA) PP-3-02-07 (AEC 2018), Compliant Ordnance Lifecycle for Warfighter Readiness. This program is sponsored by the 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 they are fielded. Continuous phased 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 have been found in soil, air, surface, and groundwater samples, creating environmental problems and interfering with training activities.

DoD is identifying replacements for substances causing environmental and/or occupational health hazards. The purpose of this toxicology study was to evaluate COGA, IBF-OH, IBF-Ome,

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and PCL mutagenicity, acute aquatic toxicity, and skin sensitization hazard using *in vitro* methods and following Good Laboratory Practice (GLP) regulations.

1.3 Conclusions

This study reports the *in vitro* toxicity evaluation in the Ames assay for mutagenicity, the Microtox Acute Aquatic Toxicity assay, and the skin sensitization assay battery of replacement candidates for isocyanate-based binders. COGA, PCL, and IBF-Ome were not mutagenic in the Ames assay, while IBF-OH was mutagenic in TA100 without the S9 liver fraction. In the acute aquatic toxicity assay, COGA was moderately toxic (Globally Harmonized System (GHS) 2), IBF-OH was slightly toxic (GHS 3), and IBF-Ome and PCL were nontoxic with an EC₅₀ above the limit for GHS categorization (UNECE 2015). COGA and PCL were negative in both skin sensitization assays (h-CLAT and DPRA). The skin sensitization potential of IBF-OH and IBF-Ome could not be determined due to compound interference with the assay constituents. Key toxicity values for the four compounds under evaluation and the baseline isocyanate binders are provided in Table 1. Where available, the hazard codes for toluene diisocyanate (TDI) and hexamethylene diisocyanate (HMDI) are included in Table 1 (NCBI 2020).

Table 1. Comparative Toxicity for Isocyanate and Non-isocyanate Binders

Compound	Genotoxicity	Skin sensitization	Acute mammalian toxicity	Acute aquatic toxicity
HMDI	negative	H315 ^c , H317 ^c	300–2,000 mg/kg ^a ; GHS 4; H331 ^c	GHS no category; practically nontoxic ^b
TDI	H351 ^c	H315 ^c , H317 ^c	68–500 mg/kg ^a ; GHS 3; H330 ^c	GHS 2; moderate toxicity ^b
COGA	negative	Negative	660–1,010 mg/kg; GHS 4	GHS 2; moderate toxicity ^b
IBF-OH	positive (-S9)	indeterminant	256 mg/kg; GHS 3	GHS 3; slightly toxic ^b
IBF-Ome	negative	indeterminant	256 mg/kg; GHS 3	GHS no category; practically nontoxic ^b
PCL	negative	negative	660–1,010 mg/kg; GHS 4	GHS no category; practically nontoxic ^b
Hazard Color key				
	Highest	Moderate-high	Low-moderate	Negligible –low

Legend:

HMDI = Hexamethylene diisocyanate

TDI = Toluene diisocyanate

mg/kg = milligrams per kilogram

GHS = Globally Harmonized System

Notes:

^a. Database values for acute oral toxicity from animal exposures (NCBI, 2020)

^b. EPA hazard categories:

^c. = H-codes from PubChem. H315 = causes skin irritation; H317 = may cause allergic skin reaction; H330 = fatal if inhaled; H331 = toxic if inhaled; H351 = suspected of causing cancer

1.4 Recommendations

COGA, PCL, IBF-OH, and IBF-Ome have moderate to low toxicity. The bacterial mutagenicity test data indicate IBF-OH is a mutagen that may be detoxified to a nonmutagenic metabolite in living systems. IBF-OH and IBF-Ome were not successfully assessed for skin sensitization potential due to interference with both of the assays. The predicted acute oral and acute aquatic toxicities are comparable or better than the chemicals they are proposed to replace. Overall, these proposed chemicals may provide moderate improvements in toxicological concerns over the isocyanates to which they are compared (HMDI and TDI), with the primary concerns being genotoxicity and skin sensitization for IBF-OH and skin sensitization for IBF-Ome. Non-aqueous skin sensitization assessments (e.g., the local lymph node assay) may be necessary to better characterize the potential for skin sensitization. IBF-OH has the potential to bioaccumulate and its low water solubility and high organic carbon to water partition coefficient (K_{oc}) suggests it could accumulate in soils and sediments. COGA and PCL may be highly mobile in aquatic environments, so additional aquatic toxicity testing (fish, algae, and invertebrate) may be necessary. Acute and 14-day rat studies are recommended to reduce uncertainty and better characterize toxicity, with the addition of a micronucleus assay to further investigate potential genotoxicity.

2 REFERENCES, ACRONYMS AND ABBEVIATIONS

See Appendix A for list of references. See the Glossary for the definitions of acronyms and abbreviations.

3 AUTHORITY

This project was funded under Military Interdepartmental Purchase Request No. W74RDV90166292. This toxicology report addresses, in part, the ESOH requirements outlined in DoDD 4715.23, *Integrated Recycling and Solid Waste Management* (DoD 2018), AR 200-1, *Environmental Protection and Enhancement* (DA 2007b); AR 40-5, *Army Public Health Program* (DA 2020); and AR 70-1, *Army Acquisition Policy* (DA 2018). It was conducted as part of an ongoing effort by SERDP.

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 to avoid unnecessary costs, conserve physical resources, and sustain the health of those potentially exposed. A goal of the SERDP program is to investigate new compounds for operational and/or environment, safety, and occupational health issues. The candidates under development as new non-isocyanate binder replacements energetics include COGA, IBF-OH, IBF-Ome, and PCL.

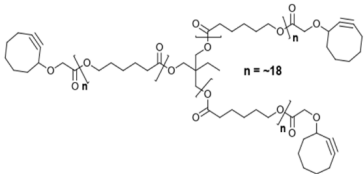
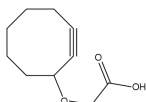
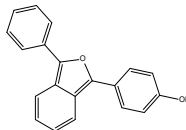
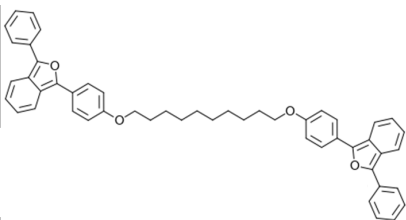
National defense requires the development of unique energetic compounds to perform specialized mission requirements. These requirements also include the sustainable use of these materials in the environment, particularly during training operations. The use of isocyanate-containing binders is a concern due to their ability to cause dermal and respiratory sensitization, resulting in an asthma-like syndrome from HMDI exposure (Mobay Inc. 1988). Additionally some are suspected of being carcinogens, such as TDI (ATSDR 2018).

The Centers for Disease Control and Prevention (CDC), Agency for Toxic Substances and Disease Registry (ATSDR) has developed an intermediate inhalation minimum risk level (MRL) for HMDI of 3×10^{-5} parts per million (ppm) based on nasal irritation and hyperplasia found in a rodent study (Mobay Inc. 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 3×10^{-6} 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 (U.S. Army Center for Health Promotion and Preventive Medicine (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 level. *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 Toxicology Directorate (TOX) of the U.S. Army Public Health Center (APHC) has been tasked with generating *in vitro* toxicity data for COGA, IBF-OH, IBF-Ome, and PCL to determine their potential negative human and environmental effects. The data from these studies informs recommendations for the continued development and additional toxicity testing of these binder replacements that supports the appropriate hazard classification and exposure guidance. Prior to conducting *in vitro* testing, *in silico* methods are used to estimate physical and chemical properties (EpiSuite™, (U.S. Environmental Protection Agency (EPA), 2015)) and toxicity (Ecological Structure Activity Relationship (ECOSAR) and Toxicity Prediction by Komputer Assisted Technology (TOPKAT) (BIOVIA 2015; EPA 2015)). The ECOSAR and TOPKAT are quantitative structure-activity relationship (QSAR) programs and provide rapid predictions for aquatic toxicity and human health. The full QSAR report was included with each *in vitro* protocol modification; Table 2 provides summary *in silico* data for COGA and IBF-OH. The polymeric structure of PCL and IBF-Ome were inadequately modeled; however, the monomer parent for each can be used for reference.

Table 2. Candidate Compounds Physical Chemical Properties, Structure and QSAR Toxicity Estimates

Estimate	QSAR-TOPKAT												ECOSAR	EPI Suite					
	Rat Oral LD50	Rat Chronic LOAEL	Rat Inhalational LC50	Skin Irritancy	Skin Sensitization	Ocular Irritancy	Developmental Toxicity Potential	Ames Mutagenicity	Weight of Evidence Carcinogenicity	Aerobic Biodegradability	Fathead Minnow LC50	Daphnia EC50	Fathead Minnow LC50	Daphnia EC50	Green Algae EC50	Aqueous solubility (mg/L; 25°C)	log Kow	log Koc	Henry's Law Constant (atm-m3/mol) @ 25°C
COGA	4	5	3	neg.	1	pos.	neg.	neg.	yes	3	3					3456	1.9	1.1	9.06E-09
confidence	vLC	HC	HC	vLC	HC	HC	vLC	HC	HC	HC	vLC								
IBF-OH	4	5	neg.	neg.	3	neg.	pos.	pos.	no	1	1	1	1	1	0.95	5.6	6.5	3.22E-10	
confidence	HC	vLC	LC	HC	HC	HC	HC	HC	vLC	vLC	vLC								
PCL																			
IBF-OME																			
Notes: confidence levels for the TOPKAT predictions are categorized as high confidence (HC), low confidence (LC) and very low confidence (vLC). Confidence levels for ECOSAR are not available. Red = highest concern, Orange = moderate-high concern, Yellow = Low-moderate concern, Green = negligible-to-low concern, Blue = data unavailable, insufficient or reliable, Grey = not applicable																			
   																			

The Ames test for mutagenicity (Ames et al. 1975) is a method widely accepted for evaluation of mutagenic potential by the EPA, the U.S. Food and Drug Administration (FDA), and the Organization for Economic Co-operation and Development (OECD) (OECD 1997; EPA 2012; FDA 2007). Historically, the mutagenicity of test materials has been evaluated in the agar plate-based Ames assay (Ames et al. 1975). The bacteria used in this test, *Salmonella typhimurium* and *Escherichia coli* (*E.coli*), have point mutations in the Histidine (*His*) and Tryptophan (*Trp*) operons, rendering the bacteria incapable of producing the amino acids his or trp, respectively. A chemical's mutagenic potential is assessed by exposing His or Trp negative (*His*- or *Trp*-) organisms to varying concentrations of a test chemical and selecting for reversion events. Reversion occurs primarily through two molecular mechanisms: single base substitutions or

frameshift mutations within the *His* or *Trp* operons. Xenometrix has developed a proprietary MPF™ Ames test that provides a convenient, high-throughput capability for mutagenicity testing (Xenometrix 2012). The test uses the same strains of bacteria required by the EPA (*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and a composite of *E. coli* pKM101/*uvrA* strains), and reduces the assay to a simple, non-agar, 384-well plate methodology that is expedient and cost effective.

The Microtox test system 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, also known as *Photobacterium phosphoreum*, Northern Regional Research Laboratory (NRRL) number B-11177). The marine bacterial bioluminescence is tied directly to cellular respiration, which is fundamental to cellular metabolism and associated life processes. These nonpathogenic, 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 effective concentration of 50% (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 comparing with other bioassays. The bacterial bioluminescence aquatic toxicity test has been validated by the industrial, academic, and governmental testing communities and achieved official "Standards Status" in several countries including an ASTM Standard (D-5660; withdrawn), ISO 11348-3 and Standard Method 8050 in the U.S., AFNOR T90-320 in France, NVN 6516 (withdrawn) in the Netherlands, and DIN 38412 in Germany.

There are multiple skin sensitization assays that can be utilized to determine skin sensitization hazard in a tiered testing strategy that forms a defined approach. The EPA has recently accepted two defined approaches for submission and registration to predict hazard. In one, the h-CLAT is the first in a tiered strategy, where a positive result allows for a hazard determination to be made and no further testing is required (EPA 2018). During a skin sensitizing reaction, activated dendritic cells migrate to the lymph node where the major histocompatibility complexes which they are presenting activate T-cells and T-cell proliferation. Secondary exposure to the chemical will then result in inflammation and an allergic reaction. Using adverse outcome pathway analysis, four key events for skin sensitization were identified (OECD 2012). *In vitro* assays for each step have been developed and validated. The h-CLAT is an *in vitro* assay for key event 3 that measures the test chemical mediated dendritic cell activation via increased expression of CD54 and CD86 on the cell surface (OECD 2012). The presence of CD54 and CD86 proteins on the cell surface is detected with flow cytometry using fluorescent labelled antibodies specific for CD54 and CD86 (Ashikaga et al. 2010); European Center for the Validation of Alternative Methods (ECVAM), Database on Alternative Methods to Animal Experimentation (ECVAM 2014; OECD 2018). The threshold criteria for a positive reaction in h-CLAT requires either a 2-fold induction of CD54 or a 1.5-fold induction of CD86 compared to solvent controls. The h-CLAT can also be utilized to predict acute oral toxicity from the cytotoxicity data produced in the course of conducting the assay.

In the absence of a positive result in the h-CLAT, a second assay should be conducted, either in a tiered approach or as a best-of-three approach (Kleinstreuer et al. 2018) (EPA 2018) (Kleinstreuer et al. 2018; EPA 2018). The DPRA fulfills either of these approaches. The DPRA assesses the first key event in the adverse outcome pathway, formation of haptens through

covalent binding of chemicals to proteins, which induces an immune response, leading to downstream effects such as the activation of dendritic cells as described in the previous paragraph. The DPRA is an *in chemico* assay that measures the decay of cysteine or lysine containing polypeptide sequences via high-performance liquid chromatography with ultraviolet detection (HPLC-UV). The threshold for a positive reaction requires a decrease in amino acid signal of approximately 6.5% compared to control samples.

This report describes the toxic effect of COGA, IBF-OH, IBF-Ome, and PCL in the Ames mutagenicity assay, the Microtox assay, and the skin sensitization defined approach. Table 3 identifies the critical events and dates of these studies.

Table 3. Critical Events

Critical Event	Date of Event (Ames)	Date of Event (Microtox)	Date of Event (h-CLAT)	Date of Event (DPRA)
Type-Protocol Modification Approved	20 June 2019	16 July 2019	9 July 2019	25 June 2019
Study Start Date	20 June 2019	16 July 2019	9 July 2019	9 July 2019
Experimental Start Date	20 June 2019	25 July 2019	9 July 2019	24 August 2020
Experimental Completion Date	8 August 2020	6 August 2019	19 August 2019	12 September 2020
Study Completion Date	March 2021	May 2020	March 2021	March 2021

Legend:

h-CLAT = human cell line activation test

DPRA = direct peptide reactivity assay

5 MATERIALS

5.1 Test Substance

NALAS Engineering, Centerbrook, Connecticut, completed synthesis of COGA (Chemical Abstract Service Registry Number® (CASRN) 917756-42-4), IBF-Ome (CASRN not Found), IBF-OH (CASRN not found), and PCL (CASRN not found). Purity analysis for these compounds was not available.

The COGA was soluble at 200 milligrams per milliliter (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 2017b, 2019b). 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.

5.2 Test System (Ames)

Xenometrix MPF™ kits were obtained from ANIARA (West Chester, Ohio). The kit contained quality assurance certificates for the *Salmonella* and *E.coli* strains. Appendix D provides a list of the kit reagents and supplementary reagents with expiration dates. All reagents were stored in refrigerators and freezers in accordance with directions in the kit as described in the Toxicology Standing Operating Procedure (TOX SOP) 068 (APHC 2017b). BacTiter-Glo kits were obtained from Promega Corporation (Madison, Wisconsin), and contains a reagent that becomes luminescent when activated by incubation with adenosine triphosphate (ATP) in lysed bacteria.

5.3 Test System (Microtox)

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 *Aliivibrio fischeri* (*A. fischeri*). Appendix D provides a list of media, solutions, 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). The positive control (zinc sulfate) was purchased from Millipore Sigma (St. Louis, Missouri). Each vial of lyophilized *A. fischeri* was tested against the standard following reconstitution. Only vials with a calculated EC₅₀ of 2–10 milligrams per liter (mg/L) at 15 minutes were qualified for further use.

5.4 Skin Sensitization Assays

5.4.1 Test System (h-CLAT)

THP-1 cells were acquired from the American Type Tissue Collection (Manassas, Virginia). Appendix D provides a list of media, solutions and other necessary test materials with expiration dates and lot numbers. All tissue culture reagents were acquired from Gibco, a subsidiary of ThermoFisher (Waltham, Massachusetts). Cells were cultured in Gibco RPMI 1640 containing 10% fetal bovine serum, 100 units per milliliter (U/mL) penicillin, 100 µg/mL streptomycin and 0.05 millimolar (mM) 2-mercaptoethanol. All cells, reagents, and chemicals were stored according to manufacturer's instructions (APHC 2019a). The positive controls for the reactivity check were 2,4-dinitrochlorobenzene (DNCB) and nickel sulfate (NiSO₄). DNCB was used as the positive control for the full test. Lactic acid (LA) was the negative control for the reactivity check. All chemicals were obtained from Millipore Sigma.

5.4.2 Test System (DPRA)

Polypeptide sequences were synthesized and obtained from RS Synthesis (Lexington, Kentucky). Sequences were as follows: Ac-RFAAKAA-COOH (776.2 gram mole (g/mol)) and Ac-RFAACAA-COOH (751.9 g/mol). Appendix D provides a list of chemicals, buffers, and other necessary test materials with expiration dates and lots numbers. All chemicals and buffers were stored according to manufacturer instructions and were obtained from Millipore Sigma. (APHC 2019a). Cinnamaldehyde (100 mM stock) was the positive control for the assay. All chemicals were obtained from Millipore Sigma.

5.5 Quality Assurance

The APHC policy requires that all experiments and studies conducted by any element of 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:

- Code of Federal Regulations (CFR), Title 40, *Protection of Environment*, Part 792, *Good Laboratory Practice Standards* (CFR 1989).

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. Appendix B provides a Quality Assurance Statements, which 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 the additional Quality Assurance/GLP requirement of archives location as well as the names of personnel contributing to the performance of this study.

6 METHODS

6.1 Ames

The experimental design and general procedures of this study were conducted under the APHC TOX SOP 068 for the Xenometrix MPF™ Ames Test Kit (APHC 2017b). The test kit is designed to determine the mutagenicity of a test material in compliance with the EPA (EPA, 2012) and OECD guidelines for the Bacterial Reverse Mutation Test (OECD 1997), APHC TOX Type Protocol: *"The Ames Test For Mutagenicity # 2758-70-iv19-04-01"* (APHC 2019b), and modifications (H-COGA, I-IBF-OH, J-IBF-Ome, and K-PCL). 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.

6.1.1 Sample Preparation

Solutions of 50 mg/mL COGA, 10 mg/mL IBF-OH, 12.5 mg/mL IBF-Ome and 6.2 mg/mL PCL in dimethyl sulfoxide (DMSO) were first serially diluted in DMSO [six (6) half-log, (1:3.12), serial dilutions] and then further diluted 25X in Ames Exposure Medium without apparent precipitation. These final diluted solutions represent the exposure concentrations with final concentrations ranging from 0.84 to 2000 micrograms per milliliter (µg/mL) (nominal).

6.1.2 Validation of Cell Growth

The frozen bacterial stocks were thawed according to TOX SOP 068 (APHC 2017b) and expanded in Xenometrix growth medium with shaking at approximately 37 Celsius (°C) overnight with or without ampicillin, as appropriate for each strain. The following morning, evidence of sufficient bacterial growth was assessed measuring the absorbance at 600 nanometers (nm) (optical density at 600 nm (OD₆₀₀); BioMate™ 3S spectrophotometer, Fisher Scientific) OD₆₀₀ ≥ 2.0 indicated sufficient density for the Xenometrix Ames Assay, although lower OD₆₀₀ values may also be acceptable. Per personal conversation with the manufacturer, sub-threshold growth were corrected by proportionately increasing the volume of each bacterial suspension to the final incubation solution to provide an appropriate density of bacteria.

6.1.3 Compound Incubation

The bacterial suspensions were diluted with Xenometrix incubation media and aliquoted to wells containing serial dilutions of 25X concentrates of the test material. The suspensions were incubated with shaking for 90 minutes at approximately 37 °C with and without the inclusion of S9 liver extract (±S9); inclusion of S9 determines if a mutagen is generated from the parent compound due to metabolism by liver enzymes. At the end of the incubation, each well was diluted 11-fold with purple Indicator Medium and 50 microliter (µL) aliquots were distributed appropriately into 384-well plates. Plates were incubated for 2 days at approximately 37 °C and then scored for presence of revertant colonies (i.e., evidence of a positive mutagenic event). Positive wells were indicated by a color change (purple to yellow) or a visible bacterial colony. The indicator media turns from purple to yellow due to a pH change resulting from active metabolism of revertant bacteria.

6.1.4 Criteria for a Positive Mutagenic Event

Positive controls appropriate for each strain were included alongside the test material to assure the assay was valid for each test; positive controls are tested in triplicate for each strain with and without the S9 microsomal fraction. The assay as a whole is considered valid if both the number of control background reversions and the number of positive control reversions are within prescribed limits (APHC 2017b, 2019b).

There are several criteria for determining a positive result: the number of reversions induced by the test compound is at least 2-fold above the background control. There is a concentration-related increase over the range tested, and/or there is a reproducible increase at one or more concentrations in the number of revertant colonies per plate in at least one strain with or without metabolic activation system. Biological relevance of the results should be considered first. Statistical methods may be used as an aid in evaluating the test results. However, statistical significance should not be the only determining factor for a positive response. Results from test substances that do not meet the above criteria are considered nonmutagenic (Xenometrix 2012).

Although most experiments will give clearly positive or negative results, in rare cases the data set will preclude making a definite judgment about the activity of the test substance. Results may remain equivocal or questionable regardless of the number of times the experiment is

repeated. Positive results from the bacterial reverse mutation test indicate that a substance induces point mutations by base substitutions and/or frame shifts in the genome of *Salmonella*. Negative results indicate that under the test conditions the test substance is not mutagenic in the tested species.

6.1.5 Determination of Cytotoxicity

Coincident with the test incubation, a plate is prepared for determination of cytotoxicity of the test material using ATP luminescence. After a 90-min incubation at approximately 37 °C, samples from the cytotoxicity plate are aliquoted to a 96-well plate. An equal volume of luminescent reagent is added to each well according to the method described for the BacTiter-Glo Microbial Cell Viability Assay and bioluminescence is measured via plate reader.

6.1.6 Data Analysis

Raw scores for color change (purple to yellow) were entered into an excel spreadsheet containing statistical algorithms to calculate mean, S.D. baseline and fold change. These data were then graphed using GraphPad Prism® version 7 (GraphPad Software, San Diego California).

6.2 Microtox

6.2.1 Experimental Design

The experimental design and general procedures of this study were conducted under the APHC TOX SOP 037 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.

6.2.2 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. Each compound was serially diluted 1:2 in DMSO and further diluted 1:100 in diluent. A total of eight concentrations were tested for the range finding. Reconstituted *A. fischeri* was 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. (Newark, Delaware). The EC₅₀ from the range finding determined the final test concentration range (See Appendix F for final chemical specific ranges).

6.2.3 Cytotoxicity Test

In instances where the range-finding test does not produce an EC₅₀, the cytotoxicity test is used to verify the range-finding data using the methodology described in paragraph 6.2.2.

6.2.4 Data Analysis

Raw luminescence data were recorded at 5, 15, and 30 minutes by the Microtox analyzer. As no EC₅₀ was found, no further analysis was necessary. All data (prints and files) were archived.

6.2.5 Derivation of Ecotoxicity Hazard Values

Data from Microtox tests were aligned with the aquatic toxicity criteria of the EPA, the OECD and the GHS to categorize the potential ecotoxicity of candidate test articles (Table 4) (OECD 2001; UNECE 2015; EPA 2017).

Table 4. Ecotoxicity Assessment Scale

LC ₅₀ or EC ₅₀ Concentration Range (mg/L)	Hazard Categories (EPA 2017)	Hazard Classes (OECD 2001)	Acute Aquatic Toxicity GHS (UNECE 2015)
< 0.01	Super Toxic	Acute Toxicity I (very toxic to aquatic life)	Acute Cat. 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 Cat. 2
10 to 100	Slightly Toxic	Acute Toxicity III (harmful to aquatic life)	Acute Cat. 3
100 to 1,000	Practically Nontoxic	—	—
> 1,000	Relatively Harmless	—	—

Legend:

LC₅₀ = median (50%) lethal concentration

EC₅₀ = median (50%) effect concentration

mg/L = milligrams per liter

EPA = U.S. Environmental Protection Agency

OECD = Organization for Economic Co-operation and Development

UNECE = United Nations Economic Commission for Europe

6.3 Skin Sensitization Assays

6.3.1 h-CLAT

The assay was conducted in compliance with the APHC TOX Type Protocol: *In Vitro* Skin Sensitization Parts 1-3 (APHC 2019a). In the absence of an SOP, testing was performed according to ECVAM DB-ALM protocol number 158 and OECD Guideline 442E (ECVAM 2014; OECD 2018).

6.3.1.1 Buffers

FACS buffer was prepared with PBS and 0.1% (w/v) BSA the day before use and stored at $+4 \pm 2$ °C. Blocking solution was made up in 1% (w/v) globulins in PBS stocks as needed, with stock being used within 1 week and stored at $+4 \pm 2$ °C. Blocking solution for use on the day of the experiment was diluted to a 0.1% solution in FACS buffer immediately prior to use. Propidium iodide (PI) was diluted to 12.5 µg/mL in PBS on the day of the experiment and maintained on ice.

6.3.1.2 Tissue Culture

Tissue culture media was prepared as described in paragraph 5.4 and maintained at $+4 \pm 2$ °C. Media was pre-warmed at room temperature prior to use for each cell plating and passage. Cells were maintained at $1.5 \times 10^5 - 8 \times 10^5$ cells/mL 37 °C, 5% CO₂. Cells were passaged every 2–3 days for no more than 30 passages or 60 days. Prior to passage or test plating, cell density was determined by counting with the TC-20 automated cell counter (Bio-Rad, Inc., Hercules, California). Cell viability was determined by Trypan blue staining (Bio-Rad, Inc.). For all testing (reactivity check, range finding and h-CLAT), cells were plated into 24-well plates at a density of 1×10^6 cells/well in 0.5 mL (i.e., 2×10^6 cells/mL). For maintenance, cells were plated at $1.5\text{--}2.0 \times 10^5$ cells/mL in 25–40 mL media depending on the timing of subsequent tests.

6.3.1.3 Reactivity Check

The reactivity check prior to full testing is used to confirm cell viability and induction of CD54 and CD86. Two weeks post thaw, a reactivity check of cells sampled from each propagation flask was performed using the control compounds DNCB, NiSO₄ and LA. DNCB was prepared as a 20 mg/mL stock solution in DMSO and stored at $+4 \pm 2$ °C in the dark. NiSO₄ was prepared as a 10 mg/mL stock solution in saline and stored at room temperature protected from light. LA was freshly prepared as a 100 mg/mL solution in saline. From these stock solutions additional dilutions were made so that the tested concentrations were 3.3–4 µg/mL DNCB, 100 µg/mL NiSO₄, and 1,000 µg/mL for LA. A 100% cytotoxic DNCB concentration (0.2 mg/mL) was added to one well as a positive control. Negative controls (diluted DMSO and saline) were also included. After all dosing solutions were distributed to the test wells, the test plate was incubated (approximately 37°C / 5% CO₂) for 24 hours. Cells were then collected, processed, stained with PI and antibodies [anti-IgG1 (isotype control), anti-CD54 and anti-CD86], and analyzed by flow cytometry (see paragraphs 6.3.6 and 6.3.7). Criteria for a successful reactivity check requires the positive controls DNCB and NiSO₄ induce CD54 and CD86 (Relative Fluorescence Intensity (RFI) criteria exceed: CD54 ≥ 200 and CD86 ≥ 150) and the negative

control, LA, does not induce CD54 or CD86 or reduce viability by more than 50% [target ~75% viability (CV75)]. When the cell sample meets these criteria, the remainder of cells from its propagation flask are used for testing chemicals. Propagation flasks can be resampled if the first sample fails the reactivity check to confirm no or poor reactivity. A second fail is cause to discard that flask and thaw a new lot of cells.

6.3.1.4 Range Finding

The range finding test is used to bracket the appropriate dose range for the full test using only the percent viability endpoint. 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. Eight serial dilutions (1:2, diluent = DMSO) were performed for each chemical. Each dilution was subsequently diluted 1:250 into tissue culture media (=final serial dilution). Pooled THP-1 cells were plated at 1×10^6 cells/well (24-well plate). An equal volume (0.5 mL) of each final serial dilution was added to the appropriate test wells. Negative (vehicle) control and cytotoxicity ('dead cell') positive controls were also included on each test plate. Cells were incubated for 24 hours (approximately 37°C /5% CO₂). After the 24 hour incubation, cells were collected and processed for staining with PI. Briefly, cells were transferred to 5 mL tubes, centrifuged (200 x g; +4 °C), and supernatants were discarded. Each pellet was resuspended in 0.6 mL cold FACS buffer and 0.2 mL of each sample was transferred to new tubes and washed by centrifuging (200 x g; +4 °C), decanting the supernatant, and resuspending the pellet in 0.2 mL FACS buffer. The wash step was repeated one time. The final pellets were resuspended in 0.4 mL FACS buffer and stained with 20 µL 12.5 µg PI/mL solution. Samples were maintained on ice in the dark and analyzed by flow cytometry (see paragraph 6.3.7). Percent viability (ratio of live cells to total acquired cells) was utilized to determine the CV75. Where CV75 was not achieved due to compound toxicity, additional range finding tests with lower compound concentrations were conducted until the CV75 was identified. If cytotoxicity was not observed at the maximum concentration, then, by default, the maximum dose is used as the highest dose in the h-CLAT.

6.3.1.5 h-CLAT Test

In the full test, the CV75 from the range finding test is used to develop the dose range and represents the 2nd highest dose of an 8-dose treatment. For the range finding assay, no cytotoxicity was observed; therefore, the top dose was the solubility limit of each compound and the same as the range finding. Compounds were solubilized in DMSO as in section 6.3.1.4 (500x). Eight 1:1.2 serial dilutions of each compound were subsequently diluted 1:250 in complete media and added in equal volume to test wells containing 0.5 mL medium and 1×10^6 cells per well (24-well plate). Three concentrations of DNCB were prepared from the 20 mg/mL stock solution and added to the appropriate wells containing 1×10^6 cells (final concentrations 0.003, 0.004 and 0.0048 mg/mL DNCB in medium). A DMSO vehicle control was prepared as was a "dead cell" control containing 10 µL of the 20 mg/mL DMSO stock. Cells were incubated for 24 hours and processed for IgG1, CD54, and CD86 staining and analysis by flow cytometry, see paragraphs 6.3.6 and 6.3.7.

6.3.1.6 Antibody Staining

Cells from each test well were transferred to individual 5 mL tubes and collected by centrifugation (250 x g/5 min/+4 °C). The supernatants were discarded and the pellets were resuspended in 1 mL cold FACS buffer and washed 2x. Cells were then incubated with blocking solution (0.6 mL 0.1% blocking buffer) for 15 minutes at $+4 \pm 2$ °C. Following blocking, samples were prepared in triplicate (i.e., split into 3 aliquots) of 180–200 µL each in a round-bottom 96-well plate, centrifuged (250 x g/5 min/+4 °C) and blocking buffer decanted. Samples were resuspended in 50 µL FACS buffer containing either IgG1, CD54, or CD86 antibodies as per the ECVAM protocol and gently vortexed, incubated at $+4 \pm 2$ °C in the dark for 30 min. and washed twice in FACS buffer (ECVAM 2014). Samples were transferred to FACS analysis tubes between washes. Following the final wash, all samples were resuspended in 0.4 mL FACS buffer, stained with 20 µL PI and mixed by vortexing. All samples were maintained on ice or at +4 °C throughout the staining process.

6.3.1.7 Flow Cytometry

The fluorescence intensities of the labeled cells were analyzed by flow cytometry using a BD FACSVerse™ flow cytometer and captured/analyzed with BD FACSuite™ v1.0.5. The acquisition channels were FL1 (fluorescein isothiocyanate (FITC) emission wavelength 530 ± 15 nm) and FL2 (PI emission wavelength >650 nm). PI stained untreated cells were used to determine the correct voltages for the forward scatter and side scatter channels. The dead cell and media controls were used to gate live (PI negative) versus dead (PI positive) cells. For each sample, 10,000 live or 30,000 total counts (whichever count was acquired first) in the PI channel were acquired, and the geometric mean fluorescence intensity (MFI) for FITC was calculated. The cell viability for each test concentration was determined from the isotype (IgG1) stained subpopulations, which were co-stained with PI as per paragraph 6.3.6.

6.3.1.8 Data Analysis

If the RFI for any concentration exceeded the positive criteria ($CD54 \geq 200$ and $CD86 \geq 150$), the EC_{200} and EC_{150} were calculated using the validated calculation spreadsheet. If the EC_{200} or EC_{150} fell below the lowest dose, the values were extrapolated according to the ECVAM protocol (ECVAM 2014). Two independent experiments were completed for each compound; the data from these two experiments were sufficient to determine sensitization and a third experiment was not necessary.

6.3.1.9 Criteria for a Valid Assay

The following criteria establish a valid assay:

- Cell viability of medium and DMSO controls was more than 90%.
- RFI values for the DNCB control for both CD54 and CD86 exceeded the positive criteria ($CD54 \geq 200$ and $CD86 \geq 150$).
- RFI values for the DMSO solvent control did not exceed positive criteria.

- The MFI ratio of both CD54 and CD86 to isotype control for DMSO and media controls exceeded 105%.
- The cell viability of at least four doses was greater than 50%.

6.3.1.10 Cell-based Acute Oral Toxicity Estimate (CAOTE)

The IC₅₀ is equivalent to the EC₅₀ (level at which cytotoxicity was induced by 50%) and was extrapolated from the cell viability of the range-finding experiments. From this IC₅₀, the following prediction algorithm was used to predict the acute oral rodent toxicity:

$$\log LD_{50} \text{ (mg/kg)} = 0.372 \log IC_{50} \text{ (}\mu\text{g/mL)} + 2.024$$

This algorithm is based upon a rat-only weight regression as demonstrated in the validation project for the Neutral Red Uptake assay, an alternative cytotoxicity assay. This model was applied to the THP-1 cells of the uptake in order to determine a hazard category and not to provide an LD₅₀ point estimate of rodent acute oral toxicity. The calculated LD₅₀ was assigned a GHS category for acute oral toxicity (Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) 2006; UNECE 2015).

6.3.2 DPRA

The assay was conducted in compliance with the APHC TOX Type Protocol: *In Vitro* Skin Sensitization Parts 1–3 (APHC 2019b). In the absence of an SOP, testing was performed according to ECVAM DB-ALM protocol number 154 and OECD Guideline 442C (ECVAM 2012; OECD 2015).

6.3.2.1 Buffers

A 100 mM, pH 7.5 sodium phosphate buffer was prepared using both sodium phosphate dibasic heptahydrate and sodium phosphate monobasic monohydrate 1 day prior to use and stored at +4 °C. Ammonium acetate (100 mM, pH 10.2) buffer was also prepared 1 day prior to use and stored for up to 2 weeks at +4 °C. All buffers were prepared with NanoPure water (ThermoScientific, Waltham, Massachusetts).

6.3.2.2 Sample Preparation

100 mM solutions of each compound and cinnamaldehyde (0.5 mL for lysine or 1.25 mL for cysteine experiments) were prepared in an appropriate solvent. COGA was prepared in acetonitrile, PCL in 1:1 DMSO: acetonitrile, and IBF-OH in DMSO. IBF-Ome was not solubilizable in any of the preferred solvents for DPRA. Peptides were prepared in a stock solution of 0.667 mM in sodium phosphate (cysteine) or ammonium acetate (lysine) buffers. Controls consisted of peptide and buffer only and co-elution (test chemical only). Samples were prepared in triplicate according to Table 5.

Table 5. ECVAM, DB-ALM Method Summary No. 154: DPRA – Summary (2012): Prescribed Sample Preparation

1:10 Ratio, Cysteine Peptide 0.5 mM Peptide, 5 mM test chemical	1:50 Ratio, Lysine Peptide 0.5 mM Peptide, 25 mM test chemical
• 750 µL Cysteine peptide solution (or pH 7.5 phosphate buffer for Co-elution controls) • 200 µL Test Solvent • 50 µL Test chemical solution (or solvent for reference controls)	• 750 µL Cysteine peptide solution (or pH 10.2 ammonium acetate buffer for Co-elution controls) • 250 µL Test chemical solution (or solvent for reference controls)

In accordance with the protocol, the standard curve was prepared using a 20% acetonitrile buffer solution and the samples were prepared in a 25% acetonitrile buffer solution. The dilution buffer is prepared in advance and the peptide is diluted to 0.534 mM (10:8 dilution) and a serial 1:2 dilution was performed for a total of six standards plus a buffer-only sample.

Standard curve and reference controls were incubated in the dark at room temperature for approximately 21 hours prior to the start of sample analysis, this allowed for test chemical and positive control sample analysis to begin approximately 24 hours after incubation started. Each peptide was analyzed two times.

6.3.2.3 Sample Analysis

The sample analyses were performed by HPLC UV-Vis detection. Chromatographic separation was achieved using a reverse phase C-18 HPLC column under gradient conditions. The aqueous mobile phase consists of 0.1% trifluoroacetic acid (TFA) in deionized water; the organic mobile phase consists of 0.085% TFA in acetonitrile. Column flow was maintained at 0.35 mL/min; the gradient was linear from 10% to 25% acetonitrile over 10 min., followed by a step to 90% acetonitrile. Injection volume was 10 µL. Cysteine and Lysine calibration standards, ranging in concentration from 0.000 to 0.534 mM, were prepared at seven concentrations by serial dilution of a 0.667 mM stock standard, in phosphate or ammonium acetate buffer, respectively. Quantitation was performed at 220 nm.

6.3.2.4 Data Analysis

The concentration of the peptide was determined from the absorbance at 220 nm by determining the area under the curve and matching it to a standard curve. Depletion of the peptide was determined by dividing the peak area of the test chemical by the mean peak area of the reference controls. The percent depletion was calculated by subtracting from 1 and multiplying by 100.

6.3.2.5 Criteria for a Valid Assay

The following criteria establish a valid assay:

- Standard curve $r^2 > 0.990$.

- Mean peptide concentration in Reference Control A = 0.50 ± 0.05 mM.
- Percent depletion in the positive control must fall into the following boundaries:
 - Cysteine: 60.8–100%.
 - Lysine: 40.2–69.4%.
- Maximum standard deviations for positive control and test chemical replicates cannot exceed the following:
 - Cysteine: 14.9%.
 - Lysine: 11.6%.
- The coefficient of variation (CV) of the peptide peak areas for Reference Controls B and C must be < 15.0%.

6.4 Concentration Verification of Test Compounds

At the end of each test day for each assay, samples of the final serial dilution were collected and stored at -80 °C for analysis by the APHC Client Services Division. A method for detecting COGA was developed with a limit of quantitation (LOQ) = 5 µg/mL. COGA concentration verification analyses were conducted in two separate instances (Aug 2020 and Jan 2021). The August 2020 analyses were performed on COGA samples from the Ames TA100, Microtox, and h-CLAT tests that were conducted in 2019. The results from the analyses were 10-fold lower than expected for the TA100 test, 1000-fold lower than expected for the Microtox tests, and as expected for the h-CLAT test. As these test stocks were prepared by different personnel and on different days, the professional opinion of the PIs of this study is that either a systemic error occurred during the concentration verification analyses or there was degradation of the sample in the months between the collection and analysis dates. Based on the PI review, the measured concentrations will not be used and the nominal COGA concentration is used for the Ames-TA100, Microtox, and h-CLAT tests. Methods for IBF-OH and IBF-Ome could not be established due to compound instability in the solvents. The method for PCL was developed but the LOQ (=150 µg/mL) was above the sample concentrations. Thus, the nominal concentrations for IBF-OH, OBF-Ome and PCL are used for reporting data. The measured concentrations of COGA will be used only from Jan 2021 analyses and when there is a greater than 10% difference between the measured and nominal values. It will be noted in the text where nominal versus measured COGA concentrations were used. The final report for COGA analysis, communications regarding compound stability, and PCL LOQ are maintained in the digitally archived records.

7 RESULTS AND DISCUSSION

7.1 Ames Assay

Appendix E provides the raw data and calculations for the Ames data for the COGA, IBF-OH, IBF-Ome and PCL experiments. Nominal concentrations are used for the graph axes. Table 6 shows the nominal and measured concentrations for COGA.

Table 6. Nominal and Measured COGA Test Concentrations

strain	TA98		TA100		TA1535		TA1537		E.coli mix	
Compound	nominal	measured	nominal	measured	nominal	measured	nominal	measured	nominal	measured
COGA µg/mL	169	120	169	No data	169	170	169	200	169	170
Extrapolated Test conc.	4.8, 15.4, 49.3, 158.0, 506.6 and 1623.6 µg/mL		Use nominal		Use nominal		8, 25.6, 82.2, 263.4, 844.2, and 2705.9 µg/mL		Use nominal	

In the TA100 test of IBF-Ome and PCL, the highest dose tested was 50 µg/mL due to solubility concerns. Subsequently, the highest dose tested for IBF-Ome was 500 µg/mL and 250 µg/mL for PCL.

7.1.1 Cytotoxicity of Test Compounds

In conjunction with the mutagenicity tests, the cytotoxicity of COGA, IBF-OH, IBF-Ome and PCL was evaluated. Cytotoxicity is indicated when bacterial viability is reduced by greater than 20% compared to controls. Figure 1 shows the data from the cytotoxicity tests.

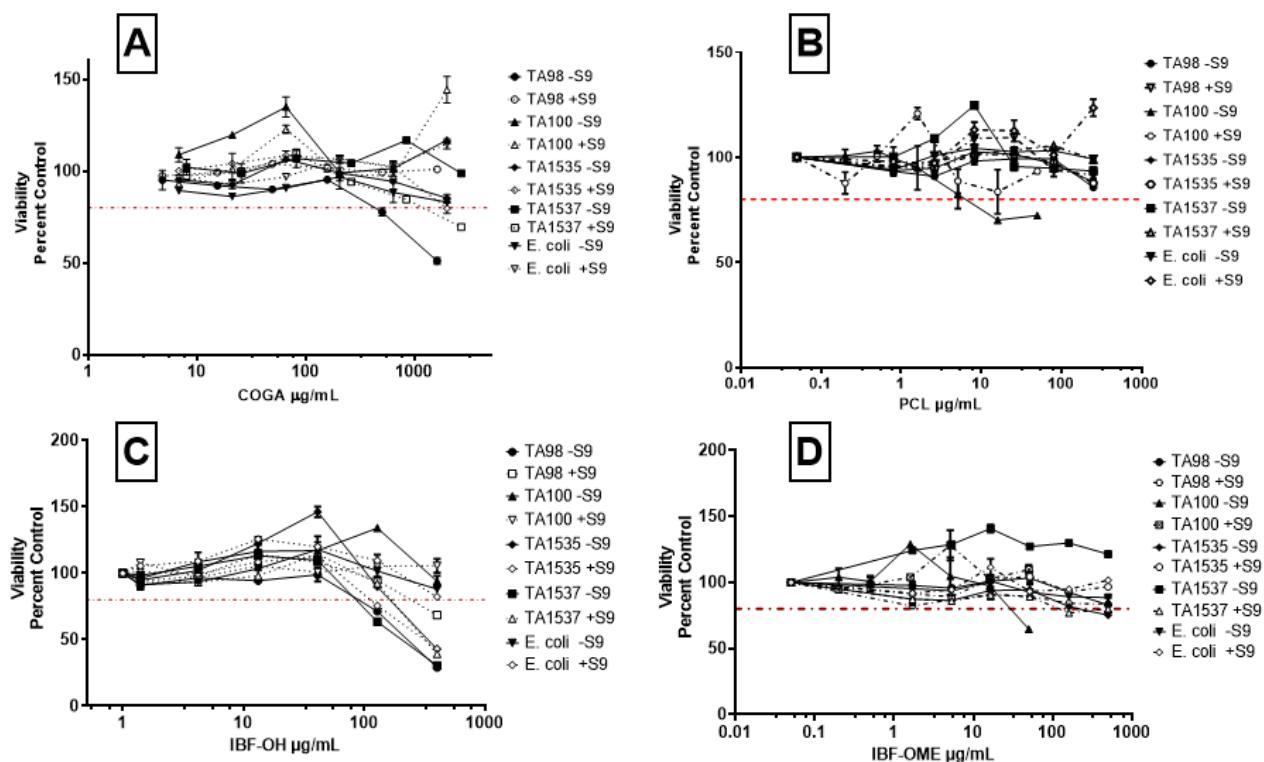


Figure 1. Tester strains cytotoxicity reported as percent viability compared to control (vehicle treated). Horizontal dashed line is 80% viability; below 80% the compound is considered cytotoxic. **A:** COGA was cytotoxic to TA98 -S9 $\leq 506.6 \mu\text{g/mL}$, TA1537 +S9 $\leq 2706 \mu\text{g/mL}$ and TA1535 +S9 $\leq 2000 \mu\text{g/mL}$; **B:** PCL was cytotoxic to TA100 -S9 $\leq 16 \mu\text{g/mL}$; **C:** IBF-OH was cytotoxic to TA98 -S9 $\leq 128 \mu\text{g/mL}$, +S9 $\leq 400 \mu\text{g/mL}$, TA1535 -S9 (400 $\mu\text{g/mL}$) / +S9 ($\leq 120 \mu\text{g/mL}$), and TA1537 -S9 ($\leq 128 \mu\text{g/mL}$), +S9 ($\leq 120 \mu\text{g/mL}$); and **D:** IBF-Ome was cytotoxic to TA100 -S9 $\leq 50 \mu\text{g/mL}$ and TA1535 -S9 $\leq 500 \mu\text{g/mL}$.

7.1.2 Mutagenicity of Test Compounds

The mutagenicity thresholds for tested compounds are shown in Table 7. The COGA, IBF-OME and PCL did not induce revertants in TA98, 100, 1537, 1537 and *E.coli* up to their respective solubility limits in either the absence (-S9) or presence (+S9) of liver microsomes. IBF-OME and PCL were tested at lower concentration range (highest concentration was 50 $\mu\text{g/mL}$) in TA100. Subsequently, both compounds were observed to be soluble as high as 500 $\mu\text{g/mL}$ and the test range was adjusted accordingly. The IBF-OH induced a dose dependent increase in revertants in the TA100 strain in the absence of S9 microsomes at and above 13.2 $\mu\text{g/mL}$. IBF-OH was not mutagenic in the presence of S9 and was not mutagenic in the other tester strains (TA98, TA1535, TA1537 and *E.coli*.)

Table 7. Threshold Mutagenicity in µg/mL of Test Compounds to Bacterial Tester Strains

Comp./	TA100		TA98		TA1535		TA1537		E.coli	
highest tested conc.	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
COGA 2000 µg/mL	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
IBF-OH 400 µg/mL	13.2	neg	neg	neg	neg	neg	neg	neg	neg	neg
IBF-Ome 500 µg/mL	neg*	neg*	neg	neg	neg	neg	neg	neg	neg	neg
PCL 250 µg/mL	neg*	neg*	neg	neg	neg	neg	neg	neg	neg	neg
* =highest concentration 50 µg/mL										

7.2 Microtox Toxicity and Risk Assessment

Toxicity of the test compounds to marine bacteria, *A. fischeri*, was measured by the Microtox acute toxicity test system at 5, 15, and 30 min. For each test compound, three individual experiments were performed in duplicate. Table 6 presents the toxicity data (EC₅₀ and the 95% Confidence Interval) and risk hazard. Appendix F: Figures – Microtox presents the analyzed data. 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 8. Microtox Toxicity and Hazard Assessment

Compound	Microtox EC ₅₀ (mg/L) ^a [95% CI]			Hazard Categories (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	—	—

Legend:

EC₅₀ = median (50%) effect concentration

mg/L = milligrams per liter

CI = Confidence Interval

EPA = U.S. Environmental Protection Agency

OECD = Organization for Economic Co-operation and Development

GHS = Global Harmonization System

Note:

^a The value of EC₅₀ at 15 minutes is used for the risk assessment.

7.3 Skin Sensitization Assays

7.3.1 h-CLAT

7.3.1.1 Reactivity Check

The THP-1 cells were checked and verified for reactivity to DNCB, NiSO₄, and lack of reactivity to LA. Cells reacted as expected with DNCB and NiSO₄ eliciting positive reactions for both CD54 and CD86 while LA was negative. See Appendix G for data.

7.3.1.2 Range-finding Assay

Two independent dose finding assays were completed in order to determine the CV75 each compound in THP-1 cells. Cytotoxicity was not observed in the dosing range, therefore a CV75 could not be experimentally determined. Therefore, the top dose for the full assay was set at the test maximum of 500 mg/mL. Appendix G contains the raw data for the Range Finding.

7.3.1.3 h-CLAT

Two independent h-CLAT assessments were completed for each compound. COGA, PCL, and IBF-Ome were negative for both CD86 and CD54 expression, indicating a negative test, and they are not likely to be sensitizers, however a confirmatory assessment in the DPRA is required according to the Defined Approach. Assessment of IBF-OH was not possible due to interference of the compound with the FITC fluorophores, further assessment must be made by DPRA. Appendix G contains the raw data. Data are reported for the nominal concentrations of the compounds due to the fact that as of the writing of this report, concentration verification has not yet been completed by APHC Client Services Division.

7.3.1.4 CAOTE

Mammalian acute oral toxicity was not able to be accurately predicted using data collected in the h-CLAT due to a lack of cytotoxicity. TOPKAT modeling for these compounds predicted that COGA and PCL will have low predicted oral toxicity, while IBF-OH and IBF-Ome will have moderate oral toxicity, GHS category 3 (UNECE 2015).

7.3.2 DPRA

Three independent experiments were completed for COGA. For PCL, two independent experiments were completed. COGA and PCL were negative in the DPRA and are thus not considered to be skin sensitizers. IBF-OH had one cysteine assay completed, however there was a precipitate that formed, limiting the ability to complete the assay in full. It was positive in this one assay; it is likely that IBF-OH is a skin sensitizer, which is in agreement with the TOPKAT model predications. IBF-Ome was insoluble in any of the recommended solvents for the assay, and thus could not be analyzed in the assay. Therefore, due to their insoluble nature, a complete assessment could not be made for IBF-OH and IBF-Ome. Appendix H contains the raw data.

8 CONCLUSIONS

In this study, COGA, PCL, IBF-OH and IBF-Ome were evaluated for mutagenicity, acute aquatic toxicity, and skin sensitization using QSAR and *in vitro* approaches. Using QSAR, COGA and IBF-OH, the parent compounds for PCL and IBF-Ome, respectively, were evaluated for acute and chronic oral toxicity, inhalation toxicity, developmental, mutagenicity (Ames), carcinogenicity, dermal and ocular irritation and skin sensitization. The larger branched compounds, PCL and IBF-Ome were not consistently modeled due to structural features that were not within the databases making the predictions for PCL and IBF-Ome unreliable. The results for toxicity evaluations provided in this report are summarized in Table 9.

Table 9. Toxicity evaluation for COGA, PCL, IBF-OH and IBF-Ome.

	QSAR-TOPKAT												ECOSAR			IN VITRO			
	Rat Oral LD50	Rat Chronic LOAEL	Rat Inhalational LC50	Skin Irritancy	Skin Sensitization	Ocular Irritancy	Developmental Toxicity Potential	Ames Mutagenicity	Weight of Evidence Carcinogenicity	Aerobic Biodegradability	Fathead Minnow LC50	Daphnia EC50	Fathead Minnow LC50	Daphnia EC50	Green Algae EC50	Rat Oral LD50 (CAOTE)	Ames Mutagenicity	Skin Sensitization	Fathead Minnow LC50
COGA	4		5	3	neg.	1	pos.	neg.	neg.	yes	3	3				5	neg.		3
confidence	vLC	vLC	HC	HC	vLC	HC	HC	vLC	HC	HC	HC	vLC							
IBF-OH	4		5	neg.	neg.	3	neg.	pos.	pos.	no	1	1	1	1	1	5	pos.	pos.	3
confidence	HC	vLC	LC	HC	HC	HC	HC	HC	HC	vLC	vLC	vLC							
PCL																5 neg.			
IBF-OME																5 neg.			
Notes: confidence levels for the TOPKAT predictions are categorized as high confidence (HC), low confidence (LC) and very low confidence (vLC). Confidence levels for ECOSAR are not available.																			

COGA is predicted to be a severe ocular irritant and potentially a developmental/reproductive toxicant. It is predicted to be negative in the Ames assay and tested negative in the Ames screen. COGA was predicted to be non-sensitizing and was not sensitizing in the in vitro tests. IBF-OH is predicted to be mutagenic and carcinogenic in the QSAR; IBF-OH tested positive for mutagenicity in the Ames screen. IBF-OH was predicted to be a skin sensitizer, but could not be confirmed as such due to an interaction with the test systems. It is likely positive after one successful DPRA assessment.

PCL and IBF-Ome in silico predictions were not reliable. Both tested negative in the Ames screen and were not sensitizers (or they were not compatible with the test). The predicted and tested aquatic toxicity for COGA were in agreement; COGA is a moderate aquatic hazard (GHS Cat 3). The predicted aquatic toxicity for IBF-OH was high, GHS Cat 1. The Microtox results for IBF-OH scored it as GHS Cat 3. Both compounds were scored as “practically non-toxic”. The poor solubility of these compounds likely further reduces their potential aquatic hazard.

9 RECOMMENDATIONS

The NALAS compounds, COGA, PCL, IBF-OH, and IBF-Ome, may present moderate to low toxicity and reduce toxicological concerns compared to the isocyanates (HMDI and TDI) that they are in development to replace. *In silico* and *in vitro* acute oral and acute aquatic toxicity estimates are similar or better for these replacement chemicals than the isocyanates. The primary concerns for IBF-OH are potential genotoxicity and skin sensitization. There is a concern for skin sensitization with IBF-Ome, as well. Due to chemical incompatibility with the test system, it was not possible to assess the skin sensitization of IBF-OH and IBF-Ome. It is recommended that non-aqueous skin sensitization assessments be completed (e.g. the local lymph node assay) to determine if IBF-OH and IBF-Ome are in fact skin sensitizers. IBF-OH also presents a bioaccumulation concern due to its low water solubility and high organic carbon to water partition coefficient (K_{OC}), which suggests it could be sequestered in soils and sediments. COGA and PCL may be mobile in aquatic environments, so additional aquatic toxicity testing (fish, algae, and invertebrates) should be considered. To further characterize potential toxicities, it may be necessary to complete acute and subacute repeat-dose (14- or 28-day) rat studies, with an addition of the micronucleus assay to further investigate the potential for genotoxicity.

10 POINTS OF CONTACT

Drs. Emily N. Reinke and Valerie H. Adams were the Study Directors and are the points of contact for this project. They may be reached at DSN 584-3980 or commercial 410-436-3980.

Submitted by: U.S. Army Public Health Center
Health Effects Division
MCHB-PH-HEF
Aberdeen Proving Ground, MD 21010-5403
410-436-3980

Prepared by:

REINKE.EMILY.NI

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Date: 2022.05.19 10:22:53 -04'00'

Emily N. Reinke, Ph.D., D.A.B.T.
Biologist
HEF
APHC

Date

ADAMS.VALERI

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ADAMS.VALERIE.H.117498571
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Valerie H. Adams, Ph.D., D.A.B.T.
Biologist
HEF
APHC

Date

Approved by:

QUINN.MICHAEL

L.J.1282372092

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QUINN.MICHAEL.J.1282372092
Date: 2022.05.19 15:05:55
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Michael J. Quinn Jr., Ph.D.
Division Chief
HEF
APHC

Date

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Date: 2022.05.23 08:57:23 -04'00'

Mark S. Johnson, Ph.D., D.A.B.T.
Director, Toxicology Directorate
APHC

Date

APPENDIX A

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Appendix B
Quality Assurance Statements
Ames Assay

Toxicity Study No. S.0002728- January 2022

Appendix B
QUALITY ASSURANCE STATEMENT
AMES ASSAY

For: Toxicology Study No. S.0002728- January 2022 AMES assay of the test articles listed, the following critical phases were inspected/audited by the Quality Safety Accreditation and Regulatory Compliance Office (QSARC):

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Type Protocol Good Laboratory Practice Standard Review	03/31/2016	03/31/2016
Test Article Specific Protocol Modification Review	02/15/2018	02/15/2018

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
In-Vitro AMES Assay - Reagents, Working Solutions Bacteria Storage	05/02/2018	05/05/2018
In-Vitro AMES Assay - Reagents, Working Solutions Bacteria Storage	05/02/2018	05/05/2018
In-Vitro AMES Assay – Compliance with GLP requirements for Test Facility SOPs	05/02/2018	05/05/2018
In-Vitro AMES Assay – Calibration Verification of Equipment Used during assay	05/02/2018	05/05/2018
Study Raw Data Good Laboratory Practice Standard Review	04/22/2022	04/22/2022
Final Study Good Laboratory Practice Standard Report Review	04/22/2022	04/22/2022

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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Michael P. Kefauver
GLP Quality Assurance Specialist, QSARC

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Microtox Assay

Toxicology Study No. S.0065662, August 2019

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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Michael P. Kefauver
GLP Quality Assurance Specialist, QSARC

04/21/2020

Date

Skin Sensitization Assays

No. S.0002728, February 2022

Quality Assurance Statement

Skin Sensitization Assay

For: Toxicology Study No. S.0002728- January 2022 Skin Sensitization Testing of the test articles listed following critical phases were inspected/audited by the Quality Safety Accreditation and Regulatory Compliance Office (QSARC):

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Test Article Specific Protocol Modification Review	N/A	N/A

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
In-Vitro Skin Sensitization h-CLAT Assay - Preparation of Stock and Working Solutions	7/10/2019	9/23/2019
In-Vitro Skin Sensitization h-CLAT Assay - Cell Suspension and Exposure	7/10/2019	9/24/2019
In-Vitro Skin Sensitization h-CLAT Assay - Reagents, Working Solutions and Cell Suspension Storage	7/10/2019	9/23/2019
In-Vitro Skin Sensitization h-CLAT Assay – Compliance with GLP requirements for Test Facility SOPs	7/10/2019	9/24/2019
In-Vitro Skin Sensitization h-CLAT Assay – Calibration Verification of Equipment Used during assay	7/10/2019	9/23/2019
Study Raw Data Good Laboratory Practice Standard Review	04/22/2022	04/22/2022
Final Study Good Laboratory Practice Standard Report Review	04/22/2022	04/22/2022

Note: 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. A review of the raw data and records indicates the above mentioned report accurately reflects the raw data and study as it was conducted. 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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Michael P. Kefauver
GLP Quality Assurance Specialist, QSARC

04/22/2022
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 Toxicology Directorate, APHC, for a minimum of 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 Toxicology Directorate for a minimum of 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.00058221.3-21, Protocol Nos. 0FMA-92-iv17-03-01T-W, 2758-70-iv19-04-01-H,I,J,K and 49-iv19-03-01H-K.

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., Toxicology Director; Michael J. Quinn, Ph.D., Division Chief, Health Effects Division (HEF).

Study Directors: Valerie H. Adams Ph.D., D.A.B.T and Emily N. Reinke, Ph.D., D.A.B.T HEF.

Technical staff: Lindsay A. Holden, Ph.D., HEF and Taryn Brown, ORISE Fellow.

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

APPENDIX D

STUDY REAGENTS

Table D-1. Ames Test Reagents

Ames Reagents	Source	Lot #	Exp Date
AG-TA98 <i>hisD3052 Salmonella typhimurium</i>	Xenometrix	27a	2/28/21
AG-TA100 <i>hisG46 Salmonella typhimurium</i>	Xenometrix	23b	1/1/21
AG-TA1535 <i>his G46 Salmonella typhimurium</i>	Xenometrix	17c	5/28/21
AG-TA1537 <i>hisC3076 Salmonella typhimurium</i>	Xenometrix	21d	4/28/21
<i>E. coli</i> wp2 [pKM101] <i>trpE65 Escherichia coli</i>	Xenometrix	P19	7/31/21
<i>E. coli</i> wp2 <i>uvrA trpE65 Escherichia coli</i>	Xenometrix	U20	7/31/21
S9-Postmitochondrial Supernatant (Aroclor)	Xenometrix	4106	05/31/22
Ampicillin	Xenometrix	191	8/31/21
N4-aminocytidine	Xenometrix	NA079541202	n/a
2-aminoanthracene	Xenometrix	1701-1804	04/28/21
2-nitrofluorene	Xenometrix	1550-1901	1/28/22
4-nitroquinoline-N-oxide	Xenometrix	1801-1902	03/28/22
9-aminoacridine	Xenometrix	1018-1810	10/28/21
Ames MPF Exposure Medium	Moltox	52878	7/23/20
Ames MPF Exposure Medium	Xenometrix	MC04819P	6/30/21
Ames MPF Reversion Indicator Medium	Xenometrix	MC06879P	6/30/21
Ames MPF Growth Medium	Xenometrix	MC02717P	4/30/21
<i>E. coli</i> Reversion Indicator Medium	Xenometrix	ND09642P	11/30/21
<i>E. coli</i> Exposure Medium	Xenometrix	MC04819P	6/30/21
Ames MPF S9 Buffer Salts, ,	Xenometrix	MC09053P	1/31/22
S9-100/1537 Booster Solution	Xenometrix	X1909BSA	9/28/22
Glucose-6-phosphate/ S9-G-6-P	Xenometrix	X1810SGPA	10/31/20
NADP/ S9-NADP	Xenometrix	X1810SNAA	10/31/21

Table D-2. 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-Aldrich	RNBG8238	06/2020
Phenol	Sigma-Aldrich	BCBW8224	N/A
Modern Water Microtox Reconstitution Solution	Modern Water	18C4048	03/2021

Table D-3. h-CLAT Test Reagents

Reagent	Supplier	Product Number	Lot Number	Expiration Date
THP-1	ATCC	TIB-202	62996831	N/A
RPMI-1640	Gibco	22400	2052771	03-30-2020
FBS	Gibco	16140	1939739	11-30-2022
2-Mercaptoethanol	Gibco	21985	1922541	11-30-2020
Penicillin-Streptomycin ^a	HyClone	SV30010	J170027	06-30-2019 ^a
Saline	Sigma	S8776	RNBD7305	N/A
DMSO (TC)	Sigma	D2438	RNBG4916	02-2020
Globulins	Sigma	G2388	017K7650V	N/A
BSA Fraction V	EMD Millipore	12660	3035346	N/A
D-PBS	Gibco	14190	1897013 1855254	07-30-2020 03-30-2020
Propidium Iodide	Sigma	P4864	MKBR1007V	N/A
CD54 Antibody, ICAM-1 Clone 6.5B5, FITC	Dako	F714301-8	20051521	09-2020
CD86 Antibody, Hu Fun-1, FITC	BD	555657	6348610	11-30-2021
IgG1 (mouse), FITC	Dako	X092701-2	20036430	07-31-2019
Flow Cytometer Beads	BD	650622	81165	05-31-2020
Sheath Fluid	BD	342003	0000221830	02-04-2022
2,4-dinitrochlorobenzene (DNCB)	Sigma	237329	BCBN7826V	N/A
Nickel Sulfate (NiSO ₄)	Sigma	656895	MKBT0269V	N/A
Lactic Acid (LA)	Sigma	W261106	MKBR4746V	N/A

Note:

^a Penicillin-Streptomycin did have an expiration date preceding the usage in the testing during the reactivity check and early growth of the cell stock; however, media stock had been appropriately temperature maintained and was within a month of expiration. Professional experience has shown that this reagent maintains full effectiveness past the expiration date.

Table D-4. DPRA Test Reagents

Reagent	Supplier	Product Number	Lot Number	Expiration Date
Lysine Peptide	RS Synthesis	N/A	RS2464040620-2	
Cysteine Peptide	RS Synthesis	N/A	RS2464040620-1	
Acetonitrile	Fisher Chemical	A955-4	172559	01/30/24
Cinnamaldehyde	Sigma-Aldrich	W228613	MKBX8146V	02/2021
Sodium phosphate monobasic monohydrate	Sigma-Aldrich	S9638-250G	SLBP0799V	
Sodium phosphate dibasic heptahydrate	Sigma-Aldrich	S9390-500G	SLBQ2859V	
Ammonium acetate	Sigma-Aldrich	238074-500G	MKBW7225V	
Ammonium hydroxide	Sigma-Aldrich	32145-500ML	19296MJ	

APPENDIX E

COGA, IBF-OH, IBF-OME AND PCL AMES ASSAY RAW DATA

TA98

Compound: COGA				Spontaneous				COGA				Assay Date: 11/19/2020				
TA 98 -S9				TA 98 -S9				TA 98 -S9				TA 98 -S9				
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t test p-value (unpaired, 1 sided)
6.8	3	4	3	0	4			0	9	3.44	1.59	5.03	0.97	0.66	0.4552	
21.1	4	1	4	3	4			6.76483215	3	3.33	0.58		0.87	0.60	0.3446	
65.9	0	0	2	4	5			21.1602763	3	3.00	1.73		0.19	0.13	0.0102 Cytotoxic effect?	
205.5	3	2	5	2				66.8516821	3	0.67	1.15		0.97	0.66	0.4596	
641.0	0	0	1	2				205.456936	3	3.33	1.53		0.68	0.46	0.1462	
2000	0	5	1	4				641.025641	3	2.33	1.15		0.58	0.40	0.1432	
Pos. Control	42	42	43					2000	3	2.00	2.65		0.58	0.40	0.1343	
								Pos. Control	3	42.33	0.58					
Compound: COGA				Spontaneous				COGA				Assay Date: 11/19/2020				
TA 98 +S9				TA 98 +S9				TA 98 +S9				TA 98 +S9				
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t test p-value (unpaired, 1 sided)
6.8	3	0	1	3	1			0	9	1.89	1.05	2.94	0.71	0.45	0.2453	
21.1	3	1	0	2	2			6.76483215	3	1.33	1.53		0.71	0.45	0.2453	
65.9	1	6	4	3	1			21.1602763	3	1.33	1.53		1.94	1.25	0.0497	
205.5	2	0	0	3				66.8516821	3	3.67	2.52		0.35	0.23	0.0596	
641.0	0	2	1	0				205.456936	3	0.67	1.15		0.53	0.34	0.1151	
2000	3	0	2	2				641.025641	3	1.00	1.00		1.06	0.68	0.4381	
Pos. Control	48	48	48					2000	3	2.00	1.00					
								Pos. Control	3	48.00	0.00					
Compound: IBF-OH				Spontaneous				IBF-OH				Assay Date: 11/19/2020				
TA 98 -S9				TA 98 -S9				TA 98 -S9				TA 98 -S9				
Conc. (l)	Replicate #1	Replicate #2	Replicate #3	Conc. (l)	Replicate #1	Replicate #2	Replicate #3	Conc. (l)	n	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t test p-value (unpaired, 1 sided)
1.4	1	2	3	0	4			0	9	3.44	1.59	5.03	0.58	0.40	0.0884	
4.2	5	4	3	3	4			1.35296643	3	2.00	1.00		1.16	0.79	0.2942	
13.2	2	1	2	4	5			4.22125262	3	4.00	1.00		0.48	0.33	0.0474 Cytotoxic effect?	
41.1	1	5	3	5				13.1703164	3	1.67	0.58		1.45	0.99	0.0975	
128.2	1	2	0	2				41.0913872	3	5.00	2.00		0.29	0.20	0.0168 Cytotoxic effect?	
400	0	0	0	4				128.205128	3	1.00	1.00		0.00	0.00	0.9022 Cytotoxic effect?	
Pos. Control	43	37	44					400	3	0.00	0.00					
								Pos. Control	3	41.33	3.79					
Compound: IBF-OH				Spontaneous				IBF-OH				Assay Date: 11/19/2020				
TA 98 +S9				TA 98 +S9				TA 98 +S9				TA 98 +S9				
Conc. (l)	Replicate #1	Replicate #2	Replicate #3	Conc. (l)	Replicate #1	Replicate #2	Replicate #3	Conc. (l)	n	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t test p-value (unpaired, 1 sided)
1.4	2	1	3	3	1			0	9	1.89	1.05	2.94	1.06	0.68	0.4381	
4.2	1	0	1	2	2			1.35296643	3	2.00	1.00		0.35	0.23	0.0451 Cytotoxic effect?	
13.2	1	3	6	3	1			4.22125262	3	0.67	0.58		0.82	0.60	0.2671	
41.1	2	1	1	3				13.1703164	3	3.33	2.52		0.81	0.45	0.0686	
128.2	2	4	10	0				41.0913872	3	1.33	0.58		0.71	0.45	0.2069	
400	0	0	1	2				128.205128	3	5.33	4.16		2.62	1.81	0.0164	
Pos. Control	48	48	48					400	3	0.67	0.58		0.35	0.23	0.0451 Cytotoxic effect?	
								Pos. Control	3	48.00	0.00					
Compound: IBF-OME				Spontaneous				IBF-OME				Assay Date: 11/19/2020				
TA 98 -S9				TA 98 -S9				TA 98 -S9				TA 98 -S9				
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t test p-value (unpaired, 1 sided)
1.7	5	4	3	5	3			0	9	4.89	1.76	6.55	0.82	0.60	0.2175	
5.3	7	1	4	5	6			1.69120804	3	4.00	1.00		0.82	0.60	0.2671	
16.5	4	1	4	5	6			5.27658908	3	4.00	3.00		0.81	0.45	0.0686	
51.4	3	3	1	4				16.4628065	3	3.00	1.73		0.48	0.35	0.0218 Cytotoxic effect?	
160.3	6	1	1	2				51.3642341	3	2.33	1.15		0.75	0.55	0.1832	
500	3	4	5	8				160.25641	3	3.67	2.52		0.92	0.60	0.2175	
Pos. Control	46	41	41					500	3	4.00	1.00					
								Pos. Control	3	42.67	2.89					
Compound: IBF-OME				Spontaneous				IBF-OME				Assay Date: 11/19/2020				
TA 98 +S9				TA 98 +S9				TA 98 +S9				TA 98 +S9				
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t test p-value (unpaired, 1 sided)
1.7	0	2	1	3	2			0	9	1.76	0.97	2.75	0.56	0.36	0.1301	
5.3	4	4	0	2	2			1.69120804	3	1.00	1.00		1.50	0.97	0.1733	
16.5	1	3	2	0	3			5.27658908	3	2.67	2.31		1.13	0.73	0.3791	
51.4	1	2	0	1				16.4628065	3	2.00	1.00		0.56	0.36	0.1301	
160.3	0	0	1	1				51.3642341	3	1.00	1.00		0.19	0.12	0.0199 Cytotoxic effect?	
500	1	3	1	2				160.25641	3	0.33	0.58		0.94	0.61	0.4362	
Pos. Control	48	48	48					500	3	1.67	1.15					
								Pos. Control	3	48.00	0.00					

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Compound: PCL				PCL				Assay Date: 11/19/2020					
TA 98 -S9				TA 98 -S9									
Conc. (l)	Replicate #1	Replicate #2	Replicate #3	TA 98 -S9	Conc. (l)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold Increase (over zero value)	Fold Increase (over baseline)	t-test p-value (unpaired, 1 sided)
0.8	2	5	1	0 ✓ 4	0	9	3.44	1.59	5.03				
2.6	3	2	1	3 ✓ 4	0.84560402	3	2.67	2.08		0.77	0.53	0.2540	
8.2	4	2	2	4 ✓ 5	2.63828454	3	2.00	1.00		0.58	0.40	0.0884	
25.7	1	1	4	5 ✓	8.23144777	3	2.67	1.15		0.77	0.53	0.2292	
80.1	5	2	1	2 ✓	25.682117	3	2.00	1.73		0.58	0.40	0.1053	
250	2	2	1	4 ✓	80.1282051	3	2.67	2.08		0.77	0.53	0.2540	
Pos. Control	42	36	36		250	3	1.67	0.58		0.48	0.33	0.0472	Cytotoxic effect?
Compound: PCL				PCL				Assay Date: 11/19/2020					
TA 98 +S9				TA 98 +S9									
Conc. (l)	Replicate #1	Replicate #2	Replicate #3	TA 98 +S9	Conc. (l)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold Increase (over zero value)	Fold Increase (over baseline)	t-test p-value (unpaired, 1 sided)
0.8	0	1	0	3 ✓ 1	0	9	1.89	1.05	2.94				
2.6	1	2	2	2 ✓ 2	0.84560402	3	0.33	0.58		0.18	0.11	0.0191	Cytotoxic effect?
8.2	1	2	1	3 ✓ 1	2.63828454	3	1.67	0.58		0.88	0.57	0.3701	
25.7	0	1	1	3 ✓	8.23144777	3	1.33	0.58		0.71	0.45	0.2069	
80.1	0	0	2	0 ✓	25.682117	3	0.67	0.58		0.35	0.23	0.0451	Cytotoxic effect?
250	2	2	2	2 ✓	80.1282051	3	0.67	1.15		0.35	0.23	0.0595	
Pos. Control	48	48	48		250	3	2.00	0.00		1.06	0.68	0.4316	

Toxicology Study No. S. 0058638-18, May 2022

Compound: PCL				PCL				Assay Date: 2/20/2020			
TA 100 -S9				TA 100 -S9				TA 100 -S9			
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
0.2	4	7	5	0	6	4.67	2.73	7.40	1.14	0.72	0.3559
0.5	3	1	2	3	0	5.33	1.53	0.43	0.27	0.0778	0.1249
1.6	1	5	1	4	0.52765691	3	2.00	1.00	0.50	0.32	0.1321
5.1	2	3	3	5	1.64028955	3	2.67	0.58	0.50	0.32	0.0998
16.0	3	2	2	3	5.13642341	3	2.67	0.58	0.71	0.45	0.2333
50	3	2	5	3	16.025641	3	2.33	1.53			
Pos. Control	45	47	43	Pos. Control	3	46.00	2.00				

Compound: PCL				PCL				Assay Date: 2/20/2020			
TA 100 +S9				TA 100 +S9				TA 100 +S9			
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
0.2	5	7	4	0	6	3.50	1.38	4.88	1.52	1.09	0.0556
0.5	4	1	2	3	0	5.33	1.53	0.67	0.48	0.1421	0.2645
1.6	2	4	7	2	0.52765691	3	2.33	1.53	0.76	0.55	0.1804
5.1	3	3	3	5	1.64028955	3	4.33	2.52	0.86	0.61	0.2992
16.0	3	4	2	3	5.13642341	3	2.67	0.58	1.33	0.96	0.1923
50	5	2	2	2	16.025641	3	3.00	1.00			
Pos. Control	8	15	14	Pos. Control	3	12.33	3.79				

TA1535

Compound: COGA				COGA				Assay Date: 12/2/2020			
TA 1535 -S9				TA 1535 -S9				TA 1535 -S9			
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
6.8	3	4	3	0	9	3.44	1.59	5.03	0.97	0.66	0.4552
21.1	4	1	4	3	6.76483215	3	3.33	0.58	0.87	0.60	0.3448
65.9	0	0	2	4	21.1062763	3	3.00	1.73	0.19	0.13	0.0102
205.5	3	2	5	5	65.8515821	3	0.67	1.15	0.97	0.66	0.4598
641.0	3	3	1	2	205.456936	3	3.33	1.53	0.68	0.46	0.1482
2000	0	5	1	4	641.025641	3	2.33	1.15	0.58	0.40	0.1343
Pos. Control	42	42	43	Pos. Control	3	42.33	0.58				

Compound: COGA				COGA				Assay Date: 12/2/2020			
TA 1535 +S9				TA 1535 +S9				TA 1535 +S9			
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
6.8	3	0	1	0	9	1.89	1.05	2.94	0.71	0.45	0.2453
21.1	3	1	0	3	6.76483215	3	1.33	1.53	0.71	0.45	0.2453
65.9	1	6	0	3	21.1062763	3	1.33	1.53	0.94	1.25	0.1407
205.5	2	0	0	0	65.8515821	3	3.67	2.52	0.35	0.23	0.0595
641.0	0	2	1	0	205.456936	3	0.67	1.15	0.53	0.34	0.1151
2000	3	1	2	2	641.025641	3	1.00	1.00	1.06	0.68	0.4381
Pos. Control	48	48	48	Pos. Control	3	48.00	0.00				

Compound: IBF-OH				IBF-OH				Assay Date: 12/2/2020			
TA 1535 -S9				TA 1535 -S9				TA 1535 -S9			
Conc. (l)	Replicate #1	Replicate #2	Replicate #3	Conc. (l)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
1.4	1	2	3	0	9	3.44	1.59	5.03	0.58	0.40	0.0884
4.2	5	4	2	3	1.35206643	3	2.00	1.00	1.16	0.79	0.2942
13.2	4	1	3	4	4.22125526	3	4.00	1.00	0.48	0.33	0.0473
41.1	7	5	3	5	13.1703164	3	1.67	0.58	1.45	0.99	0.0971
128.2	1	2	0	2	41.0913872	3	5.00	2.00	0.29	0.20	0.0189
400	0	0	0	4	128.205128	3	1.00	1.00	0.00	0.00	0.6092
Pos. Control	43	37	44	Pos. Control	3	41.33	3.79				

Compound: IBF-OH				IBF-OH				Assay Date: 12/2/2020			
TA 1535 +S9				TA 1535 +S9				TA 1535 +S9			
Conc. (l)	Replicate #1	Replicate #2	Replicate #3	Conc. (l)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
1.4	2	1	3	0	9	1.89	1.05	2.94	1.06	0.68	0.4381
4.2	1	0	1	3	1.35206643	3	2.00	1.00	0.35	0.23	0.0451
13.2	1	3	6	3	4.22125526	3	0.67	0.58	1.76	1.13	0.0851
41.1	2	3	1	0	13.1703164	3	3.33	2.52	0.71	0.45	0.2069
128.2	2	4	10	0	41.0913872	3	1.33	0.58	2.82	1.81	0.0164
400	0	1	1	0	128.205128	3	5.33	4.16	0.35	0.23	0.0451
Pos. Control	48	48	48	Pos. Control	3	48.00	0.00				

Compound: IBF-OME				IBF-OME				Assay Date: 12/2/2020			
TA 1535 -S9				TA 1535 -S9				TA 1535 -S9			
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
1.7	4	3	2	0	9	2.00	1.22	3.22	1.50	0.93	0.1168
5.3	0	1	1	4	1.69120804	3	3.00	1.00	0.33	0.21	0.0630
16.5	3	18	13	2	5.27656908	3	0.67	0.58	5.67	3.51	0.0019
51.4	1	2	1	0	16.4628955	3	11.33	7.64	0.67	0.41	0.1976
160.3	4	2	1	3	51.3642341	3	1.33	0.58	1.17	0.72	0.3633
500	0	1	2	3	160.25641	3	2.33	1.53	0.50	0.31	0.1168
Pos. Control	48	48	48	Pos. Control	3	48.00	0.00				

Compound: IBF-OME				IBF-OME				Assay Date: 12/2/2020			
TA 1535 +S9				TA 1535 +S9				TA 1535 +S9			
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
1.7	1	1	0	0	9	0.75	1.00	0.83	0.67	0.41	0.4185
5.3	0	1	0	1	1.69120804	3	0.67	0.58	0.33	0.21	0.2089
16.5	0	1	0	0	5.27656908	3	0.33	0.58	0.33	0.21	0.2089
51.4	0	0	1	2	16.4628955	3	0.33	0.58	0.33	0.21	0.2089
160.3	0	0	1	0	51.3642341	3	0.33	0.58	0.33	0.21	0.2089
500	0	0	0	0	160.25641	3	0.33	0.58	0.00	0.00	0.0743
Pos. Control	26	26	30	Pos. Control	3	27.33	2.31				

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Compound: PCL				PCL				Assay Date: 12/8/2020			
TA 1537 -S9				TA 1537 -S9				TA 1537 -S9			
Conc. (l)	Replicate #1	Replicate #2	Replicate #3	Conc. (l)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
0.8	2	5	1	0	9	3.44	1.53	5.83	0.77	0.53	0.2540
2.6	3	2	1	0.84560402	3	2.67	2.08	0.58	0.58	0.40	0.0894
8.2	4	2	2	2.5328454	3	2.67	1.15	0.77	0.53	0.2252	
25.7	1	1	4	8.23144777	3	2.00	1.73	0.58	0.40	0.1053	
80.1	5	2	1	25.682117	3	2.67	2.08	0.77	0.53	0.2540	
250	2	2	1	80.120261	3	1.67	0.58	0.48	0.33	0.0471	Cytotoxic effect?
Pos. Control	42	36	36	250	3	38.00	3.46				

TA1537

Compound: COGA				COGA				Assay Date: 12/8/2020			
TA 1537 -S9				TA 1537 -S9				TA 1537 -S9			
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
6.8	1	3	1	0	9	2.44	1.74	4.18	0.68	0.40	0.2465
21.1	2	2	3	6.76483215	3	1.67	1.15	0.58	0.40	0.4186	
65.9	2	1	2	21.10870763	3	2.67	0.58	1.09	0.64	0.0730	
205.5	3	1	2	65.8515821	3	3.00	0.00	1.23	0.72	0.3020	
641.0	0	2	1	205.456936	3	2.00	1.00	0.82	0.48	0.3446	
2000	3	2	1	641.025641	3	1.00	1.00	0.41	0.24	0.1053	
Pos. Control	48	48	48	2000	3	3.00	1.00	0.82	0.48	0.3446	

Compound: IBF-OH				IBF-OH				Assay Date: 12/8/2020			
TA 1537 -S9				TA 1537 -S9				TA 1537 -S9			
Conc. (l)	Replicate #1	Replicate #2	Replicate #3	Conc. (l)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
1.4	2	3	4	0	9	2.67	1.73	2.40	1.13	0.72	0.3090
4.2	2	2	2	1.35296643	3	3.00	1.00	1.00	0.64	0.4185	
13.2	1	2	2	4.22125526	3	2.67	0.58	1.13	0.72	0.3090	
41.1	1	1	2	13.1703164	3	3.00	1.00	0.38	0.24	0.0970	
128.2	4	2	4	41.0913872	3	1.00	0.00	1.25	0.80	0.2175	
400	2	1	1	128.205128	3	3.33	1.15	0.50	0.32	0.1570	
Pos. Control	48	48	48	400	3	3.33	0.58				

Compound: IBF-OME				IBF-OME				Assay Date: 12/8/2020			
TA 1537 -S9				TA 1537 -S9				TA 1537 -S9			
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	mean # pos. Wells	Corr. mean	SD	Base line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
1.7	5	1	0	0	9	1.56	1.74	3.30	1.29	0.61	0.3701
5.3	0	3	1	1.69120804	3	2.00	2.65	0.86	0.40	0.4242	
16.5	1	1	2	5.27656908	3	1.33	1.53	0.86	0.40	0.4185	
51.4	0	3	2	16.4628955	3	1.33	0.58	1.07	0.51	0.4619	
160.3	2	0	1	51.3642341	3	1.67	1.53	0.64	0.30	0.3090	
500	1	4	0	160.25641	3	1.00	1.00	1.07	0.51	0.4619	
Pos. Control	48	48	45	500	3	1.67	2.08	1.07	0.51	0.4645	

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Compound: PCL				PCL								Assay Date:		12/8/2020	
TA 1537 -S9				Spontaneous TA 1537 -S9				TA 1537 -S9				Assay Date:		12/8/2020	
Conc. ()	Replicate #1	Replicate #2	Replicate #3	TA 1537 -S9	Conc. ()	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)		
0.8	1	3	5	0	0	1	2.67	1.73	4.40		1.13	0.72	0.3262		
2.6	0	5	1	3	4		0.84560402	3	3.00	2.00	0.75	0.48	0.3701		
8.2	2	2	2	4	1		2.63828454	3	2.00	2.65	0.75	0.48	0.3387		
25.7	1	1	2	5			8.23144777	3	2.00	0.00	0.50	0.32	0.1578		
80.1	3	2	2	6			25.682117	3	1.33	0.58	0.86	0.50	0.4596		
250	3	3	3	7			80.1282051	3	2.33	0.58	1.13	0.72	0.3020		
Pos. Control	48	48	48	8			250	3	3.00	0.00					
Pos. Control				Pos. Control	3		48.00	0.00	0.00						
Compound: PCL				PCL								Assay Date:		12/8/2020	
TA 1537 +S9				Spontaneous TA 1537 +S9				TA 1537 +S9				Assay Date:		12/8/2020	
Conc. ()	Replicate #1	Replicate #2	Replicate #3	TA 1537 +S9	Conc. ()	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)		
0.8	1	2	1	3	0	0	0	9	2.33	1.50	0.57	0.34	0.0943		
2.6	3	1	1	2	4		0.84560402	3	1.33	3.83	0.71	0.47	0.1774		
8.2	3	2	3	3	4		2.63828454	3	1.67	1.15	1.14	0.62	0.4020		
25.7	2	3	3	4			8.23144777	3	2.67	0.58	0.75	0.29	0.1160		
80.1	2	3	3	5			25.682117	3	1.00	1.29	0.75	0.29	0.1160		
250	3	2	3	6			80.1282051	3	2.00	1.00	0.86	0.50	0.2754		
Pos. Control	41	41	40	7			250	3	3.67	1.15	1.57	0.92	0.1265		
Pos. Control				Pos. Control	3		40.67	0.58	0.58						

E.coli

Compound: COGA			
EC Combo -S9			
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3
6.8	6	7	5
21.1	5	5	7
65.9	5	0	7
205.5	2	5	2
641.0	6	5	3
2000	7	7	9
Pos. Control	43	47	45

Spontaneous

EC Combo -S9	
5	5
4	6
4	8
6	
4	
5	

COGA			
EC Combo -S9			
Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean SD
0	9	5.22	1.30
6.76483215	3	6.00	1.00
21.1062763	3	5.67	1.15
65.8515821	3	4.00	3.61
205.456936	3	3.00	1.73
641.025641	3	4.67	1.53
2000	3	7.67	1.15
Pos. Control	3	45.00	2.00

Assay Date: 12/16/2020

Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
6.52	1.15	0.92	0.1858
	1.09	0.87	0.3060
	0.77	0.61	0.1892
	0.57	0.46	0.0192
	0.89	0.72	0.2754
	1.47	1.18	0.0082

Cytotoxic effect?

Compound: COGA			
EC Combo -S9			
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3
6.8	8	5	5
21.1	6	5	8
65.9	6	4	7
205.5	7	5	7
641.0	6	9	8
2000	8	3	8
Pos. Control	10	16	13

Spontaneous

EC Combo -S9	
5	5
7	4
4	4
9	
5	

COGA			
EC Combo -S9			
Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean SD
0	9	5.33	1.66
6.76483215	3	6.00	1.73
21.1062763	3	6.33	1.53
65.8515821	3	5.67	1.53
205.456936	3	6.33	1.15
641.025641	3	7.67	1.53
2000	3	6.33	2.89
Pos. Control	3	13.00	3.00

Assay Date: 12/16/2020

Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
6.99	1.13	0.86	0.2817
	1.19	0.91	0.1900
	1.06	0.81	0.3829
	1.19	0.91	0.1810
	1.44	1.10	0.0289
	1.19	0.91	0.2316

Compound: IBF-OH			
EC Combo -S9			
Conc. (l)	Replicate #1	Replicate #2	Replicate #3
1.4	6	12	6
4.2	4	2	11
13.2	8	4	6
41.1	5	10	4
128.2	4	4	7
400	1	0	3
Pos. Control	45	48	45

Spontaneous

EC Combo -S9	
5	5
4	6
4	8
6	
4	
5	

IBF-OH			
EC Combo -S9			
Conc. (l)	n	mean # pos. Wells	Corr. mean SD
0	9	5.22	1.30
1.35296643	3	8.00	3.46
4.22125526	3	6.67	4.73
13.1703164	3	6.00	2.00
41.0913872	3	6.33	3.21
128.205128	3	5.00	1.73
400	3	1.33	1.53
Pos. Control	3	46.00	1.73

Assay Date: 12/16/2020

Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
6.52	1.53	1.23	0.0285
	1.09	0.87	0.3940
	1.15	0.92	0.2226
	1.21	0.97	0.1944
	0.96	0.77	0.4082
	0.26	0.20	0.0000

Cytotoxic effect?

Compound: IBF-OH			
EC Combo +S9			
Conc. (l)	Replicate #1	Replicate #2	Replicate #3
1.4	5	6	2
4.2	5	5	2
13.2	5	7	2
41.1	5	8	7
128.2	3	4	6
400	3	6	4
Pos. Control	24	20	17

Spontaneous

EC Combo +S9	
5	5
7	4
4	4
9	
5	

IBF-OH			
EC Combo +S9			
Conc. (l)	n	mean # pos. Wells	Corr. mean SD
0	9	5.33	1.66
1.35296643	3	4.33	2.08
4.22125526	3	3.33	1.53
13.1703164	3	4.67	2.52
41.0913872	3	6.67	1.53
128.205128	3	4.33	1.53
400	3	4.33	1.53
Pos. Control	3	20.33	3.51

Assay Date: 12/16/2020

Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
6.99	0.81	0.62	0.2059
	0.63	0.48	0.0480
	0.88	0.67	0.3015
	1.25	0.95	0.1244
	0.81	0.62	0.1900
	0.81	0.62	0.1900

Cytotoxic effect?

Compound: COGA				COGA				Assay Date: 12/16/2020				
EC Combo +S9				EC Combo +S9				t-test p-value (unpaired, 1 sided)				
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
6.8	8	5	5	0	9	5.33	1.66	6.59		1.13	0.86	0.2817
21.1	6	5	8	6.76483215	3	6.00	1.73			1.19	0.91	0.1900
65.9	6	4	7	21.1062763	3	6.33	1.53			1.06	0.81	0.3829
205.5	7	5	7	65.8515821	3	5.67	1.53			1.19	0.91	0.1810
641.0	6	9	8	205.456936	3	6.33	1.15			1.44	1.10	0.0239
2000	8	3	8	641.025641	3	7.67	1.53			1.19	0.91	0.2316
Pos. Control				Pos. Control								
Pos. Control				Pos. Control								

Compound: IBF-OH				IBF-OH				Assay Date: 12/16/2020				
EC Combo -S9				EC Combo -S9				t-test p-value (unpaired, 1 sided)				
Conc. ()	Replicate #1	Replicate #2	Replicate #3	Conc. ()	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
1.4	6	12	6	0	9	5.22	1.30	6.52		1.53	1.23	0.0286
4.2	4	2	11	1.35296643	3	8.00	3.46			1.09	0.87	0.3940
13.2	8	4	6	4.22125526	3	5.67	4.73			1.15	0.92	0.2226
41.1	5	10	4	13.1703164	3	6.00	2.00			1.21	0.97	0.1944
128.2	4	4	7	41.0913872	3	6.33	3.21			0.96	0.77	0.4082
400	1	0	3	128.205128	3	5.00	1.73			0.26	0.20	0.0008
Pos. Control				45	48	45	400	3	1.33	1.53		
Pos. Control				Pos. Control	3	46.00	1.73					

Compound: IBF-OH				IBF-OH				Assay Date: 12/16/2020				
EC Combo +S9				EC Combo +S9				t-test p-value (unpaired, 1 sided)				
Conc. ()	Replicate #1	Replicate #2	Replicate #3	Conc. ()	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
1.4	5	6	2	0	9	5.33	1.66	6.59		0.81	0.62	0.2059
4.2	3	5	2	1.35296643	3	4.33	2.08			0.63	0.48	0.0480
13.2	5	7	2	4.22125526	3	3.33	1.53			0.88	0.67	0.3015
41.1	5	8	7	13.1703164	3	4.67	2.52			1.25	0.95	0.1244
128.2	3	4	6	41.0913872	3	6.67	1.53			0.81	0.62	0.1900
400	3	6	4	128.205128	3	4.33	1.53			0.81	0.62	0.1900
Pos. Control	24	20	17	400	3	4.33	1.53					
Pos. Control				Pos. Control	3	20.33	3.51					

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Compound: IBF-OME				IBF-OME				Assay Date: 12/16/2020			
EC Combo -S9				EC Combo -S9							
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
1.7	5	5	9	0	9	6.11	1.62	7.73	1.04	0.82	0.4275
5.3	4	2	1	1.69120804	3	6.33	2.31		0.38	0.30	0.0027
16.5	11	5	4	5.27656908	3	2.33	1.53		1.09	0.86	0.3580
51.4	5	4	5	16.4628955	3	6.67	3.79		0.76	0.60	0.0854
160.3	9	7	7	51.3642341	3	4.67	0.58		1.25	0.99	0.0797
500	9	4	5	160.25641	3	7.67	1.15		0.98	0.78	0.4653
Pos. Control	47	48	47	500	3	6.00	2.65				
				Pos. Control	3	47.33	0.58				
Compound: IBF-OME				IBF-OME				Assay Date: 12/16/2020			
EC Combo +S9				EC Combo +S9							
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3	Conc. (µg/ml)	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
1.7	2	6	5	0	9	6.11	2.03	8.14	0.71	0.53	0.1100
5.3	3	8	6	1.69120804	3	4.33	2.08		0.93	0.70	0.3806
16.5	5	10	7	5.27656908	3	5.67	2.52		1.20	0.90	0.2052
51.4	3	5	10	16.4628955	3	7.33	2.52		0.98	0.74	0.4733
160.3	7	6	2	51.3642341	3	6.00	3.61		0.82	0.61	0.2296
500	4	4	7	160.25641	3	5.00	1.73		0.82	0.61	0.2089
Pos. Control	15	19	15	Pos. Control	3	16.33	2.31				
Compound: PCL				PCL				Assay Date: 12/16/2020			
EC Combo -S9				EC Combo -S9							
Conc. (l)	Replicate #1	Replicate #2	Replicate #3	Conc. (l)	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
0.8	7	9	8	0	9	5.22	1.30	6.52	1.53	1.23	0.0037
2.6	10	7	8	0.84560402	3	8.00	1.00		1.60	1.28	0.0031
8.2	6	6	7	2.63828454	3	8.33	1.53		0.96	0.77	0.4062
25.7	5	5	8	8.23144777	3	5.00	1.73		1.15	0.92	0.2118
80.1	7	3	5	25.682117	3	6.00	1.73		0.96	0.77	0.4125
250	6	6	4	80.1282051	3	5.00	2.00		1.02	0.82	0.4452
Pos. Control	44	46	47	250	3	5.33	1.15				
				Pos. Control	3	45.67	1.53				
Compound: PCL				PCL				Assay Date: 12/16/2020			
EC Combo +S9				EC Combo +S9							
Conc. (l)	Replicate #1	Replicate #2	Replicate #3	Conc. (l)	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
0.8	7	6	6	0	9	5.33	1.66	6.99	1.19	0.91	0.1713
2.6	10	5	7	0.84560402	3	6.33	0.58		1.38	1.05	0.0691
8.2	5	9	12	2.63828454	3	7.33	2.52		1.63	1.24	0.0216
25.7	6	5	5	8.23144777	3	8.67	3.51		1.00	0.76	0.5000
80.1	4	8	5	25.682117	3	5.33	0.58		1.06	0.81	0.3905
250	9	8	11	80.1282051	3	5.67	2.08		1.69	1.29	0.0041
Pos. Control	26	19	15	250	3	3.00	1.73				
				Pos. Control	3	28.00	5.57				

APPENDIX F

COGA MICROTOX TEST DATA TABLES AND CALCULATIONS

Table F-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:

^a Corrected 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. 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*

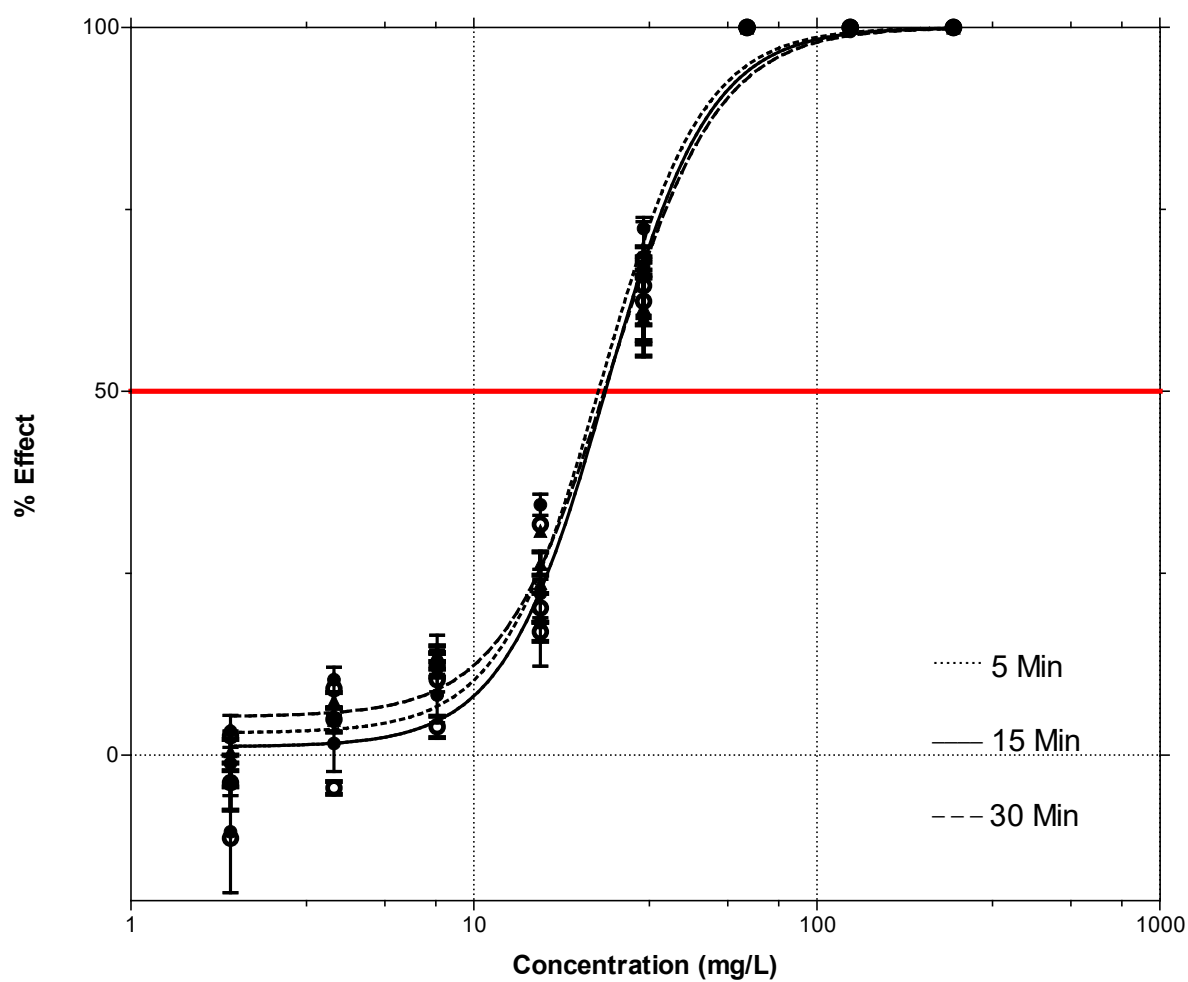


Figure F-1 – Microtox toxicity of COGA: Red line represents median effect.

IBF-OH MICROTOX TEST DATA TABLES AND CALCULATIONS

Table F-3. 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 F-4. 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:

^a EC₅₀ values calculated for the marine bacteria, *A. fischeri*

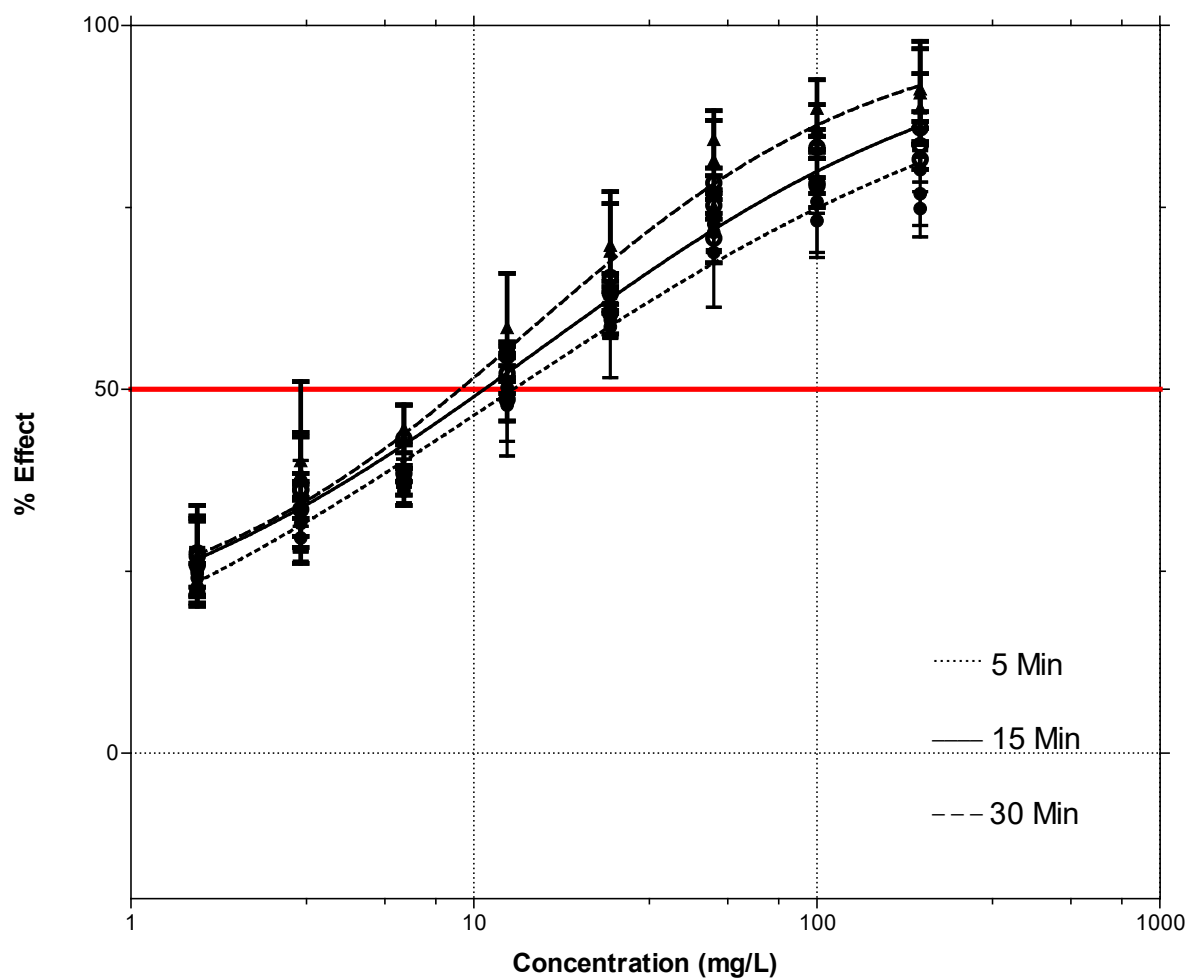


Figure F-2 – Microtox Toxicity of IBF-OH – Red line represents median effect

IBF-Ome MICROTOX TEST DATA TABLES AND CALCULATIONS

Table F-5. 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-6. 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*

PCL MICROTOX TEST DATA TABLES AND CALCULATIONS

Table F-7. 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 F-8. 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*

APPENDIX G

SKIN SENSITIZATION TEST RAW DATA

h-CLAT Data

Table G-1. Reactivity Check

Test article	Concentration (mg/mL)	Viability (% alive)	% Change (CD86)	% Change (CD54)	Positive (CD86/CD54)	Pass/Fail
Media		95.92	1	1	N/N	Pass
Saline		96.13	1	1	N/N	Pass
DMSO		95.25	1	1	N/N	Pass
DNCB	0.0033	82	268.9	662.6	Y/Y	Pass
	0.0040	75.89	156.8	520.1	Y/Y	Pass
NiSO ₄	0.10	73.52	231.6	2624.6	Y/Y	Pass
Lactic Acid	1	95.77	87.5	112.8	N/N	Pass

Legend:

mg/mL = milligrams per milliliter

N = No

Y = Yes

DMSO = dimethyl sulfoxide

DNCB = 2,4-dinitrochlorobenzene

NiSO₄ = nickel sulfate

Reactivity Check Raw Data

Experiment:
hCLAT Reactivity check 7-9-19
Protocol 49-iv19-03-01 Mod A,
CBRN

Cytometer: BD FACSVers

Cytometer SN: Z6511530048

Statistics					
Name	Events	% Parent	% Grandparent	% Total	FITC-A Geo Mean
Live Cells:All Events	10,000	---	---	100.00	876
Live Cells:Live cells	9,599	95.99	---	95.99	843
Dead Cells:All Events	10,000	---	---	100.00	1,877
Dead Cells:Live cells	136	1.36	---	1.36	1,275
IgG Media:All Events	10,423	---	---	100.00	904
IgG Media:Live cells	9,998	95.92	---	95.92	869
IgG Saline:All Events	10,398	---	---	100.00	887
IgG Saline:Live cells	9,996	96.13	---	96.13	850
IgG DMSO:All Events	10,499	---	---	100.00	902
IgG DMSO:Live cells	10,000	95.25	---	95.25	855
IgG Lactio Acid:All Events	10,442	---	---	100.00	869
IgG Lactio Acid:Live cells	10,000	95.77	---	95.77	830
IgG DNCB 1:All Events	12,195	---	---	100.00	1,177
IgG DNCB 1:Live cells	10,000	82.00	---	82.00	1,031
IgG DNCB 2:All Events	13,175	---	---	100.00	1,260
IgG DNCB 2:Live cells	9,998	75.89	---	75.89	1,078
IgG DNCB 3:All Events	14,280	---	---	100.00	1,364
IgG DNCB 3:Live cells	10,000	70.03	---	70.03	1,073
IgG Nickel II Sulfate:All Events	13,602	---	---	100.00	1,019
IgG Nickel II Sulfate:Live cells	10,000	73.52	---	73.52	914
CD86 Media :All Events	10,524	---	---	100.00	2,485
CD86 Media :Live cells	10,000	95.02	---	95.02	2,246
CD54 Media:All Events	10,542	---	---	100.00	1,174
CD54 Media:Live cells	10,000	94.86	---	94.86	1,115
CD86 Saline:All Events	10,423	---	---	100.00	2,298
CD86 Saline:Live cells	9,996	95.90	---	95.90	2,099
CD54 Saline:All Events	10,424	---	---	100.00	1,102
CD54 Saline:Live cells	10,000	95.93	---	95.93	1,061
CD86 DMSO:All Events	10,514	---	---	100.00	2,740
CD86 DMSO:Live cells	10,000	95.11	---	95.11	2,458
CD54 DMSO:All Events	10,480	---	---	100.00	1,118
CD54 DMSO:Live cells	10,000	95.42	---	95.42	1,069
CD86 Lactio Acid:All Events	10,493	---	---	100.00	2,128
CD86 Lactio Acid:Live cells	10,000	95.30	---	95.30	1,923
CD54 Lactio Acid:All Events	10,474	---	---	100.00	1,124
CD54 Lactio Acid:Live cells	10,000	95.47	---	95.47	1,068
CD86 DNCB 1:All Events	12,478	---	---	100.00	6,679
CD86 DNCB 1:Live cells	10,000	80.14	---	80.14	5,342
CD54 DNCB 1:All Events	12,620	---	---	100.00	2,467
CD54 DNCB 1:Live cells	10,000	79.24	---	79.24	2,449
CD86 DNCB 2:All Events	13,141	---	---	100.00	5,571
CD86 DNCB 2:Live cells	9,982	75.96	---	75.96	3,591
CD54 DNCB 2:All Events	13,284	---	---	100.00	2,227
CD54 DNCB 2:Live cells	9,942	74.84	---	74.84	2,191
CD86 DNCB 3:All Events	14,930	---	---	100.00	5,320
CD86 DNCB 3:Live cells	10,000	66.98	---	66.98	1,982
CD54 DNCB 3:All Events	15,987	---	---	100.00	1,759
CD54 DNCB 3:Live cells	10,000	62.55	---	62.55	1,419
CD86 Nickel II Sulfate:All Events	14,628	---	---	100.00	5,955
CD86 Nickel II Sulfate:Live cells	10,000	68.36	---	68.36	3,806
CD54 Nickel II Sulfate:All Events	15,792	---	---	100.00	5,403
CD54 Nickel II Sulfate:Live cells	9,886	62.60	---	62.60	6,452

Table G-2. Range Finding

7/25/2019		PI- Dose Finding		
	Stock (mg/mL)	Test Concentration in DMSO(mg/mL)	Viability	CV75
DMSO		0	95.98	N/A
COGA Run #1	1.56	0.00312	96.14	
	3.13	0.00625	96.32	
	6.25	0.0125	96.17	
	12.5	0.025	95.68	
	25	0.05	95.98	
	50	0.1	96.22	
	100	0.2	96.42	
	200	0.4	96.04	
7/30/2019		PI- Dose Finding		
	Stock (mg/mL)	Test Concentration in DMSO(mg/mL)	Viability	CV75
DMSO		0	95.76	N/A
COGA Run #2	1.56	0.00312	95.64	
	3.13	0.00625	95.79	
	6.25	0.0125	96.26	
	12.5	0.025	95.74	
	25	0.05	95.59	
	50	0.1	95.81	
	100	0.2	96.12	
	200	0.4	96.45	
7/25/2019		PI- Dose Finding		
	Stock (mg/mL)	Test Concentration in DMSO(mg/mL)	Viability	CV75
DMSO		0	95.98	N/A
IBF-OH Run #1		0	95.98	
	0.156	0.000313	98.17	
	0.312	0.000625	98.35	
	0.625	0.00125	98.12	
	1.25	0.0025	97.94	
	2.5	0.005	98.07	
	5	0.01	97.1	
	10	0.02	95.54	
7/30/2019		PI- Dose Finding		

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	Stock (mg/mL)	Test Concentration in DMSO(mg/mL)	Viability	CV75
DMSO		0	95.76	
IBF-OH Run #2	0.156	0.000313	98.49	0.025
	0.312	0.000625	98.49	
	0.625	0.00125	98.29	
	1.25	0.0025	98.94	
	2.5	0.005	98.04	
	5	0.01	96.42	
	10	0.02	86.2	
	20	0.04	50.93	
7/25/2019		PI- Dose Finding		
	Stock (mg/mL)	Test Concentration in DMSO(mg/mL)	Viability	CV75
DMSO		0	95.98	
IBF-Ome Run #1	0.00977	1.95E-05	95.49	N/A
	0.0195	3.91E-05	95.64	
	0.0391	0.0000781	95.21	
	0.0781	0.000156	95.67	
	0.156	0.000313	94.9	
	0.313	0.000625	95.62	
	0.625	0.00125	95.46	
	1.25	0.0025	95.04	
7/30/2019		PI- Dose Finding		
	Stock (mg/mL)	Test Concentration in DMSO(mg/mL)	Viability	CV75
DMSO		0	95.76	
IBF-Ome Run #2	0.00977	1.95E-05	95.59	N/A
	0.0195	3.91E-05	95.33	
	0.0391	0.0000781	95.63	
	0.0781	0.000156	95.51	
	0.156	0.000313	95.29	
	0.313	0.000625	95.5	
	0.625	0.00125	94.59	
	1.25	0.0025	94.87	
7/25/2019		PI- Dose Finding		
	Stock (mg/mL)	Test Concentration in DMSO(mg/mL)	Viability	CV75

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DMSO		0	95.98	
PCL Run #1	0.00977	1.95E-05	95.86	N/A
	0.0195	3.91E-05	95.33	
	0.0391	0.0000781	95.74	
	0.0781	0.000156	95.97	
	0.156	0.000313	95.7	
	0.313	0.000625	95.14	
	0.625	0.00125	95.65	
	1.25	0.0025	95.45	
7/30/2019		PI- Dose Finding		
	Stock (mg/mL)	Test Concentration in DMSO(mg/mL)	Viability	CV75
DMSO		0	95.76	
PCL Run #2	0.00977	1.95E-05	95.68	N/A
	0.0195	3.91E-05	95.86	
	0.0391	0.0000781	95.3	
	0.0781	0.000156	95.96	
	0.156	0.000313	95.42	
	0.313	0.000625	95.47	
	0.625	0.00125	95.57	
	1.25	0.0025	95.72	

Legend:

PI = Propidium iodide

DMSO = dimethyl sulfoxide

mg/mL = milligrams per milliliter

CV75 = 75% cell viability

Toxicology Study No. S. 0058638-18, May 2022

Range Finding Experiment 1 Raw Data

Experiment:

hCLAT range finding COGA PCL IBF-OHMPK IBF-Ome #1 07-21

Cytometer: BD FACSVers

Cytometer SN: Z6511530048

Protocol # 49-IV19-03-01

Mods H, I, J, K

Statistics						
Name	Events	% Parent	% Grandparent	% Total	FSC-A Mean	SSC-A Mean
Live cells:P1	9,628	96.28	***	96.28	57,619	55,353
Dead cells:P1	156	0.60	***	0.60	37,185	115,047
Media:P1	10,000	95.53	***	95.53	55,591	55,436
DMSO:P1	10,000	95.98	***	95.98	55,468	54,795
COGA 1:P1	10,000	96.04	***	96.04	54,094	56,013
COGA 2:P1	10,000	96.42	***	96.42	51,776	54,727
COGA 3:P1	10,000	96.22	***	96.22	52,949	55,528
COGA 4:P1	10,000	95.98	***	95.98	51,460	55,390
COGA 5:P1	10,000	95.68	***	95.68	53,196	55,782
COGA 6:P1	10,000	96.17	***	96.17	53,756	55,673
COGA 7:P1	10,000	96.32	***	96.32	54,109	54,672
COGA 8:P1	10,000	96.14	***	96.14	54,233	54,121
PCL 1:P1	10,000	95.45	***	95.45	58,378	54,960
PCL 2:P1	10,000	95.65	***	95.65	53,779	55,200
PCL 3:P1	10,000	95.14	***	95.14	54,082	55,850
PCL 4:P1	10,000	95.70	***	95.70	52,429	57,527
PCL 5:P1	10,000	95.97	***	95.97	52,313	57,009
PCL 6:P1	10,000	95.74	***	95.74	52,569	55,579
PCL 7:P1	10,000	95.33	***	95.33	51,798	55,624
PCL 8:P1	10,000	95.86	***	95.86	52,212	55,961
IBF-OHMPK 1:P1	255	0.85	***	0.85	16,564	13,902
IBF-OHMPK 2:P1	412	1.37	***	1.37	19,043	11,839
IBF-OHMPK 3:P1	244	0.81	***	0.81	19,218	13,035
IBF-OHMPK 4:P1	134	0.45	***	0.45	25,548	23,093
IBF-OHMPK 5:P1	6,468	24.69	***	24.69	46,617	42,332
IBF-OHMPK 6:P1	10,000	82.71	***	82.71	50,411	51,096
IBF-OHMPK 7:P1	10,000	93.92	***	93.92	51,290	53,937
IBF-OHMPK 8:P1	10,000	94.98	***	94.98	51,012	55,428
IBF-Ome 1:P1	10,000	95.04	***	95.04	52,887	55,589
IBF-Ome 2:P1	10,000	95.46	***	95.46	52,237	56,282
IBF-Ome 3:P1	10,041	95.62	***	95.62	51,773	56,184
IBF-Ome 4:P1	10,000	94.90	***	94.90	51,667	56,745
IBF-Ome 5:P1	10,000	95.67	***	95.67	51,528	57,185
IBF-Ome 6:P1	10,026	95.21	***	95.21	52,956	57,077
IBF-Ome 7:P1	10,000	95.64	***	95.64	53,242	57,107
IBF-Ome 8:P1	10,000	95.49	***	95.49	51,833	56,442

Statistics						
Name	Events	% Parent	% Grandparent	% Total	FSC-A Mean	SSC-A Mean
Live cells:P1	9,628	96.28	***	96.28	57,619	55,353
Dead cells:P2	24,649	94.26	***	94.26	51,181	114,143
IBF-OHMPK 1:P3	25,792	85.97	***	85.97	59,460	59,917
IBF-OHMPK 2:P3	28,662	95.54	***	95.54	58,492	60,153
IBF-OHMPK 3:P3	29,130	97.10	***	97.10	55,257	60,216
IBF-OHMPK 4:P3	29,420	98.07	***	98.07	54,028	60,900
IBF-OHMPK 5:P3	25,663	97.94	***	97.94	52,230	55,612
IBF-OHMPK 6:P3	11,863	98.12	***	98.12	52,323	57,261
IBF-OHMPK 7:P3	10,471	98.35	***	98.35	52,021	56,739
IBF-OHMPK 8:P3	10,336	98.17	***	98.17	51,625	57,951

Note: It appears that the IBF-OHMPK (which is visibly a neon green/yellow color) is interfering with the PI absorbance. Therefore, I've gated out dead cells (P2) and the live, but shifted absorbance IBF-OHMPK cells (P3) for population viability determination.

-LAH

Toxicology Study No. S. 0058638-18, May 2022

Experiment:

ICLAT range finding COGA PCL IBF-OHMPK IBF-Ome #1 07-21

Cytometer: BD FACSVers

Cytometer SN: Z6511530048

Protocol # 49-IV19-03-01

Mods H, I, J, K

Statistics						
Name	Events	% Parent	% Grandparent	% Total	FSC-A Mean	SSC-A Mean
Live cells: All Events	10,000	***	***	100.00	57,674	57,521
Dead cells: All Events	26,150	***	***	100.00	51,414	116,060
Media: All Events	10,468	***	***	100.00	55,543	57,327
DMSO: All Events	10,419	***	***	100.00	55,527	56,839
COGA 1: All Events	10,412	***	***	100.00	54,241	58,294
COGA 2: All Events	10,371	***	***	100.00	51,871	56,552
COGA 3: All Events	10,393	***	***	100.00	53,023	57,452
COGA 4: All Events	10,419	***	***	100.00	51,532	57,427
COGA 5: All Events	10,451	***	***	100.00	53,201	57,715
COGA 6: All Events	10,398	***	***	100.00	53,929	57,673
COGA 7: All Events	10,382	***	***	100.00	54,109	56,116
COGA 8: All Events	10,401	***	***	100.00	54,390	56,001
PCL 1: All Events	10,477	***	***	100.00	58,333	57,335
PCL 2: All Events	10,455	***	***	100.00	53,914	57,462
PCL 3: All Events	10,511	***	***	100.00	54,261	58,484
PCL 4: All Events	10,449	***	***	100.00	52,750	60,088
PCL 5: All Events	10,420	***	***	100.00	52,478	59,301
PCL 6: All Events	10,445	***	***	100.00	52,718	58,014
PCL 7: All Events	10,490	***	***	100.00	51,977	57,893
PCL 8: All Events	10,432	***	***	100.00	52,404	58,403
IBF-OHMPK 1: All Events	30,000	***	***	100.00	59,446	68,177
IBF-OHMPK 2: All Events	30,000	***	***	100.00	58,840	63,561
IBF-OHMPK 3: All Events	30,000	***	***	100.00	55,450	62,445
IBF-OHMPK 4: All Events	30,000	***	***	100.00	53,926	61,884
IBF-OHMPK 5: All Events	26,202	***	***	100.00	51,928	56,250
IBF-OHMPK 6: All Events	12,090	***	***	100.00	52,049	57,685
IBF-OHMPK 7: All Events	10,647	***	***	100.00	51,733	57,013
IBF-OHMPK 8: All Events	10,529	***	***	100.00	51,399	58,537
IBF-Ome 1: All Events	10,522	***	***	100.00	52,879	58,125
IBF-Ome 2: All Events	10,476	***	***	100.00	52,185	58,340
IBF-Ome 3: All Events	10,501	***	***	100.00	52,000	58,606
IBF-Ome 4: All Events	10,537	***	***	100.00	51,694	59,059
IBF-Ome 5: All Events	10,453	***	***	100.00	51,728	59,622
IBF-Ome 6: All Events	10,530	***	***	100.00	52,906	59,106
IBF-Ome 7: All Events	10,456	***	***	100.00	53,384	59,406
IBF-Ome 8: All Events	10,472	***	***	100.00	51,828	58,374

Toxicology Study No. S. 0058638-18, May 2022

Range Finding Experiment 2 Raw Data

Experiment: NCIAT range finding COGA PCL/IBF-OHMPK IBF-Ome #2 07-31 Cytometer: BD FACSVerser Cytometer SN: Z6511530048
 Protocol 49-IV19-03-01
 Mods H, I, J, K

Statistics					
Name	Events	% Parent	% Grandparent	% Total	Propidium Iodide-A Geo Mean
Live cells:live	9,640	96.40	***	96.40	326
Dead cells:live	436	1.51	***	1.51	813
Media:live	10,000	95.91	***	95.91	314
DMSO:live	10,000	95.76	***	95.76	301
COGA 1:live	10,000	96.45	***	96.45	380
COGA 2:live	10,000	96.12	***	96.12	353
COGA 3:live	10,000	95.81	***	95.81	342
COGA 4:live	10,000	95.59	***	95.59	325
COGA 5:live	10,000	95.74	***	95.74	312
COGA 6:live	10,000	96.26	***	96.26	309
COGA 7:live	10,000	95.79	***	95.79	301
COGA 8:live	10,000	95.64	***	95.64	301
PCL 1:live	10,000	95.72	***	95.72	302
PCL 2:live	10,000	95.57	***	95.57	300
PCL 3:live	10,000	95.47	***	95.47	301
PCL 4:live	10,000	95.42	***	95.42	299
PCL 5:live	10,000	95.96	***	95.96	303
PCL 6:live	10,000	95.30	***	95.30	302
PCL 7:live	10,000	95.86	***	95.86	303
PCL 8:live	10,000	95.68	***	95.68	302
IBF-OHMPK 1:live	197	0.66	***	0.66	734
IBF-OHMPK 2:live	181	0.60	***	0.60	623
IBF-OHMPK 3:live	197	0.66	***	0.66	545
IBF-OHMPK 4:live	189	0.63	***	0.63	712
IBF-OHMPK 5:live	5,818	19.39	***	19.39	1,176
IBF-OHMPK 6:live	10,000	85.40	***	85.40	832
IBF-OHMPK 7:live	9,994	94.19	***	94.19	558
IBF-OHMPK 8:live	10,000	95.66	***	95.66	411
IBF-Ome 1:live	10,000	94.87	***	94.87	308
IBF-Ome 2:live	10,000	94.59	***	94.59	301
IBF-Ome 3:live	10,000	95.50	***	95.50	304
IBF-Ome 4:live	10,000	95.29	***	95.29	301
IBF-Ome 5:live	10,000	95.51	***	95.51	302
IBF-Ome 6:live	10,000	95.63	***	95.63	305
IBF-Ome 7:live	10,000	95.33	***	95.33	305
IBF-Ome 8:live	10,000	95.59	***	95.59	297

Statistics					
Name	Events	% Parent	% Grandparent	% Total	Propidium Iodide-A Geo Mean
Live cells:live	9,640	96.40	***	96.40	326
Dead cells:dead	27,320	94.66	***	94.66	20,464
IBF-OHMPK 1:IBF-OHMPK live	15,278	50.93	***	50.93	14,912
IBF-OHMPK 2:IBF-OHMPK live	25,859	86.20	***	86.20	12,720
IBF-OHMPK 3:IBF-OHMPK live	28,926	96.42	***	96.42	7,846
IBF-OHMPK 4:IBF-OHMPK live	29,413	98.04	***	98.04	4,341
IBF-OHMPK 5:IBF-OHMPK live	29,681	98.94	***	98.94	1,987
IBF-OHMPK 6:IBF-OHMPK live	11,510	98.29	***	98.29	958
IBF-OHMPK 7:IBF-OHMPK live	10,451	98.49	***	98.49	611
IBF-OHMPK 8:IBF-OHMPK live	10,296	98.49	***	98.49	445

Note: It appears that the IBF-OHMPK (which is visibly a neon green/yellow color) is interfering with the PI absorbance. Therefore, I've gated out dead cells (dead) and the live, but shifted absorbance IBF-OHMPK cells (IBF-OHMPK live) for population viability determination. There is some overlap between the dead and IBF-OHMPK live populations, which I believe is an artifact of being conservative (i.e., assuming overlapping population = dead). See lab notebook for cell counts that show IBF-OHMPK 1 and 8 as 98-99% live.

-LAH

Operator: Lindsay A. Holden, PhD

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Software: BD FACSuite v1.0.5
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Toxicology Study No. S. 0058638-18, May 2022

Experiment:
hCLAT range finding COGA PCL IBF-OHMPK IBF-Ome #2 07-30

Cytometer: BD FACSVerser

Cytometer SN: Z6511530048

Protocol 49-IV19-03-01

Mods H, I, J, K

Statistics					
Name	Events	% Parent	% Grandparent	% Total	Propidium Iodide-A Geo Mean
Live cells:All Events	10,000	***	***	100.00	359
Dead cells:All Events	28,861	***	***	100.00	18,748
Media:All Events	10,426	***	***	100.00	340
DMSO:All Events	10,443	***	***	100.00	335
COGA 1:All Events	10,368	***	***	100.00	410
COGA 2:All Events	10,404	***	***	100.00	381
COGA 3:All Events	10,437	***	***	100.00	377
COGA 4:All Events	10,461	***	***	100.00	365
COGA 5:All Events	10,445	***	***	100.00	338
COGA 6:All Events	10,389	***	***	100.00	335
COGA 7:All Events	10,439	***	***	100.00	332
COGA 8:All Events	10,456	***	***	100.00	326
PCL 1:All Events	10,447	***	***	100.00	329
PCL 2:All Events	10,464	***	***	100.00	328
PCL 3:All Events	10,474	***	***	100.00	335
PCL 4:All Events	10,480	***	***	100.00	331
PCL 5:All Events	10,421	***	***	100.00	331
PCL 6:All Events	10,493	***	***	100.00	338
PCL 7:All Events	10,432	***	***	100.00	328
PCL 8:All Events	10,452	***	***	100.00	332
IBF-OHMPK 1:All Events	30,000	***	***	100.00	24,788
IBF-OHMPK 2:All Events	30,000	***	***	100.00	14,814
IBF-OHMPK 3:All Events	30,000	***	***	100.00	8,042
IBF-OHMPK 4:All Events	30,000	***	***	100.00	4,479
IBF-OHMPK 5:All Events	30,000	***	***	100.00	2,034
IBF-OHMPK 6:All Events	11,710	***	***	100.00	985
IBF-OHMPK 7:All Events	10,611	***	***	100.00	621
IBF-OHMPK 8:All Events	10,454	***	***	100.00	451
IBF-Ome 1:All Events	10,541	***	***	100.00	356
IBF-Ome 2:All Events	10,572	***	***	100.00	342
IBF-Ome 3:All Events	10,471	***	***	100.00	336
IBF-Ome 4:All Events	10,494	***	***	100.00	335
IBF-Ome 5:All Events	10,470	***	***	100.00	338
IBF-Ome 6:All Events	10,457	***	***	100.00	338
IBF-Ome 7:All Events	10,490	***	***	100.00	341
IBF-Ome 8:All Events	10,461	***	***	100.00	332

Operator: Lindsay A. Holden, PhD

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Software: BD FACSuite v1.0.5
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Toxicology Study No. S. 0058638-18, May 2022

h-CLAT COGA Experiment 1 Raw Data

Experiment: hCLAT COGA IBF-OHMPK #1 08-02-2019

Protocol 49-IV19-03-01

Cytometer: BD FACSVerser

Cytometer SN: Z6511530048

Mods H, J

Statistics						
Name	Events	% Parent	% Grandparent	% Total	FITC-A Geo Mean	Propidium Iodide-A Geo Mean
Live cells:Live	27,495	91.65	***	91.65	902	335
Dead cells:Live	355	1.38	***	1.38	989	491
Media IgG:Live	10,000	92.50	***	92.50	833	315
Media CD86:Live	10,000	92.27	***	92.27	2,420	307
Media CD54:Live	10,000	92.30	***	92.30	1,059	312
DMSO IgG:Live	10,000	93.00	***	93.00	800	301
DMSO CD86:Live	10,031	90.28	***	90.28	2,762	297
DMSO CD54:Live	10,000	92.19	***	92.19	1,023	296
DNCB 1 IgG:Live	9,941	65.79	***	65.79	896	368
DNCB 1 CD86:Live	9,901	64.59	***	64.59	6,249	364
DNCB 1 CD54:Live	9,981	63.96	***	63.96	2,595	358
DNCB 2 IgG:Live	9,933	73.58	***	73.58	1,017	373
DNCB 2 CD86:Live	9,752	71.23	***	71.23	7,194	370
DNCB 2 CD54:Live	9,929	72.51	***	72.51	1,998	376
DNCB 3 IgG:Live	9,936	73.45	***	73.45	951	376
DNCB 3 CD86:Live	9,935	68.51	***	68.51	7,858	366
DNCB 3 CD54:Live	9,902	72.79	***	72.79	1,852	367
COGA 1 IgG:Live	10,003	93.90	***	93.90	879	329
COGA 1 CD86:Live	10,014	93.47	***	93.47	2,887	324
COGA 1 CD54:Live	10,000	92.96	***	92.96	1,180	329
COGA 2 IgG:Live	10,000	93.08	***	93.08	884	330
COGA 2 CD86:Live	10,106	92.82	***	92.82	2,799	322
COGA 2 CD54:Live	10,000	93.50	***	93.50	1,164	326
COGA 3 IgG:Live	10,000	93.48	***	93.48	876	326
COGA 3 CD86:Live	10,039	93.07	***	93.07	2,981	320
COGA 3 CD54:Live	10,000	93.44	***	93.44	1,145	326
COGA 4 IgG:Live	10,022	93.66	***	93.66	858	321
COGA 4 CD86:Live	10,186	93.01	***	93.01	3,078	312
COGA 4 CD54:Live	10,341	93.90	***	93.90	1,136	317
COGA 5 IgG:Live	10,000	93.75	***	93.75	852	319
COGA 5 CD86:Live	10,046	92.92	***	92.92	3,001	312
COGA 5 CD54:Live	10,002	93.54	***	93.54	1,085	320
COGA 6 IgG:Live	10,000	93.81	***	93.81	824	318
COGA 6 CD86:Live	10,000	92.34	***	92.34	2,896	312
COGA 6 CD54:Live	10,000	93.29	***	93.29	1,122	314
COGA 7 IgG:Live	10,029	92.10	***	92.10	817	312
COGA 7 CD86:Live	10,010	93.02	***	93.02	3,061	305
COGA 7 CD54:Live	10,007	93.88	***	93.88	1,112	308
COGA 8 IgG:Live	10,000	93.52	***	93.52	843	318
COGA 8 CD86:Live	10,008	93.18	***	93.18	2,377	313
COGA 8 CD54:Live	10,000	93.22	***	93.22	1,205	317
IBF-OHMPK 1 IgG:Live	253	0.87	***	0.87	3,306	351
IBF-OHMPK 1 CD86:Live	210	0.70	***	0.70	5,155	448
IBF-OHMPK 1 CD54:Live	215	0.72	***	0.72	4,093	440
IBF-OHMPK 2 IgG:Live	173	0.58	***	0.58	4,237	438
IBF-OHMPK 2 CD86:Live	183	0.61	***	0.61	5,236	473
IBF-OHMPK 2 CD54:Live	175	0.58	***	0.58	4,374	472
IBF-OHMPK 3 IgG:Live	189	0.63	***	0.63	3,447	346
IBF-OHMPK 3 CD86:Live	193	0.64	***	0.64	5,589	486
IBF-OHMPK 3 CD54:Live	182	0.61	***	0.61	4,513	452
IBF-OHMPK 4 IgG:Live	162	0.54	***	0.54	3,868	427
IBF-OHMPK 4 CD86:Live	195	0.65	***	0.65	5,228	446
IBF-OHMPK 4 CD54:Live	194	0.65	***	0.65	4,308	446
IBF-OHMPK 5 IgG:Live	133	0.44	***	0.44	4,177	459
IBF-OHMPK 5 CD86:Live	154	0.51	***	0.51	5,631	498
IBF-OHMPK 5 CD54:Live	181	0.60	***	0.60	3,933	475
IBF-OHMPK 6 IgG:Live	148	0.49	***	0.49	4,080	492
IBF-OHMPK 6 CD86:Live	176	0.59	***	0.59	5,645	501
IBF-OHMPK 6 CD54:Live	212	0.71	***	0.71	4,915	518
IBF-OHMPK 7 IgG:Live	157	0.52	***	0.52	5,265	572
IBF-OHMPK 7 CD86:Live	202	0.67	***	0.67	5,569	469
IBF-OHMPK 7 CD54:Live	179	0.60	***	0.60	5,103	558
IBF-OHMPK 8 IgG:Live	159	0.53	***	0.53	5,353	520
IBF-OHMPK 8 CD86:Live	155	0.52	***	0.52	5,516	494
IBF-OHMPK 8 CD54:Live	213	0.71	***	0.71	5,598	589

Toxicology Study No. S. 0058638-18, May 2022

h-CLAT COGA Experiment 2 Raw Data

Experiment: hCLAT COGA #2 PCL #1 08-14-2019

Cytometer: BD FACSVerser

Cytometer SN: Z6511530048

Protocol # 49-IV19-03-01

Mods H, I

Statistics						
Name	Events	% Parent	% Grandparent	% Total	FITC-A Geo Mean	Propidium Iodide-A Geo Mean
live cells:live	28,983	96.61	***	96.61	843	337
dead cells:live	1,390	5.88	***	5.88	951	399
media IgG:live	10,000	96.17	***	96.17	820	326
media CD86:live	10,003	95.49	***	95.49	2,567	322
media CD54:live	10,000	95.58	***	95.58	1,031	326
DMSO IgG:live	10,000	96.02	***	96.02	773	309
DMSO CD86:live	10,007	96.07	***	96.07	2,616	297
DMSO CD54:live	10,000	96.53	***	96.53	996	307
DNCB 1 IgG:live	9,929	72.55	***	72.55	1,016	430
DNCB 1 CD86:live	9,841	70.91	***	70.91	7,306	399
DNCB 1 CD54:live	10,056	70.88	***	70.88	2,564	409
DNCB 2 IgG:live	9,897	77.42	***	77.42	1,016	425
DNCB 2 CD86:live	9,924	74.31	***	74.31	7,942	400
DNCB 2 CD54:live	9,922	75.94	***	75.94	2,001	419
DNCB 3 IgG:live	9,996	82.10	***	82.10	1,024	418
DNCB 3 CD86:live	9,874	78.83	***	78.83	7,782	398
DNCB 3 CD54:live	9,929	81.56	***	81.56	1,807	413
COGA 1 IgG:live	10,000	95.63	***	95.63	962	374
COGA 1 CD86:live	10,000	95.68	***	95.68	2,784	367
COGA 1 CD54:live	10,000	95.76	***	95.76	1,267	374
COGA 2 IgG:live	10,000	95.89	***	95.89	921	360
COGA 2 CD86:live	10,004	95.92	***	95.92	2,817	357
COGA 2 CD54:live	10,000	95.91	***	95.91	1,214	362
COGA 3 IgG:live	10,000	95.72	***	95.72	888	350
COGA 3 CD86:live	10,000	95.75	***	95.75	2,792	351
COGA 3 CD54:live	10,000	96.11	***	96.11	1,215	354
COGA 4 IgG:live	10,000	96.29	***	96.29	898	353
COGA 4 CD86:live	10,011	95.93	***	95.93	2,667	351
COGA 4 CD54:live	10,000	96.04	***	96.04	1,165	348
COGA 5 IgG:live	10,000	96.06	***	96.06	882	345
COGA 5 CD86:live	10,029	95.94	***	95.94	2,737	338
COGA 5 CD54:live	10,000	95.76	***	95.76	1,142	345
COGA 6 IgG:live	10,000	95.19	***	95.19	857	338
COGA 6 CD86:live	10,003	95.27	***	95.27	2,693	329
COGA 6 CD54:live	10,000	95.38	***	95.38	1,097	333
COGA 7 IgG:live	10,000	95.66	***	95.66	829	328
COGA 7 CD86:live	10,000	95.31	***	95.31	2,503	324
COGA 7 CD54:live	10,009	95.83	***	95.83	1,150	325
COGA 8 IgG:live	10,000	95.89	***	95.89	848	334
COGA 8 CD86:live	10,022	96.11	***	96.11	2,437	329
COGA 8 CD54:live	10,000	95.79	***	95.79	1,062	335
PCL 1 IgG:live	10,000	96.84	***	96.84	773	305
PCL 1 CD86:live	10,000	94.87	***	94.87	2,443	298
PCL 1 CD54:live	10,000	96.58	***	96.58	966	306
PCL 2 IgG:live	10,000	96.22	***	96.22	789	307
PCL 2 CD86:live	10,000	95.55	***	95.55	2,798	300
PCL 2 CD54:live	10,000	95.92	***	95.92	961	308
PCL 3 IgG:live	10,000	96.52	***	96.52	771	304
PCL 3 CD86:live	10,050	95.85	***	95.85	2,740	300
PCL 3 CD54:live	10,000	95.85	***	95.85	992	306
PCL 4 IgG:live	10,000	96.48	***	96.48	783	309
PCL 4 CD86:live	10,000	96.51	***	96.51	2,404	300
PCL 4 CD54:live	10,000	96.21	***	96.21	983	309
PCL 5 IgG:live	10,004	96.62	***	96.62	769	301
PCL 5 CD86:live	10,000	96.16	***	96.16	2,776	300
PCL 5 CD54:live	10,000	96.62	***	96.62	959	304
PCL 6 IgG:live	10,018	96.04	***	96.04	766	303
PCL 6 CD86:live	10,051	95.74	***	95.74	2,795	301
PCL 6 CD54:live	10,020	96.18	***	96.18	973	305
PCL 7 IgG:live	10,008	96.15	***	96.15	772	304
PCL 7 CD86:live	10,021	95.76	***	95.76	2,702	299
PCL 7 CD54:live	10,000	96.26	***	96.26	957	303
PCL 8 IgG:live	10,000	95.68	***	95.68	769	303
PCL 8 CD86:live	10,102	95.45	***	95.45	2,726	296
PCL 8 CD54:live	10,232	95.24	***	95.24	940	301

Operator: Lindsay A. Holden, PhD

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Software: BD FACSuite v1.0.5

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Table G-3. COGA Data Analysis

	Stock Concentration (mg/mL)	Concentration (mg/mL)	Viability (IgG)	FITC IgG	FITC CD86	RFI	% change	EC150	FITC CD54	RFI	% change	EC200
Experiment 1 8/2/2019												
Media		0.00	92.50	833.00	2420.00	1.00	100.00		1059.00	1.00	100.00	
DMSO		0.00	93.00	800.00	2762.00	1.24	123.63		1023.00	0.99	98.67	
DNCB Control	1.67	0.00	73.45	951.00	7858.00	3.52	352.04		1852.00	3.99	398.67	
	2.00	0.00	73.58	1017.00	7194.00	3.15	314.83		1998.00	4.34	434.07	
	2.40	0.00	65.79	896.00	6249.00	2.73	272.83		2595.00	7.52	751.77	
COGA	55.82	0.11	93.52	843.00	2377.00	0.78	78.19		1205.00	1.62	162.33	
	66.98	0.13	92.10	817.00	3061.00	1.14	114.37		1112.00	1.32	132.29	
	80.38	0.16	93.81	824.00	2896.00	1.06	105.61		1122.00	1.34	133.63	
	96.45	0.19	93.75	852.00	3001.00	1.10	109.53		1085.00	1.04	104.48	
	115.74	0.23	93.66	858.00	3078.00	1.13	113.15		1136.00	1.25	124.66	
	138.89	0.28	93.48	876.00	2981.00	1.07	107.29		1145.00	1.21	120.63	
	166.67	0.33	93.08	884.00	2799.00	0.98	97.60		1164.00	1.26	125.56	
	200.00	0.40	93.90	879.00	2887.00	1.02	102.34		1180.00	1.35	134.98	
Experiment 2 8/14/2019												
Media		0.00	96.17	820.00	2567.00	1.00	100.00		1031.00	1.00	100.00	
DMSO		0.00	96.02	773.00	2616.00	1.05	105.50		996.00	1.06	105.69	
DNCB Control	1.67	0.00	82.10	1024.00	7782.00	3.44	344.44		1807.00	3.46	346.46	
	2.00	0.00	77.42	1016.00	7942.00	3.53	353.01		2001.00	4.36	435.84	
	2.40	0.00	72.55	1016.00	7306.00	3.21	320.59		2564.00	6.85	684.96	
COGA	55.82	0.11	95.89	848.00	2437.00	0.86	86.22		1062.00	0.96	95.96	
	66.98	0.13	95.66	829.00	2503.00	0.91	90.83		1150.00	1.44	143.95	
	80.38	0.16	95.19	857.00	2693.00	1.00	99.62		1097.00	1.08	107.62	
	96.45	0.19	96.06	882.00	2737.00	1.01	100.65		1142.00	1.17	116.59	
	115.74	0.23	96.29	898.00	2667.00	0.96	95.98		1165.00	1.20	119.73	
	138.89	0.28	95.72	888.00	2792.00	1.03	103.31		1215.00	1.47	146.64	
	166.67	0.33	95.89	921.00	2817.00	1.03	102.88		1214.00	1.31	131.39	
	200.0	0.4	95.6	962.0	2784.0	1.0	98.9		1267.0	1.4	136.8	

Legend: mg/mL = milligrams per milliliter; IgG = Immunoglobulin G; FITC = fluorescein isothiocyanate; RFI = Relative Fluorescence Intensity
DMSO = dimethyl sulfoxide; DNCB = 2,4-dinitrochlorobenzene

Toxicology Study No. S. 0058638-18, May 2022

h-CLAT IBF-Ome Experiment 1 Raw Data

Experiment: hCLAT PCL #2 IBF-Ome #1 08-16-2019

Protocol 49-IV19-03-01

Mods I, K

Cytometer: BD FACSVerser

Cytometer SN: Z6511530048

Statistics						
Name	Events	% Parent	% Grandparent	% Total	FITC-A Geo Mean	Propidium Iodide-A Geo Mean
live cells:live	9,552	95.52	***	95.52	857	346
dead cells:live	151	1.51	***	1.51	1,151	577
media IgG:live	10,000	94.56	***	94.56	866	338
media CD86:live	10,000	94.50	***	94.50	2,764	332
media CD54:live	10,000	94.84	***	94.84	1,067	345
DMSO IgG:live	10,000	91.60	***	91.60	879	344
DMSO CD86:live	10,000	93.77	***	93.77	2,746	320
DMSO CD54:live	10,000	93.93	***	93.93	1,023	330
DNCB 1 IgG:live	10,000	62.11	***	62.11	964	372
DNCB 1 CD86:live	9,835	59.22	***	59.22	2,841	359
DNCB 1 CD54:live	10,039	59.40	***	59.40	1,521	365
DNCB 2 IgG:live	9,918	70.08	***	70.08	885	378
DNCB 2 CD86:live	10,236	68.15	***	68.15	6,935	362
DNCB 2 CD54:live	10,219	69.20	***	69.20	2,243	367
DNCB 3 IgG:live	9,929	77.01	***	77.01	923	394
DNCB 3 CD86:live	9,991	74.27	***	74.27	7,485	373
DNCB 3 CD54:live	10,112	74.76	***	74.76	1,860	398
PCL 1 IgG:live	10,000	92.15	***	92.15	833	336
PCL 1 CD86:live	10,006	92.99	***	92.99	2,895	322
PCL 1 CD54:live	10,000	92.76	***	92.76	1,009	330
PCL 2 IgG:live	10,000	94.22	***	94.22	828	324
PCL 2 CD86:live	10,186	93.80	***	93.80	2,572	319
PCL 2 CD54:live	10,083	93.17	***	93.17	1,028	327
PCL 3 IgG:live	10,017	94.66	***	94.66	818	322
PCL 3 CD86:live	10,009	94.02	***	94.02	2,615	313
PCL 3 CD54:live	10,007	93.90	***	93.90	1,002	320
PCL 4 IgG:live	10,000	94.80	***	94.80	825	322
PCL 4 CD86:live	10,048	94.22	***	94.22	2,724	319
PCL 4 CD54:live	10,000	93.53	***	93.53	987	323
PCL 5 IgG:live	10,010	94.84	***	94.84	834	318
PCL 5 CD86:live	10,026	94.84	***	94.84	2,679	315
PCL 5 CD54:live	10,000	94.22	***	94.22	980	323
PCL 6 IgG:live	10,000	94.41	***	94.41	803	319
PCL 6 CD86:live	10,000	93.04	***	93.04	2,672	316
PCL 6 CD54:live	10,000	94.72	***	94.72	991	321
PCL 7 IgG:live	10,000	93.52	***	93.52	813	319
PCL 7 CD86:live	10,000	92.97	***	92.97	2,524	319
PCL 7 CD54:live	10,002	93.25	***	93.25	1,005	324
PCL 8 IgG:live	10,000	94.76	***	94.76	808	318
PCL 8 CD86:live	10,000	93.99	***	93.99	2,727	313
PCL 8 CD54:live	10,000	93.48	***	93.48	992	327
IBF-Ome 1 IgG:live	10,000	93.84	***	93.84	864	328
IBF-Ome 1 CD86:live	10,000	92.90	***	92.90	2,826	321
IBF-Ome 1 CD54:live	10,000	94.14	***	94.14	1,013	325
IBF-Ome 2 IgG:live	10,000	93.48	***	93.48	853	324
IBF-Ome 2 CD86:live	10,000	93.65	***	93.65	2,834	317
IBF-Ome 2 CD54:live	10,000	93.56	***	93.56	1,019	322
IBF-Ome 3 IgG:live	10,000	94.16	***	94.16	860	326
IBF-Ome 3 CD86:live	10,000	93.49	***	93.49	2,616	319
IBF-Ome 3 CD54:live	10,000	93.36	***	93.36	1,023	329
IBF-Ome 4 IgG:live	10,000	94.22	***	94.22	869	325
IBF-Ome 4 CD86:live	10,000	94.38	***	94.38	2,527	312
IBF-Ome 4 CD54:live	10,000	94.68	***	94.68	1,018	323
IBF-Ome 5 IgG:live	10,000	94.52	***	94.52	848	325
IBF-Ome 5 CD86:live	10,011	94.28	***	94.28	2,595	319
IBF-Ome 5 CD54:live	10,000	95.17	***	95.17	980	322
IBF-Ome 6 IgG:live	10,000	94.77	***	94.77	838	321
IBF-Ome 6 CD86:live	10,010	93.93	***	93.93	2,773	311
IBF-Ome 6 CD54:live	10,024	94.42	***	94.42	998	326
IBF-Ome 7 IgG:live	10,000	94.80	***	94.80	828	319
IBF-Ome 7 CD86:live	10,170	94.04	***	94.04	2,509	315
IBF-Ome 7 CD54:live	10,000	93.87	***	93.87	1,001	321
IBF-Ome 8 IgG:live	10,000	94.71	***	94.71	820	317
IBF-Ome 8 CD86:live	10,010	94.05	***	94.05	2,620	311
IBF-Ome 8 CD54:live	10,000	94.86	***	94.86	986	314

Operator: Lindsay A. Holden, PhD

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Software: BD FACSuite v1.0.5

Page 1 of 2

Toxicology Study No. S. 0058638-18, May 2022

h-CLAT IBF-Ome Experiment 2 Raw Data

Experiment: hCLAT IBF-Ome #2 08-19-2019
 Cytometer: BD FACSVerser Cytometer SN: Z6511530048
 Protocol 49-IV19-03-01
 Mod K

Statistics						
Name	Events	% Parent	% Grandparent	% Total	FITC-A Geo Mean	Propidium Iodide-A Geo Mean
live cells:live	9,620	96.20	***	96.20	767	311
dead cells:live	154	1.54	***	1.54	969	481
media IgG:live	10,000	95.79	***	95.79	777	309
media CD86:live	10,009	95.67	***	95.67	2,027	299
media CD54:live	10,000	95.22	***	95.22	957	309
DMSO IgG:live	10,006	96.00	***	96.00	755	302
DMSO CD86:live	10,022	95.18	***	95.18	2,341	294
DMSO CD54:live	10,000	95.31	***	95.31	925	300
DNCB 1 IgG:live	9,923	79.21	***	79.21	886	379
DNCB 1 CD86:live	10,098	76.30	***	76.30	4,625	364
DNCB 1 CD54:live	9,938	77.01	***	77.01	2,026	366
DNCB 2 IgG:live	9,937	80.85	***	80.85	894	381
DNCB 2 CD86:live	9,986	78.93	***	78.93	4,866	362
DNCB 2 CD54:live	9,886	79.49	***	79.49	1,880	371
DNCB 3 IgG:live	9,995	81.52	***	81.52	938	403
DNCB 3 CD86:live	10,000	81.33	***	81.33	4,860	375
DNCB 3 CD54:live	9,955	80.28	***	80.28	1,833	389
IBF-Ome 1 IgG:live	10,000	95.68	***	95.68	774	294
IBF-Ome 1 CD86:live	10,017	94.63	***	94.63	2,056	290
IBF-Ome 1 CD54:live	10,004	94.88	***	94.88	986	292
IBF-Ome 2 IgG:live	10,000	94.91	***	94.91	764	292
IBF-Ome 2 CD86:live	10,000	94.63	***	94.63	1,895	285
IBF-Ome 2 CD54:live	10,007	94.86	***	94.86	964	289
IBF-Ome 3 IgG:live	10,020	96.00	***	96.00	791	299
IBF-Ome 3 CD86:live	10,074	95.20	***	95.20	1,982	287
IBF-Ome 3 CD54:live	10,000	95.47	***	95.47	971	290
IBF-Ome 4 IgG:live	10,000	95.40	***	95.40	761	290
IBF-Ome 4 CD86:live	10,005	95.31	***	95.31	2,020	288
IBF-Ome 4 CD54:live	10,000	95.11	***	95.11	969	289
IBF-Ome 5 IgG:live	10,001	95.31	***	95.31	763	292
IBF-Ome 5 CD86:live	10,000	94.79	***	94.79	1,959	292
IBF-Ome 5 CD54:live	10,000	94.22	***	94.22	951	291
IBF-Ome 6 IgG:live	10,021	95.45	***	95.45	770	295
IBF-Ome 6 CD86:live	10,009	94.71	***	94.71	1,942	286
IBF-Ome 6 CD54:live	10,000	95.39	***	95.39	966	286
IBF-Ome 7 IgG:live	10,005	96.02	***	96.02	772	298
IBF-Ome 7 CD86:live	10,000	95.21	***	95.21	1,820	296
IBF-Ome 7 CD54:live	10,000	96.04	***	96.04	936	292
IBF-Ome 8 IgG:live	10,000	94.80	***	94.80	762	293
IBF-Ome 8 CD86:live	10,000	93.46	***	93.46	1,960	293
IBF-Ome 8 CD54:live	10,008	94.73	***	94.73	915	292

Table G-4. h-CLAT IBF-Ome Data Analysis

	Stock Concentration (mg/mL)	Concentration (mg/mL)	Viability (IgG)	FITC IgG	FITC CD86	RFI	% change	EC150	FITC CD54	RFI	% change	EC200
Experiment 1 8/16/2019												
Media		0.00	94.56	866.00	2764.00	1.00	100.00		1067.00	1.00	100.00	
DMSO		0.00	91.60	879.00	2746.00	0.98	98.37		1023.00	0.72	71.64	
DNCB Control	1.67	0.00	77.01	923.00	7485.00	3.51	351.47		1860.00	4.66	466.17	
	2.00	0.00	70.08	885.00	6935.00	3.24	324.05		2243.00	6.76	675.62	
	2.40	0.00	62.11	964.00	2841.00	1.01	100.54		1521.00	2.77	277.11	
IBF-Ome	0.35	0.00	94.71	820.00	2620.00	0.96	96.41		986.00	1.15	115.28	
	0.42	0.00	94.80	828.00	2509.00	0.90	90.04		1001.00	1.20	120.14	
	0.50	0.00	94.77	838.00	2773.00	1.04	103.64		998.00	1.11	111.11	
	0.60	0.00	94.52	848.00	2595.00	0.94	93.57		980.00	0.92	91.67	
	0.72	0.00	94.22	869.00	2527.00	0.89	88.81		1018.00	1.03	103.47	
	0.87	0.00	94.16	860.00	2616.00	0.94	94.05		1023.00	1.13	113.19	
	1.04	0.00	93.48	853.00	2834.00	1.06	106.11		1019.00	1.15	115.28	
	1.25	0.00	93.84	864.00	2826.00	1.05	105.09		1013.00	1.03	103.47	
Experiment 2 8/19/2019												
Media		0.00	95.79	777.00	2027.00	1.00	100.00		957.00	1.00	100.00	
DMSO		0.00	96.00	755.00	2341.00	1.27	126.88		925.00	0.94	94.44	
DNCB Control	1.67	0.00	81.52	938.00	4860.00	2.10	210.07		1833.00	4.45	445.27	
	2.00	0.00	80.85	894.00	4866.00	2.13	212.75		1880.00	4.91	490.55	
	2.40	0.00	79.21	886.00	4625.00	2.00	200.27		2026.00	5.67	567.16	
IBF-Ome		0.00	94.80	762.00	1960.00	0.76	75.54		915.00	0.90	90.00	
		0.00	96.02	772.00	1820.00	0.66	66.08		936.00	0.96	96.47	
		0.00	95.45	770.00	1942.00	0.74	73.90		966.00	1.15	115.29	
		0.00	95.31	763.00	1959.00	0.75	75.41		951.00	1.11	110.59	
		0.00	95.40	761.00	2020.00	0.79	79.38		969.00	1.22	122.35	
		0.00	96.00	791.00	1982.00	0.75	75.09		971.00	1.06	105.88	
		0.00	94.91	764.00	1895.00	0.71	71.31		964.00	1.18	117.65	
		0.00	95.68	774.00	2056.00	0.81	80.83		986.00	1.25	124.71	

Legend:

h-CLAT = human cell line activation test; mg/mL = milligrams per milliliter; IgG = Immunoglobulin G; FITC = fluorescein isothiocyanate;

RFI = Relative Fluorescence Intensity; DMSO = dimethyl sulfoxide; DNCB = 2,4-dinitrochlorobenzene

Toxicology Study No. 0058638-18, May 2022

h-CLAT PCL Experiment 1 Raw Data

Experiment: hCLAT COGA #2 PCL #1 08-14-2019

Protocol # 49-IV19-03-01

Cytometer: BD FACSVerser

Cytometer SN: Z6511530048

Mods H, I

Statistics						
Name	Events	% Parent	% Grandparent	% Total	FITC-A Geo Mean	Propidium Iodide-A Geo Mean
live cells:live	28,983	96.61	***	96.61	843	337
dead cells:live	1,390	5.88	***	5.88	951	399
media IgG:live	10,000	96.17	***	96.17	820	326
media CD86:live	10,003	95.49	***	95.49	2,567	322
media CD54:live	10,000	95.58	***	95.58	1,031	326
DMSO IgG:live	10,000	96.02	***	96.02	773	309
DMSO CD86:live	10,007	96.07	***	96.07	2,616	297
DMSO CD54:live	10,000	96.53	***	96.53	996	307
DNCB 1 IgG:live	9,929	72.55	***	72.55	1,016	430
DNCB 1 CD86:live	9,841	70.91	***	70.91	7,306	399
DNCB 1 CD54:live	10,056	70.88	***	70.88	2,564	409
DNCB 2 IgG:live	9,897	77.42	***	77.42	1,016	425
DNCB 2 CD86:live	9,924	74.31	***	74.31	7,942	400
DNCB 2 CD54:live	9,922	75.94	***	75.94	2,001	419
DNCB 3 IgG:live	9,996	82.10	***	82.10	1,024	418
DNCB 3 CD86:live	9,874	78.83	***	78.83	7,782	398
DNCB 3 CD54:live	9,929	81.56	***	81.56	1,807	413
COGA 1 IgG:live	10,000	95.63	***	95.63	962	374
COGA 1 CD86:live	10,000	95.68	***	95.68	2,784	367
COGA 1 CD54:live	10,000	95.76	***	95.76	1,267	374
COGA 2 IgG:live	10,000	95.89	***	95.89	921	360
COGA 2 CD86:live	10,004	95.92	***	95.92	2,617	357
COGA 2 CD54:live	10,000	95.91	***	95.91	1,214	362
COGA 3 IgG:live	10,000	95.72	***	95.72	888	350
COGA 3 CD86:live	10,000	95.75	***	95.75	2,792	351
COGA 3 CD54:live	10,000	96.11	***	96.11	1,215	354
COGA 4 IgG:live	10,000	96.29	***	96.29	898	353
COGA 4 CD86:live	10,011	95.93	***	95.93	2,667	351
COGA 4 CD54:live	10,000	96.04	***	96.04	1,165	348
COGA 5 IgG:live	10,000	96.06	***	96.06	882	345
COGA 5 CD86:live	10,029	95.94	***	95.94	2,737	338
COGA 5 CD54:live	10,000	95.76	***	95.76	1,142	345
COGA 6 IgG:live	10,000	95.19	***	95.19	857	338
COGA 6 CD86:live	10,003	95.27	***	95.27	2,693	329
COGA 6 CD54:live	10,000	95.38	***	95.38	1,097	333
COGA 7 IgG:live	10,000	95.66	***	95.66	829	328
COGA 7 CD86:live	10,000	95.31	***	95.31	2,503	324
COGA 7 CD54:live	10,009	95.83	***	95.83	1,150	325
COGA 8 IgG:live	10,000	95.89	***	95.89	848	334
COGA 8 CD86:live	10,022	96.11	***	96.11	2,437	329
COGA 8 CD54:live	10,000	95.79	***	95.79	1,062	335
PCL 1 IgG:live	10,000	96.84	***	96.84	773	305
PCL 1 CD86:live	10,000	96.07	***	96.07	2,643	298
PCL 1 CD54:live	10,000	96.58	***	96.58	966	306
PCL 2 IgG:live	10,000	96.22	***	96.22	789	307
PCL 2 CD86:live	10,000	95.55	***	95.55	2,798	300
PCL 2 CD54:live	10,000	95.92	***	95.92	961	308
PCL 3 IgG:live	10,000	96.52	***	96.52	771	304
PCL 3 CD86:live	10,050	95.85	***	95.85	2,740	300
PCL 3 CD54:live	10,000	95.85	***	95.85	992	306
PCL 4 IgG:live	10,000	96.40	***	96.40	783	309
PCL 4 CD86:live	10,000	96.51	***	96.51	2,404	300
PCL 4 CD54:live	10,000	96.21	***	96.21	983	309
PCL 5 IgG:live	10,004	96.62	***	96.62	769	301
PCL 5 CD86:live	10,000	96.16	***	96.16	2,776	300
PCL 5 CD54:live	10,000	96.62	***	96.62	959	304
PCL 6 IgG:live	10,018	96.04	***	96.04	766	303
PCL 6 CD86:live	10,051	95.74	***	95.74	2,795	301
PCL 6 CD54:live	10,020	96.18	***	96.18	973	305
PCL 7 IgG:live	10,008	96.15	***	96.15	772	304
PCL 7 CD86:live	10,021	95.76	***	95.76	2,702	299
PCL 7 CD54:live	10,000	96.26	***	96.26	957	303
PCL 8 IgG:live	10,000	95.68	***	95.68	769	303
PCL 8 CD86:live	10,102	95.45	***	95.45	2,726	296
PCL 8 CD54:live	10,232	95.24	***	95.24	940	301

Operator: Lindsay A. Holden, PhD

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Software: BD FACSuite v1.0.5

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Toxicology Study No. 0058638-18, May 2022

h-CLAT PCL Experiment 2 Raw Data

Experiment: hCLAT PCL #2 IBF-Ome #1 08-16-2019

Protocol 49-IV19-03-01

Mods I, K

Cytometer: BD FACSVerser

Cytometer SN: Z6511530048

Statistics						
Name	Events	% Parent	% Grandparent	% Total	FITC-A Geo Mean	Propidium Iodide-A Geo Mean
live cells:live	9,552	95.52	***	95.52	857	346
dead cells:live	151	1.51	***	1.51	1,151	577
media IgG:live	10,000	94.56	***	94.56	866	338
media CD86:live	10,000	94.50	***	94.50	2,764	332
media CD54:live	10,000	94.84	***	94.84	1,067	345
DMSO IgG:live	10,000	91.60	***	91.60	879	344
DMSO CD86:live	10,000	93.77	***	93.77	2,746	320
DMSO CD54:live	10,000	93.93	***	93.93	1,023	330
DNCB 1 IgG:live	10,000	62.11	***	62.11	964	372
DNCB 1 CD86:live	9,835	59.22	***	59.22	2,841	359
DNCB 1 CD54:live	10,039	59.40	***	59.40	1,521	365
DNCB 2 IgG:live	9,918	70.08	***	70.08	885	378
DNCB 2 CD86:live	10,236	68.15	***	68.15	6,935	362
DNCB 2 CD54:live	10,219	69.20	***	69.20	2,243	367
DNCB 3 IgG:live	9,929	77.01	***	77.01	923	394
DNCB 3 CD86:live	9,991	74.27	***	74.27	7,485	373
DNCB 3 CD54:live	10,112	74.76	***	74.76	1,860	398
PCL 1 IgG:live	10,000	92.15	***	92.15	833	336
PCL 1 CD86:live	10,006	92.99	***	92.99	2,895	322
PCL 1 CD54:live	10,000	92.76	***	92.76	1,009	330
PCL 2 IgG:live	10,000	94.22	***	94.22	828	324
PCL 2 CD86:live	10,186	93.80	***	93.80	2,572	319
PCL 2 CD54:live	10,083	93.17	***	93.17	1,028	327
PCL 3 IgG:live	10,017	94.66	***	94.66	818	322
PCL 3 CD86:live	10,009	94.02	***	94.02	2,615	313
PCL 3 CD54:live	10,007	93.90	***	93.90	1,002	320
PCL 4 IgG:live	10,000	94.80	***	94.80	825	322
PCL 4 CD86:live	10,048	94.22	***	94.22	2,724	319
PCL 4 CD54:live	10,000	93.53	***	93.53	987	323
PCL 5 IgG:live	10,010	94.84	***	94.84	834	318
PCL 5 CD86:live	10,026	94.84	***	94.84	2,679	315
PCL 5 CD54:live	10,000	94.22	***	94.22	980	323
PCL 6 IgG:live	10,000	94.41	***	94.41	803	319
PCL 6 CD86:live	10,000	93.04	***	93.04	2,672	316
PCL 6 CD54:live	10,000	94.72	***	94.72	991	321
PCL 7 IgG:live	10,000	93.52	***	93.52	813	319
PCL 7 CD86:live	10,000	92.97	***	92.97	2,524	319
PCL 7 CD54:live	10,002	93.25	***	93.25	1,005	324
PCL 8 IgG:live	10,000	94.76	***	94.76	808	318
PCL 8 CD86:live	10,000	93.99	***	93.99	2,727	313
PCL 8 CD54:live	10,000	93.48	***	93.48	992	327
IBF-Ome 1 IgG:live	10,000	93.84	***	93.84	864	328
IBF-Ome 1 CD86:live	10,000	92.90	***	92.90	2,826	321
IBF-Ome 1 CD54:live	10,000	94.14	***	94.14	1,013	325
IBF-Ome 2 IgG:live	10,000	93.48	***	93.48	853	324
IBF-Ome 2 CD86:live	10,000	93.65	***	93.65	2,834	317
IBF-Ome 2 CD54:live	10,000	93.56	***	93.56	1,019	322
IBF-Ome 3 IgG:live	10,000	94.16	***	94.16	860	326
IBF-Ome 3 CD86:live	10,000	93.49	***	93.49	2,616	319
IBF-Ome 3 CD54:live	10,000	93.36	***	93.36	1,023	329
IBF-Ome 4 IgG:live	10,000	94.22	***	94.22	869	325
IBF-Ome 4 CD86:live	10,000	94.38	***	94.38	2,527	312
IBF-Ome 4 CD54:live	10,000	94.68	***	94.68	1,018	323
IBF-Ome 5 IgG:live	10,000	94.52	***	94.52	848	325
IBF-Ome 5 CD86:live	10,011	94.28	***	94.28	2,595	319
IBF-Ome 5 CD54:live	10,000	95.17	***	95.17	980	322
IBF-Ome 6 IgG:live	10,000	94.77	***	94.77	838	321
IBF-Ome 6 CD86:live	10,010	93.93	***	93.93	2,773	311
IBF-Ome 6 CD54:live	10,024	94.42	***	94.42	998	326
IBF-Ome 7 IgG:live	10,000	94.80	***	94.80	828	319
IBF-Ome 7 CD86:live	10,170	94.04	***	94.04	2,509	315
IBF-Ome 7 CD54:live	10,000	93.87	***	93.87	1,001	321
IBF-Ome 8 IgG:live	10,000	94.71	***	94.71	820	317
IBF-Ome 8 CD86:live	10,010	94.05	***	94.05	2,620	311
IBF-Ome 8 CD54:live	10,000	94.86	***	94.86	986	314

Operator: Lindsay A. Holden, PhD

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Software: BD FACSuite v1.0.5

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Table G-4. h-CLAT PCL Data Analysis

	Stock Concentration (mg/mL)	Concentration (mg/mL)	Viability (IgG)	FITC IgG	FITC CD86	RFI	% change	EC150	FITC CD54	RFI	% change	EC200
Experiment 1 8/14/2019												
Media		0.00	96.17	820.00	2567.00	1.00	100.00		1031.00	1.00	100.00	
DMSO		0.00	96.02	773.00	2616.00	1.05	105.50		996.00	1.06	105.69	
DNCB Control	1.67	0.00	82.10	1024.00	7782.00	3.67	366.68		1807.00	3.71	371.09	
	2.00	0.00	77.42	1016.00	7942.00	3.76	375.80		2001.00	4.67	466.82	
	2.40	0.00	72.55	1016.00	7306.00	3.41	341.29		2564.00	7.34	733.65	
PCL	0.35	0.00	95.68	769.00	2726.00	1.06	106.19		940.00	0.77	76.68	
	0.42	0.00	96.15	722.00	2702.00	1.07	107.43		957.00	1.05	105.38	
	0.50	0.00	96.04	766.00	2795.00	1.10	110.09		973.00	0.93	92.83	
	0.60	0.00	96.62	769.00	2776.00	1.09	108.90		959.00	0.85	85.20	
	0.72	0.00	96.48	783.00	2404.00	0.88	87.95		983.00	0.90	89.69	
	0.87	0.00	96.52	771.00	2740.00	1.07	106.84		992.00	0.99	99.10	
	1.04	0.00	96.22	789.00	2798.00	1.09	109.01		961.00	0.77	77.13	
	1.25	0.00	96.84	773.00	2643.00	1.01	101.47		996.00	1.00	100.00	
Experiment 2 8/16/2019												
Media		0.00	94.56	866.00	2764.00	1.00	100.00		1067.00	1.00	100.00	
DMSO		0.00	91.60	879.00	2746.00	0.98	98.37		1023.00	0.72	71.64	
DNCB Control	1.67	0.00	77.01	923.00	7485.00	3.56	356.05		1860.00	4.44	444.08	
	2.00	0.00	70.08	885.00	6935.00	3.28	328.27		2243.00	6.44	643.60	
	2.40	0.00	62.11	964.00	2841.00	1.02	101.84		1521.00	2.64	263.98	
PCL	0.35	0.00	94.76	808.00	2727.00	1.03	102.79		992.00	1.28	127.78	
	0.42	0.00	93.52	813.00	2524.00	0.92	91.64		1005.00	1.33	133.33	
	0.50	0.00	94.41	803.00	2672.00	1.00	100.11		991.00	1.31	130.56	
	0.60	0.00	94.84	834.00	2679.00	0.99	98.82		980.00	1.01	101.39	
	0.72	0.00	94.80	825.00	2724.00	1.02	101.71		987.00	1.13	112.50	
	0.87	0.00	94.66	818.00	2615.00	0.96	96.25		1002.00	1.28	127.78	
	1.04	0.00	94.22	828.00	2572.00	0.93	93.41		1028.00	1.39	138.89	
	1.25	0.00	92.15	833.00	2895.00	1.10	110.44		1009.00	1.22	122.22	

Legend: h-CLAT = human cell line activation test; mg/mL = milligrams per milliliter; IgG = Immunoglobulin G; FITC = fluorescein isothiocyanate; RFI = Relative Fluorescence Intensity; DMSO = dimethyl sulfoxide; DNCB = 2,4-dinitrochlorobenzene

IBF-OH Raw Data

Experiment: hCLAT COGA IBF-OHMPK #1 08-02-2019

Protocol 49-IV19-03-01

Mods H, J

Cytometer: BD FACSVerser

Cytometer SN: Z6511530048

Statistics						
Name	Events	% Parent	% Grandparent	% Total	FITC-A Geo Mean	Propidium Iodide-A Geo Mean
Live cells:Live	27,495	91.65	***	91.65	902	335
Dead cells:Dead	24,551	95.56	***	95.56	1,711	17,404
IBF-OHMPK 1 IgG:IBF-OHMPK	27,100	93.62	***	93.62	86,103	7,436
IBF-OHMPK 1 CD86:IBF-OHMPK	28,285	94.28	***	94.28	94,168	7,850
IBF-OHMPK 1 CD54:IBF-OHMPK	28,261	94.20	***	94.20	81,491	7,108
IBF-OHMPK 2 IgG:IBF-OHMPK	28,713	95.71	***	95.71	80,677	6,979
IBF-OHMPK 2 CD86:IBF-OHMPK	28,531	95.10	***	95.10	90,005	7,591
IBF-OHMPK 2 CD54:IBF-OHMPK	28,672	95.57	***	95.57	78,639	6,866
IBF-OHMPK 3 IgG:IBF-OHMPK	29,041	96.80	***	96.80	71,862	6,325
IBF-OHMPK 3 CD86:IBF-OHMPK	28,832	96.11	***	96.11	79,894	6,744
IBF-OHMPK 3 CD54:IBF-OHMPK	28,927	96.42	***	96.42	67,366	5,918
IBF-OHMPK 4 IgG:IBF-OHMPK	28,891	96.30	***	96.30	59,598	5,233
IBF-OHMPK 4 CD86:IBF-OHMPK	28,861	96.20	***	96.20	66,969	5,649
IBF-OHMPK 4 CD54:IBF-OHMPK	28,818	96.06	***	96.06	57,849	5,126
IBF-OHMPK 5 IgG:IBF-OHMPK	29,346	97.82	***	97.82	48,614	4,385
IBF-OHMPK 5 CD86:IBF-OHMPK	29,058	96.86	***	96.86	56,196	4,796
IBF-OHMPK 5 CD54:IBF-OHMPK	28,706	95.69	***	95.69	46,279	4,125
IBF-OHMPK 6 IgG:IBF-OHMPK	29,189	97.30	***	97.30	48,439	4,396
IBF-OHMPK 6 CD86:IBF-OHMPK	28,997	96.66	***	96.66	51,772	4,388
IBF-OHMPK 6 CD54:IBF-OHMPK	28,758	95.86	***	95.86	43,606	3,979
IBF-OHMPK 7 IgG:IBF-OHMPK	29,132	97.11	***	97.11	40,230	3,670
IBF-OHMPK 7 CD86:IBF-OHMPK	28,920	96.40	***	96.40	45,964	3,900
IBF-OHMPK 7 CD54:IBF-OHMPK	28,831	96.10	***	96.10	39,146	3,563
IBF-OHMPK 8 IgG:IBF-OHMPK	29,225	97.42	***	97.42	33,889	3,158
IBF-OHMPK 8 CD86:IBF-OHMPK	28,992	96.64	***	96.64	38,842	3,306
IBF-OHMPK 8 CD54:IBF-OHMPK	28,771	95.90	***	95.90	34,855	3,194

Note: It appears that the IBF-OHMPK (which is visibly a neon green/yellow color) is interfering with the PI and FITC absorbance. Therefore, I've gated out dead cells (dead) and the live, but shifted absorbance IBF-OHMPK cells (IBF-OHMPK) for population viability determination. There is some overlap between the dead and IBF-OHMPK live populations, which I believe is an artifact of being conservative (i.e., assuming overlapping population = dead). See lab notebook for cell counts that show live, dead, and IBF-OHMPK cell viability percentages.

-LAH

DPRA Data

Raw Data Cysteine #1

Pr NQ and OFHDA
First 100

Instrument: U3000_SYS_1B Sequence: DP-DPRA-OFHDA_OFHMBBS_PrQ-13_AUG20

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Summary							
Sequence Details							
Name:		DP-DPRA-OFHDA_OFHMBBS_PrQ-13_AUG20		Created On:		26/Feb/19 14:30:42	
Directory:		DNKIData		Created By:		US Army	
Data Vault:		ChromeleonLocal		Updated On:		17/Aug/20 08:24:34	
No. of Injections:		37		Updated By:		US Army	
By Component		Cystene					
No.	Injection Name	Ret Time min Cystene UV_VIS_1	Area mAU*min Cystene UV_VIS_1	Height mAU Cystene UV_VIS_1	Amount mM peptide Cystene UV_VIS_1	Rel.Area % Cystene UV_VIS_1	Peak Type Cystene UV_VIS_1
1	ACN blk	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	STD 7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	STD 6	10.942	1.073	10.461	0.018	100.00	M
6	STD 5	10.933	1.958	21.030	0.032	100.00	M
7	STD 4	10.933	4.252	42.545	0.069	96.44	M
8	STD 3	10.927	8.076	82.462	0.132	98.18	M
9	STD 2	10.923	15.715	154.266	0.267	98.27	M
10	STD 1	10.913	28.306	268.935	0.534	96.50	M
11	ref control A	10.897	26.874	255.608	0.500	96.89	M
12	ref control A	10.917	27.037	254.258	0.504	97.02	M
13	ref control A	10.910	26.822	253.686	0.499	96.94	M
14	ref control B	10.917	27.256	256.986	0.509	96.56	M
15	ref control B	10.908	27.188	254.557	0.507	96.43	M
16	ref control B	10.903	27.072	255.791	0.505	96.58	M
17	ref control C	10.910	26.862	253.837	0.500	97.04	M
18	Co-el OFHDA	10.900	0.152	1.068	0.004	100.00	M
19	Co-el PrQ	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
20	Cinnamaldehyde	10.928	6.066	59.331	0.098	98.05	M
21	OFHDA	10.903	24.705	234.889	0.451	96.47	M
22	PrQ	10.922	25.850	243.911	0.476	96.42	M
23	reference control C	10.910	26.558	251.530	0.493	96.96	M
24	Cinnamaldehyde	10.800	0.066	1.539	0.003	1.13	M
25	OFHDA	10.902	24.560	232.748	0.447	96.71	M
26	PrQ	10.922	25.818	241.888	0.471	96.55	M
27	reference control C	10.902	26.120	247.313	0.462	96.31	M
28	Cinnamaldehyde	10.922	5.516	54.287	0.089	98.90	M
29	OFHDA	10.918	24.168	229.861	0.439	97.23	M
30	PrQ	10.912	25.210	240.936	0.462	97.18	M
31	reference control C	10.900	26.103	247.237	0.482	96.56	M
32	Cinnamaldehyde	10.925	5.394	53.301	0.087	98.80	M
33	Reference control B	10.905	26.104	248.058	0.482	97.18	M
34	Reference control B	10.900	26.069	248.302	0.481	96.74	M
35	Reference control B	10.895	26.015	247.318	0.480	96.57	M
36	10% ACN	10.922	0.372	1.521	0.007	100.00	M
37	10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Toxicology Study No. 0058638-18, May 2022

Raw Data Lysine #1

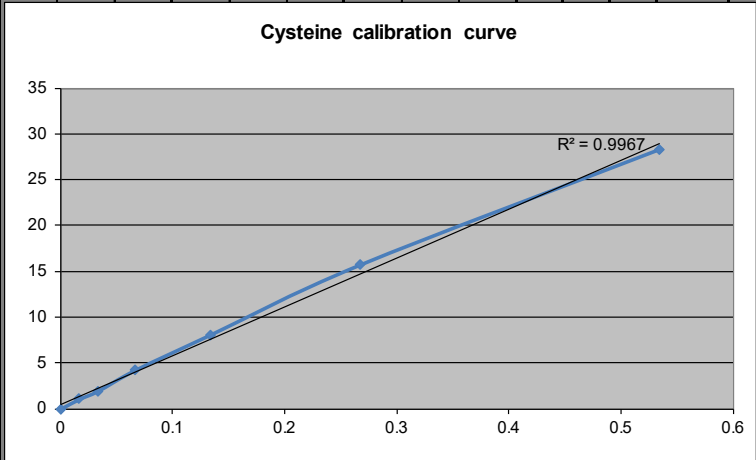
Summary *PRNQ and OFHDA first "K"*

Sample Details						
Sample ID:	DP-DPRA-OFHDA_PrQ-K-18_AUG20	Created On:	26/Feb/19 14:30:42			
File:	DNK1Data	Created By:	US Army			
Method:	Chromleon Local	Updated On:	21/Aug/20 10:00:11			
Injection:	37	Updated By:	US Army			
Component						
Lysine						
Injection Name	Ret.Time min Lysine UV_VIS_1	Area mAU*min Lysine UV_VIS_1	Height mAU Lysine UV_VIS_1	Amount mM peptide Lysine UV_VIS_1	Rel.Area % Lysine UV_VIS_1	Peak Ly UV
ACN blk	n.a.	n.a.	n.a.	n.a.	n.a.	
10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	
10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	
STD 7	8.275	31.347	266.218	0.534	100.00	E
STD 6	8.297	15.599	154.287	0.267	100.00	E
STD 5	8.307	7.747	84.787	0.134	100.00	E
STD 4	8.332	3.834	44.309	0.067	100.00	E
STD 3	8.328	1.724	21.859	0.031	100.00	E
STD 2	8.343	0.942	11.372	0.018	100.00	E
STD 1	n.a.	n.a.	n.a.	n.a.	n.a.	
ref control A	8.270	29.717	256.860	0.506	100.00	E
ref control A	8.263	29.746	258.696	0.507	100.00	E
ref control A	8.267	29.883	256.997	0.509	100.00	E
ref control B	8.265	29.537	254.245	0.503	100.00	E
ref control B	8.272	29.588	256.325	0.504	100.00	E
ref control B	8.273	29.409	255.046	0.501	99.75	E
ref control C	8.267	30.186	259.348	0.514	100.00	E
Co-el OFHDA	10.770	0.129	0.814	0.004	27.49	
Co-el PrQ	n.a.	n.a.	n.a.	n.a.	n.a.	
Cinnamaldehyde	8.320	6.872	72.020	0.119	98.97	E
OFHDA	8.203	29.445	217.510	0.502	98.99	E
PrQ	8.277	29.883	256.090	0.508	100.00	E
reference control C	8.270	29.660	257.456	0.505	99.59	E
Cinnamaldehyde	8.312	7.000	73.650	0.121	98.69	E
OFHDA	8.207	29.758	219.681	0.507	99.06	E
PrQ	8.277	29.883	256.090	0.508	100.00	E
reference control C	8.278	29.663	255.136	0.505	100.00	E
Cinnamaldehyde	8.315	7.390	76.066	0.128	98.50	E
OFHDA	8.198	29.770	218.174	0.507	98.63	E
PrQ	8.270	29.876	255.688	0.509	99.51	E
reference control C	8.277	29.666	255.296	0.506	100.00	E
Cinnamaldehyde	8.303	7.793	78.493	0.134	97.02	E
Reference control B	8.277	29.344	253.270	0.500	100.00	E
Reference control B	8.263	29.288	254.585	0.499	100.00	E
Reference control B	8.268	29.438	254.817	0.502	100.00	E
10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	
10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	

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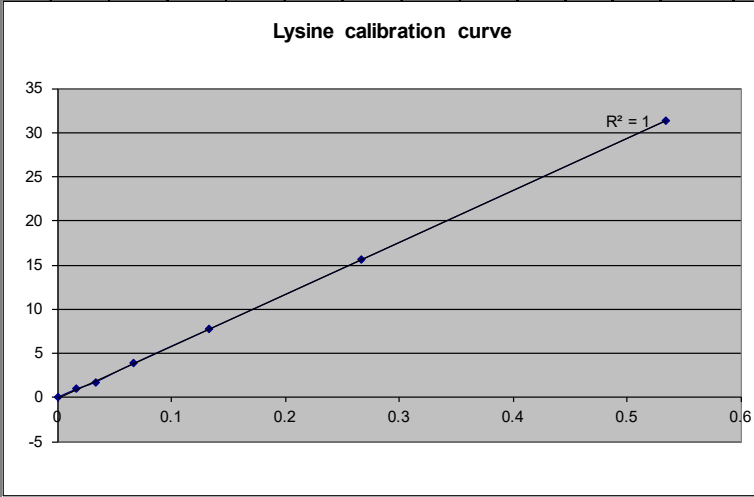
DPRA Cysteine #1 Calculations

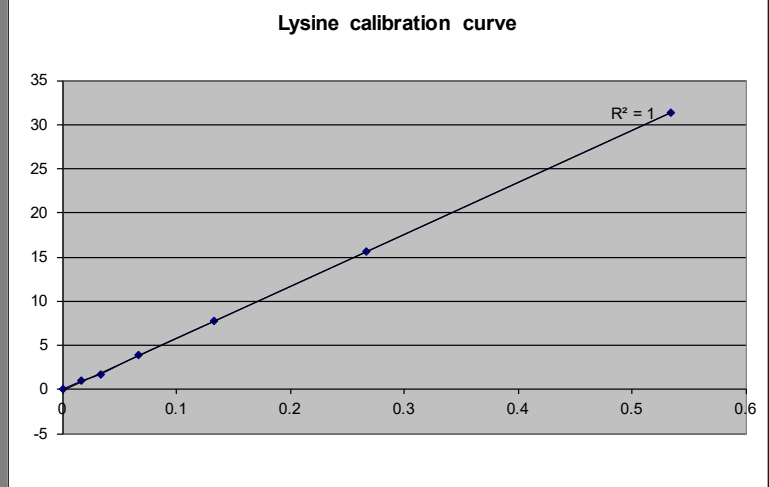
Vehicle	Code	Conc/ replicate	Cysteine																	
			Peak Area at 220 nm	Peptide Concentration (mM)	Peptide Depletion (%)	CORRECTED Peptide Depletion (%)	Peak Area			Peptide Conc			Peptide Depl.			Precipitates	Re-solub. Or Centrif.	Absorbs at 220 nm	Retent. Time similar to pept.	Co-elution
	STANDARD						mean	SD	CV	mean	SD	CV	mean	SD	CV					
		STD1	28	0.5																
		STD2	16	0.3																
		STD3	8.1	0.1																
		STD4	4.3	0.1																
		STD5	2	0																
		STD6	1.1	0																
		Dil Buff	0	0																
	REF CTRL A	r1	26.874	0.49541066						0.50	0.00	0.00								
		r2	27	0.498470249																
		r3	27	0.494434595																
	REF CTRL B	r1	27.256	0.502580986																
		r2	27.188	0.501304593																
		r3	27.072	0.499127216			27.172	0.093	0.0034	0.25	0.28	1.14								
		r4		-0.009027338																
		r5		-0.009027338																
		r6		-0.009027338																
Acetonitrile	REF CTRL C	r1	26.862	0.495185415			26.508	0.382	0.0144	0.4885	0.0072	0.0147								
		r2	27	0.489479187																
		r3	26	0.480938617																
Acetonitrile	POSITIVE CONTROL Cinnamic Aldehyde	r1	6.0066	0.103719454	0.773401406	0.773401406	5.6389	0.3243	0.0575	0.0968	0.0061	0.0629	0.7873	0.0122	0.0155					
		r2	6	0.094510654	0.791909259	0.791909259														
		r3	5	0.092220655	0.796511701	0.796511701														
Acetonitrile	CHEMICAL 1 PrNQ 1	r1	25.85	0.476189684	0.024810432	0.024810432	25.56	0.3241	0.0127	0.4707	0.0061	0.0129	0.0358	0.0122	0.3419	N	Y/N	Y/N	N	Y/N
		r2	26	0.471853703	0.033524892	0.033524892														
		r3	25	0.464176574	0.048954391	0.048954391														



Toxicology Study No. 0058638-18, May 2022

DPRA Lysine#1 Calculations

Vehicle		Code	Conc/ replicate	Lysine																	
				Peak Area at 220 nm	Peptide Concentration (mM)	Peptide Depletion (%)	CORRECTED Peptide Depletion (%)	Peak Area			Peptide Conc			Peptide Depl.			Precipitates	Re-solub. Or Centrif.	Absorbs at 220 nm	Retent. Time similar to pept.	Co-elution
								mean	SD	CV	mean	SD	CV	mean	SD	CV					
STANDARD								Lysine calibration curve 													
		STD1	31	0.5																	
		STD2	16	0.3																	
		STD3	7.7	0.1																	
		STD4	3.8	0.1																	
		STD5	1.7	0																	
		STD6	0.9	0																	
		Dil Buff	0	0																	
	REF CTRL A																				
		r1	29.717	0.506615991						0.5077	0.0015	0.003									
		r2	29.746	0.507108787																	
		r3	29.883	0.509436826																	
	REF CTRL B																				
		r1	29.537	0.503557254																	
		r2	29.588	0.504423896																	
		r3	29.409	0.501382152			29.434	0.1135	0	0.5018	0.0019	0.0038									
		r4	29.344	0.500277608																	
		r5	29.288	0.499326001																	
		r6	29.438	0.501874948																	
Acetonitrile	REF CTRL C																				
		r1	29.66	0.505647391																	
		r2	29.663	0.50569837			29.67	0.0142	0.0005	0.5058	0.0002	0.0005									
		r3	29.686	0.506089208																	
Acetonitrile	POSITIVE CONTROL																				
	Cinnamic Aldehyde																				
		r1	6.872	0.118411261	0.768382973	0.768382973															
		r2	7	0.120586363	0.764068802	0.764068802	7.0873	0.2698	0.0381	0.1221	0.0046	0.0376	0.7611	0.0091	0.0119						
		r3	7.39	0.127213627	0.750924064	0.750924064															
Acetonitrile	CHEMICAL 1																				
	PrNQ 1																				
		r1	29.826	0.508468226	-0.00526913	0	29.835	0.0378	0.0013	0.5086	0.0006	0.0013	0	0	#####	N	N	N	N	N	
		r2	29.802	0.508060395	-0.004460223	0															
		r3	29.876	0.509317875	-0.006954353	0															



QC data Cysteine and Lysine #1

Vehicle	Code	Criterion	Criterion met?
	STANDARD		
		Lysine: $r^2 > 0,99$	Lys R2 0.999955464 YES
			Lys Intercept -0.096243936
		Cysteine: $r^2 > 0,99$	Lys Slope 58.84781466
			Cys R2 0.996684435 YES
			Cys Intercept 0.480932599
			Cys Slope 53.27513012
	REF CTRL A		
		Range Mean Conc Cys [0.45 to 0.55]	YES
		Range Mean Conc Lys [0.45 to 0.55]	YES
	REF CTRL B		
		Peak Area CV ctrl B < 15% - Cys	YES
		Peak Area CV ctrl B < 15% - Lys	YES
Acetonitrile	REF CTRL C		
		Peak Area CV ctrl C < 15% - Cys	YES
		Peak Area CV ctrl C < 15% - Lys	YES
		Range Mean Conc Cys [0.45 to 0.55]	YES
		Range Mean Conc Lys [0.45 to 0.55]	YES
Acetonitrile	POSITIVE CONTROL		
	Cinnamic Aldehyde	60,8 < Mean % Depl Cys < 96,6	YES
		40,2 < Mean % Depl Lys < 69,4	Not Met
		SD % Depl Cys < 14,9	YES
		SD % Depl Lys < 11,6	YES
Acetonitrile	CHEMICAL 1		
	PrNQ 1	SD % Depl Cys < 14,9	YES
		SD % Depl Lys < 11,6	YES

Cinnamaldehyde is referred to as cinnamic aldehyde in this table. The cinnamaldehyde depletion for lysine was greater than the preferred mean percentage range. Still consider that this was a successful assay. Additionally, lysine is not required to make a call for sensitization.

Toxicology Study No. 0058638-18, May 2022

Raw Data Cysteine #2

Instrument:U3000_SYS_1B Sequence:DP-DPRA-PrQ-IAMMDA52-OFHBS-28_AUG20

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Summary							
Sequence Details							
Name:		DP-DPRA-PrQ-IAMMDA52-OFHBS-28_AUG20		Created On:	26/Feb/19 14:30:42		
Directory:		DNKIData		Created By:	US Army		
Data Vault:		ChromleonLocal		Updated On:	31/Aug/20 08:31:48		
No. of Injections:		47		Updated By:	US Army		
By Component		Cystene					
No.	Injection Name	Ret.Time min Cystene UV_VIS_1	Area mAU*min Cystene UV_VIS_1	Height mAU Cystene UV_VIS_1	Amount mM peptide Cystene UV_VIS_1	Rel.Area % Cystene UV_VIS_1	Peak Type Cystene UV_VIS_1
1	ACN blk	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	STD 7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	STD 6	10.932	1.300	11.791	0.018	100.00	M
6	STD 5	10.918	2.429	24.027	0.033	100.00	M
7	STD 4	10.922	4.731	47.919	0.066	98.47	M
8	STD 3	10.908	9.265	92.953	0.133	98.72	M
9	STD 2	10.900	17.731	173.371	0.267	97.06	M
10	STD 1	10.888	32.156	295.820	0.534	100.00	BMB*
11	ref control A	10.885	31.912	294.345	0.529	100.00	BMB*
12	ref control A	10.897	31.494	295.691	0.520	100.00	BMB*
13	ref control A	10.888	31.611	294.979	0.523	100.00	BMB*
14	ref control B	10.895	29.234	270.118	0.475	100.00	BMB*
15	ref control B	10.885	28.975	268.435	0.470	100.00	BMB*
16	ref control B	10.877	28.938	268.485	0.469	100.00	BMB*
17	ref control C	10.880	31.077	283.797	0.512	100.00	BMB*
18	Acetone	10.888	29.986	274.196	0.490	100.00	BMB*
19	DMSO	10.907	14.260	140.313	0.211	100.00	BMB*
20	Co-el PrQ	10.900	0.144	0.862	0.002	50.16	M
21	Co-el IAMMDA52	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
22	Co-el OFHMBBS	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
23	Cinnamaldehyde	10.915	7.465	73.476	0.106	98.84	M
24	PrNQ	10.883	30.718	281.527	0.504	100.00	BMB*
25	IAMMDA52	10.830	0.631	5.427	0.008	100.00	M
26	OFHMBBS	10.887	29.370	268.530	0.477	100.00	BMB*
27	reference control C	10.878	30.001	277.215	0.490	100.00	BMB*
28	Acetone	10.877	28.675	263.433	0.464	100.00	BMB*
29	DMSO	10.895	12.207	120.591	0.178	100.00	BMB*
30	Cinnamaldehyde	10.902	7.206	70.285	0.102	99.07	BM
31	PrNQ	10.888	30.625	278.525	0.503	100.00	BMB*
32	IAMMDA52	10.830	0.689	5.995	0.009	100.00	M
33	OFHMBBS	10.887	28.790	266.009	0.466	100.00	BMB*
34	reference control C	10.888	29.435	272.405	0.479	100.00	BMB*
35	Acetone	10.880	27.745	255.756	0.445	100.00	BMB*
36	DMSO	10.900	11.037	109.559	0.160	100.00	BMB*
37	Cinnamaldehyde	10.898	6.656	64.162	0.094	97.26	M
38	PrNQ	10.878	30.105	276.888	0.492	100.00	BMB*
39	IAMMDA52	10.818	0.764	6.540	0.010	100.00	M
40	OFHMBBS	10.885	28.200	261.218	0.455	98.01	M
41	reference control C	10.890	28.984	266.286	0.470	100.00	BMB*
42	Cinnamaldehyde	10.897	6.423	64.037	0.091	97.79	M
43	Reference control B	10.885	26.404	246.430	0.420	100.00	BMB*

DP-DPRA-PrQ-IAMMDA52-OFHBS-28_AUG20/Summary

Chromleon (c) Dionex
Version 7.1.1.1127

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Instrument: U3000_SYS_18 Sequence: DP-DPRA-PrQ-IAMMDA52-OFHBS-28_AUG20

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44	Reference control B	10.875	26.526	245.157	0.423	100.00	BMB*
45	Reference control B	10.882	26.401	245.491	0.420	100.00	BMB*
46	10% ACN	10.912	0.139	1.161	0.001	100.00	M
47	10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Toxicology Study No. 0058638-18, May 2022

Raw Data Lysine #2

Instrument:U3000_SYS_1B Sequence:DP-DPRA-HEATN_COGA_RUN2_OFHDA_PrNQ_Run2-K-27_Aug 20

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Summary							
Sequence Details							
Name:	DP-DPRA-HEATN_COGA_RUN2_OFHDA_PrN			Created On:	26/Feb/19 14:30:42		
Directory:	DNKData			Created By:	US Army		
Data Vault:	ChromeleonLocal			Updated On:	28/Aug/20 08:23:39		
No. of Injections:	44			Updated By:	US Army		
By Component		Lysine					
No.	Injection Name	Ret Time min Lysine UV_VIS_1	Area mAU*min Lysine UV_VIS_1	Height mAU Lysine UV_VIS_1	Amount mM peptide Lysine UV_VIS_1	Rel.Area % Lysine UV_VIS_1	Peak Type Lysine UV_VIS_1
1	ACN blk	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	STD 7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	STD 6	8.365	1.100	12.969	0.016	100.00	BMB*
6	STD 5	8.357	2.202	25.696	0.032	100.00	BMB*
7	STD 4	8.347	4.459	50.311	0.066	100.00	BMB*
8	STD 3	8.338	9.028	96.146	0.135	100.00	BMB*
9	STD 2	8.312	17.752	172.980	0.267	100.00	BMB*
10	STD 1	8.293	35.555	297.342	0.534	99.67	BMB*
11	ref control A	8.290	33.285	281.657	0.500	100.00	BMB*
12	ref control A	8.302	33.303	283.274	0.500	100.00	BMB*
13	ref control A	8.307	33.215	282.198	0.499	99.64	BMB*
14	ref control B	8.297	33.302	281.793	0.500	100.00	BMB*
15	ref control B	8.287	33.561	282.506	0.504	100.00	BMB*
16	ref control B	8.298	33.458	281.404	0.503	100.00	BMB*
17	ref control C	8.295	33.357	283.405	0.501	100.00	BMB*
18	Co Elut_OFHDA	10.753	0.090	0.713	0.000	26.53	M
19	Co Elut_HEATN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
20	Co Elut_COGA	11.393	0.081	0.323	0.000	100.00	M
21	Cinnamaldehyde	8.355	6.781	71.322	0.101	98.83	RMB*
22	HEATN	8.303	33.087	282.281	0.497	100.00	BMB*
23	COGA	8.293	32.680	279.296	0.491	100.00	BMB*
24	OFHDA	8.225	33.381	241.303	0.501	98.57	RMB*
25	PrNQ	8.290	33.543	285.097	0.504	100.00	BMB*
26	reference control C	8.287	33.288	280.429	0.500	99.73	BMB*
27	Cinnamaldehyde	8.343	6.913	72.771	0.103	97.40	BMB*
28	HEATN	8.302	33.337	281.929	0.501	100.00	BMB*
29	COGA	8.295	32.910	278.833	0.494	99.91	BMB*
30	OFHDA	8.233	32.582	240.749	0.489	98.95	BMB*
31	PrNQ	8.297	33.302	283.684	0.500	100.00	BMB*
32	reference control C	8.292	33.486	283.184	0.503	100.00	BMB*
33	Cinnamaldehyde	8.332	6.745	73.325	0.101	97.00	BMB*
34	HEATN	8.293	33.348	281.157	0.501	100.00	BMB*
35	COGA	8.287	32.640	277.651	0.490	99.32	BMB*
36	OFHDA	8.218	32.949	239.657	0.495	98.73	BMB*
37	PrNQ	8.297	33.666	282.725	0.506	100.00	BMB*
38	reference control C	8.297	33.045	283.007	0.496	99.61	BMB*
39	Cinnamaldehyde	8.345	6.964	75.421	0.104	95.26	BMB*
40	Reference control B	8.287	33.726	284.064	0.507	100.00	BMB*
41	Reference control B	8.293	33.776	285.623	0.507	99.57	BMB*
42	Reference control B	8.288	33.483	285.421	0.503	100.00	BMB*
43	10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

DP-DPRA-HEATN_COGA_RUN2_OFHDA_PrNQ_Run2-K-27_Aug 20/Summary

Chromeleon (c) Dionex
Version 7.1.1.1127

Toxicology Study No. 0058638-18, May 2022

Instrument:U3000_SYS_1B Sequence:DP-DPRA-HEATN_COGA_RUN2_OFHDA_PrNQ_Run2-K-27_Aug 20

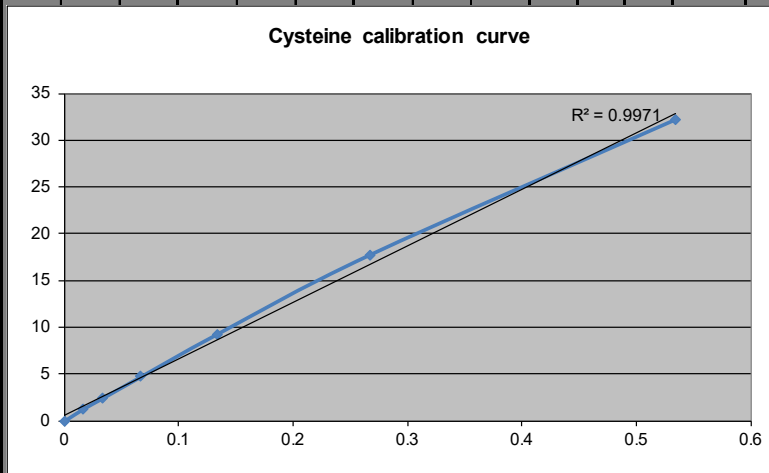
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44	10% ACN	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
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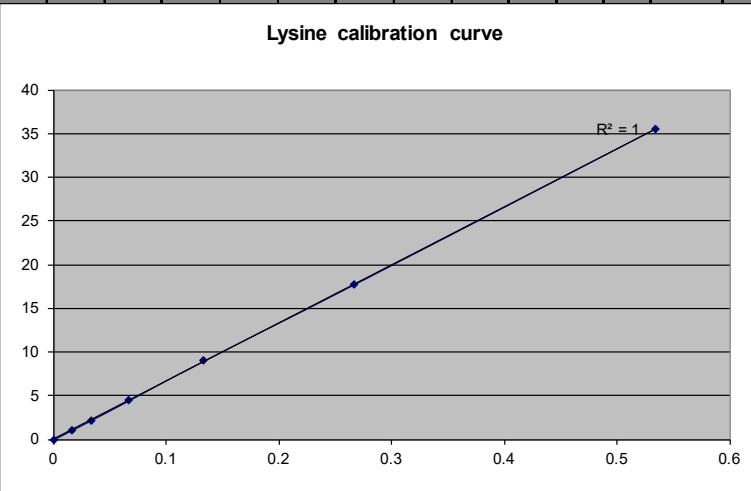
Calculations Cysteine #2

Vehicle	Code	Conc/ replicate	Cysteine																	
			Peak Area at 220 nm	Peptide Concentration (mM)	Peptide Depletion (%)	CORRECTED Peptide Depletion (%)	Peak Area			Peptide Conc			Peptide Depl.			Precipitates	Re-solub. Or Centrif.	Absorbs at 220 nm	Retent. Time similar to pept.	Co-elution
							mean	SD	CV	mean	SD	CV	mean	SD	CV					
	STANDARD																			
		STD1	32	0.5																
		STD2	18	0.3																
		STD3	9.3	0.1																
		STD4	4.7	0.1																
		STD5	2.4	0																
		STD6	1.3	0																
		Dil Buff	0	0																
	REF CTRL A																			
		r1	31.912	0.519047132						0.52	0.00	0.01								
		r2	31	0.512118757																
		r3	32	0.514058039																
	REF CTRL B																			
		r1	29.234	0.474659126																
		r2	28.975	0.470366186																
		r3	28.938	0.469752909																
		r4	26.404	0.427751713			27.746	1.4314	0.0516	0.45	0.02	0.05								
		r5	26.526	0.42977387																
		r6	26.401	0.427701987																
Acetonitrile	REF CTRL C																			
		r1	30.001	0.487372196																
		r2	29	0.477990713			29.473	0.5096	0.0173	0.4786	0.0084	0.0176								
		r3	29	0.470515362																
Acetonitrile	POSITIVE CONTROL																			
	Cinnamic Aldehyde	r1	7.206	0.109543751	0.755507804	0.755507804														
		r2	7	0.100427469	0.77416874	0.77416874	6.7617	0.4021	0.0595	0.1022	0.0067	0.0652	0.7706	0.0136	0.0177					
		r3	6	0.09656548	0.782074191	0.782074191														
Acetonitrile	CHEMICAL 1																			
	PrNQ 1	r1	30.718	0.499256512	-0.042230265	0	30.483	0.3304	0.0108	0.4954	0.0055	0.0111	0	0	#####	Y/N	Y/N	Y/N	Y/N	Y/N
		r2	31	0.497715032	-0.03907487	0														
		r3	30	0.489096002	-0.021431803	0														



Toxicology Study No. 0058638-18, May 2022

Calculations Lysine #2

Vehicle		Code	Conc/ replicate	Lysine																	
				Peak Area at 220 nm	Peptide Concentration (mM)	Peptide Depletion (%)	CORRECTED Peptide Depletion (%)	Peak Area			Peptide Conc			Peptide Depl.			Precipitates	Re-solub. Or Centrif.	Absorbs at 220 nm	Retent. Time similar to pept.	Co-elution
								mean	SD	CV	mean	SD	CV	mean	SD	CV					
STANDARD								Lysine calibration curve 													
		STD1	36	0.5																	
		STD2	18	0.3																	
		STD3	9	0.1																	
		STD4	4.5	0.1																	
		STD5	2.2	0																	
		STD6	1.1	0																	
		Dil Buff	0	0																	
	REF CTRL A																				
		r1	33.285	0.499759578																	
		r2	33.303	0.500029968						0.4995	0.0007	0.0014									
		r3	33.215	0.49870806																	
	REF CTRL B																				
		r1	33.302	0.500014946																	
		r2	33.561	0.503905562																	
		r3	33.458	0.502358329			33.551	0.177	0	0.5038	0.0027	0.0053									
		r4	33.726	0.506384139																	
		r5	33.776	0.507135224																	
		r6	33.483	0.502733871																	
Acetonitrile	REF CTRL C																				
		r1	33.357	0.500841139																	
		r2	33.288	0.499804643			33.377	0.1005	0.003	0.5011	0.0015	0.003									
		r3	33.486	0.502778936																	
Acetonitrile	POSITIVE CONTROL																				
	Cinnamic Aldehyde	r1	6.781	0.101624907	0.796836145	0.796836145															
		r2	6.913	0.103607769	0.792881325	0.792881325	6.886	0.0944	0.0137	0.1032	0.0014	0.0137	0.7937	0.0028	0.0036						
		r3	6.964	0.104373875	0.791353327	0.791353327															
Acetonitrile	CHEMICAL 1																				
	PrNQ 1	r1	33.543	0.503635172	-0.004973485	0	33.504	0.1852	0.0055	0.503	0.0028	0.0055	0.0007	0.0013	1.7321	N	Y/N	Y	N	N	
		r2	33.302	0.500014946	0.002247056	0.002247056															
		r3	33.666	0.505482839	-0.008658657	0															

Toxicology Study No. 0058638-18, May 2022

QC for Cysteine and Lysine #2

Vehicle	Code	Conc/ replicate	Mean Depletion	Reactivity Class (CYS + LYS)	Reactivity Class (CYS only)	Criterion	Criterion met?
	STANDARD						
		STD1				Lysine: r2 > 0,99	Lys R2 0.999980117 YES
		STD2					Lys Intercept 0.015785278
		STD3				Cysteine: r2 > 0,99	Lys Slope 66.57043951
		STD4					
		STD5					Cys R2 0.997097033 YES
		STD6					Cys Intercept 0.597048683
		Dil Buff					Cys Slope 60.33161425
	REF CTRL A						
		r1				Range Mean Conc Cys [0.45 to 0.55]	YES
		r2					
		r3				Range Mean Conc Lys [0.45 to 0.55]	YES
	REF CTRL B						
		r1					
		r2				Peak Area CV ctrl B < 15% - Cys	YES
		r3					
		r4				Peak Area CV ctrl B < 15% - Lys	YES
		r5					
		r6					
Acetonitrile	REF CTRL C						
		r1				Peak Area CV ctrl C < 15% - Cys	YES
		r2				Peak Area CV ctrl C < 15% - Lys	YES
		r3				Range Mean Conc Cys [0.45 to 0.55]	YES
						Range Mean Conc Lys [0.45 to 0.55]	YES
Acetonitrile	POSITIVE CONTROL Cinnamic Aldehyde						
		r1				60,8 < Mean % Depl Cys < 96,6	YES
		r2				40,2 < Mean % Depl Lys < 69,4	Not Met
		r3				SD % Depl Cys < 14,9	YES
						SD % Depl Lys < 11,6	YES
Acetonitrile	CHEMICAL 1 PrNQ 1						
		r1	0	MINIMAL	MINIMAL	SD % Depl Cys < 14,9	YES
		r2				SD % Depl Lys < 11,6	YES
		r3					

Cinnamaldehyde is referred to as cinnamic aldehyde in this table. The cinnamaldehyde depletion was outside the specified preferred range for the lysine assay. It was depleted more than the preferred range, so is considered acceptable. Additionally, lysine is not required for a sensitization call.

GLOSSARY

AERTA	Army Environmental Research and Technology Assessment
APHC	U.S. Army Public Health Center
AR	Department of the Army Regulation
ASTM	American Society for Testing and Materials
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
AFNOR	Association Française de Normalisation
BP	boiling point
BSA	Bovine serum albumin
CAOTE	Cell-based acute oral toxicity estimate
CO ₂	Carbon dioxide
CV	Coefficient of variation
CV75	cell viability at 75%
CDC	Center for Disease Control and Prevention
CASRN	Chemical Abstracts Service Registry Number
CD54	Cluster of Differentiation 54
CD86	Cluster of Differentiation 86
CFR	Code of Federal Regulations
CI	Confidence Interval
°C	degrees Celsius
COGA	Cyclooct-1-yn-3-glycolic acid
DA	Department of the Army
DIN	Deutsches Institut für Normung

DMSO	dimethyl sulfoxide
DNCB	2,4-dinitrochlorobenzene
DPRA	Direct peptide reactivity assay
DoD	Department of Defense
DoDD	Department of Defense Directive
ECOSAR	Ecological Structure Activity Relationship
EC	Effect concentration
EC ₅₀	median (50%) effect concentration
EC ₁₅₀	Effective Concentration resulting in 150% increase
EC ₂₀₀	Effective Concentration resulting in 200% increase
EPA	U.S. Environmental Protection Agency
ESOH	Environmental safety and occupational health
ECVAM	European Centre for the Validation of Alternative Method
ECVAM DB-ALM	ECVAM Database on Alternative Methods to Animal Experimentation
FACS	Fluorescence-activated cell sorting
FDA	U.S. Food and Drug Administration
FITC	Fluorescein isothiocyanate
FL1	FITC emission wavelength 530 ± 15 nm
FL2	PI emission wavelength >650 nm
GHS	Global Harmonization System
Cat	Category
GLP	Good Laboratory Practice
g	Grams

g/m ³ -h	Grams/cubic meter per hour
K _H	Henry's law constant
h-CLAT	Human cell line activation test
<i>His</i>	Histidine
<i>his</i> -	histidine or tryptophan negative
HPLC	high-performance liquid chromatography
HPLC-UV	high-performance liquid chromatography with ultraviolet detection
IC ₅₀	median (50%) inhibitory concentration
IgG1	Immunoglobulin G1
ISO	International Organization for Standardization
U	International units
kg	kilogram
LA	lactic acid
L	liter
LD ₅₀	median (50%) lethal (oral) dose
log K _{OC}	Log Organic carbon partition coefficient
log K _{OW}	Log Octanol-water partition coefficient
LOAEL	lowest-observed adverse effect level
MFI	mean fluorescence intensity
MPF	microplate format
MP	melting point
µg	micrograms
µL	microliter
µM	micromolar

mg	milligram
mL	milliliter
mmHg	millimeter of mercury
mM	millimolar
MW	molecular weight
MRL	minimum risk level
nm	nanometers
NiSO ₄	nickel sulfate
NOAEL	no-observed adverse effect level
NOEL	no-observed effect level
NRRL	Northern Regional Research Laboratory
NVN	Nederlandse voornorm
OD ₆₀₀	optical density at wavelength of 600 nm
OECD	Organization for Economic Co-operation and Development
PBS	Phosphate Buffered Solution
PPE	Personal Protective Equipment
IBF-OH	p-(3-phenyl-1-isobenzofuranyl)phenol
IBF-Ome	1-Phenyl-3-(p-{10-[3-phenyl-1-isobenzofuranyl]phenoxy]decyloxyphenyl)isobenzofuran
PCL	Polycaprolactone-cyclooctyne trimer
PI	propidium iodide
QSAR	Quantitative Structure-Activity Relationship
r ²	coefficient of determination
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine

RDT&E	research, development, testing, and evaluation
RfD	reference dose
RFI	relative fluorescence
±S9	with or without S9 liver extract
SD	standard deviation
SERDP	DoD Strategic Environmental Research and Development Program
SOP	Standard Operating Procedures
TBD	to be determined
TOPKAT	Toxicity Prediction by Komputer Assisted Technology
TOX	Toxicology Directorate
TFA	trifluoroacetic acid
TNT	trinitrotoluene
<i>Trp</i>	tryptophan
<i>Trp-</i>	tryptophan negative
UNECE	United Nations Economic Commission for Europe
VP	vapor pressure
w/v	weight per volume