

Understanding Mixtures: Assessing Chemical Compound Mixtures for Toxicological Characterization

Desiree M. Harris¹, Dylan H. Fudge², Tyler D. Goralski², Shelby O. Roe²

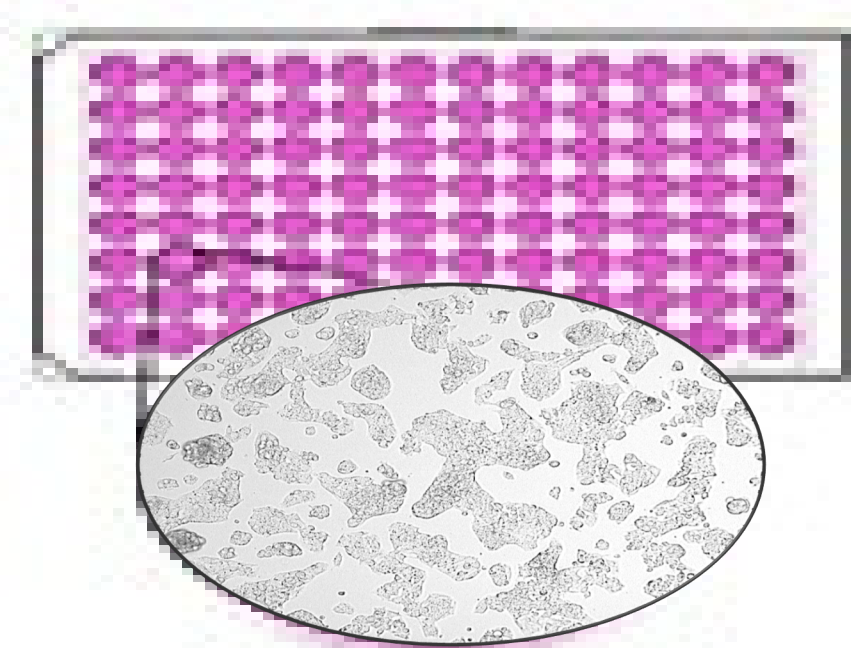
¹Precise Systems, Lexington Park, MD. ²U.S. Army Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD.

Abstract

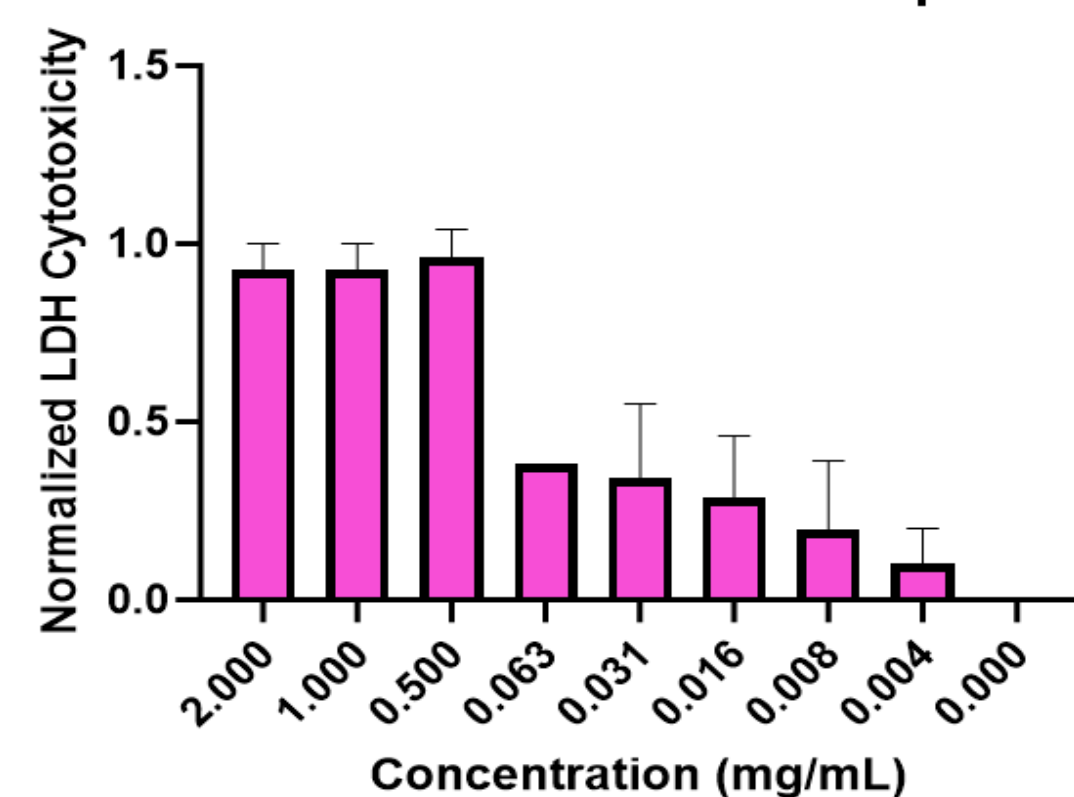
Human history contains many examples of combining chemical compounds to produce new and improved tools for our survival i.e. medicine, dyes, soaps, and metal alloys. The discovery of novel chemical mixtures has continued to modern times with beneficial drug combinations to combat cancer and deleterious combinations used for military or law enforcement purposes. Mixtures of chemical compounds and their combined effects on the human body present an extraordinarily complex problem that requires a reliable characterization method. Typically, the chemical compounds being mixed will manifest similar effects in the human body i.e. cytotoxicity, sedation, etc. The method of characterization for mixtures needs to address whether the two chemical compounds, acting together produce a combined effect that is consistent with their individual potencies, termed additive, an effect greater than additive, termed synergistic or an effect that is less than additive, termed sub-additive. There have been many methods developed to determine if a drug combination departs from additivity however the Loewe methodologies graphical representation of an isobole is the original method. For this project an isobole was established for a well-known drug combination that causes hepatotoxicity, acetaminophen and amoxicillin with clavulanate, to build the foundation for the characterization of the innumerable potential chemical combinations that present a threat to the warfighter.

Methods

HepG2 human liver carcinoma cells were seeded at 45,000 cells per well in a 96-well plate then dosed for 24-hours with a well-known liver toxicant in media supernatant collected and used to run LDH cytotoxicity assay.

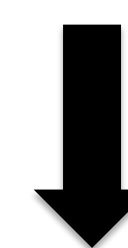
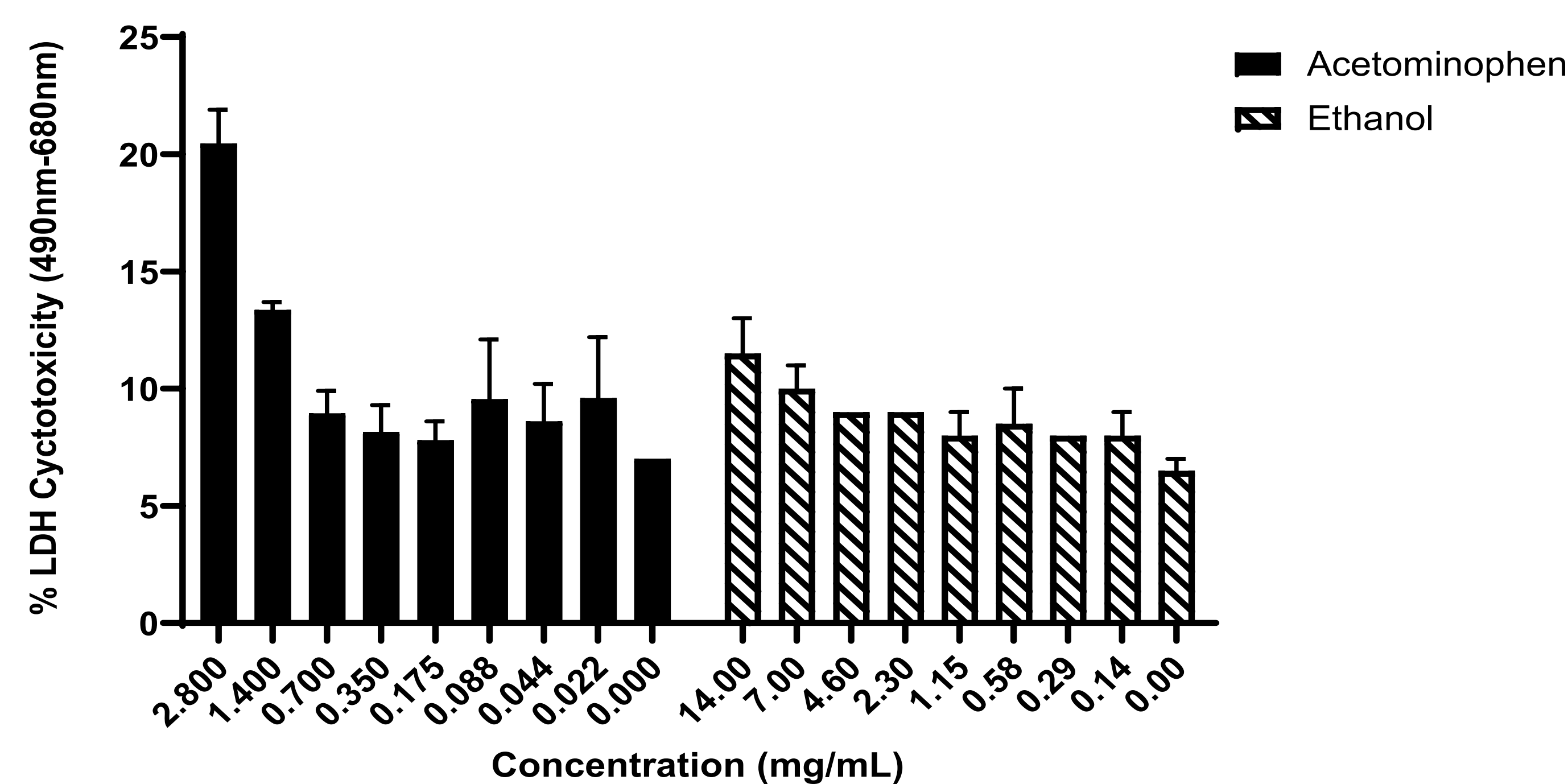


Amoxicillin Clavulanate Dose-Response in HepG2

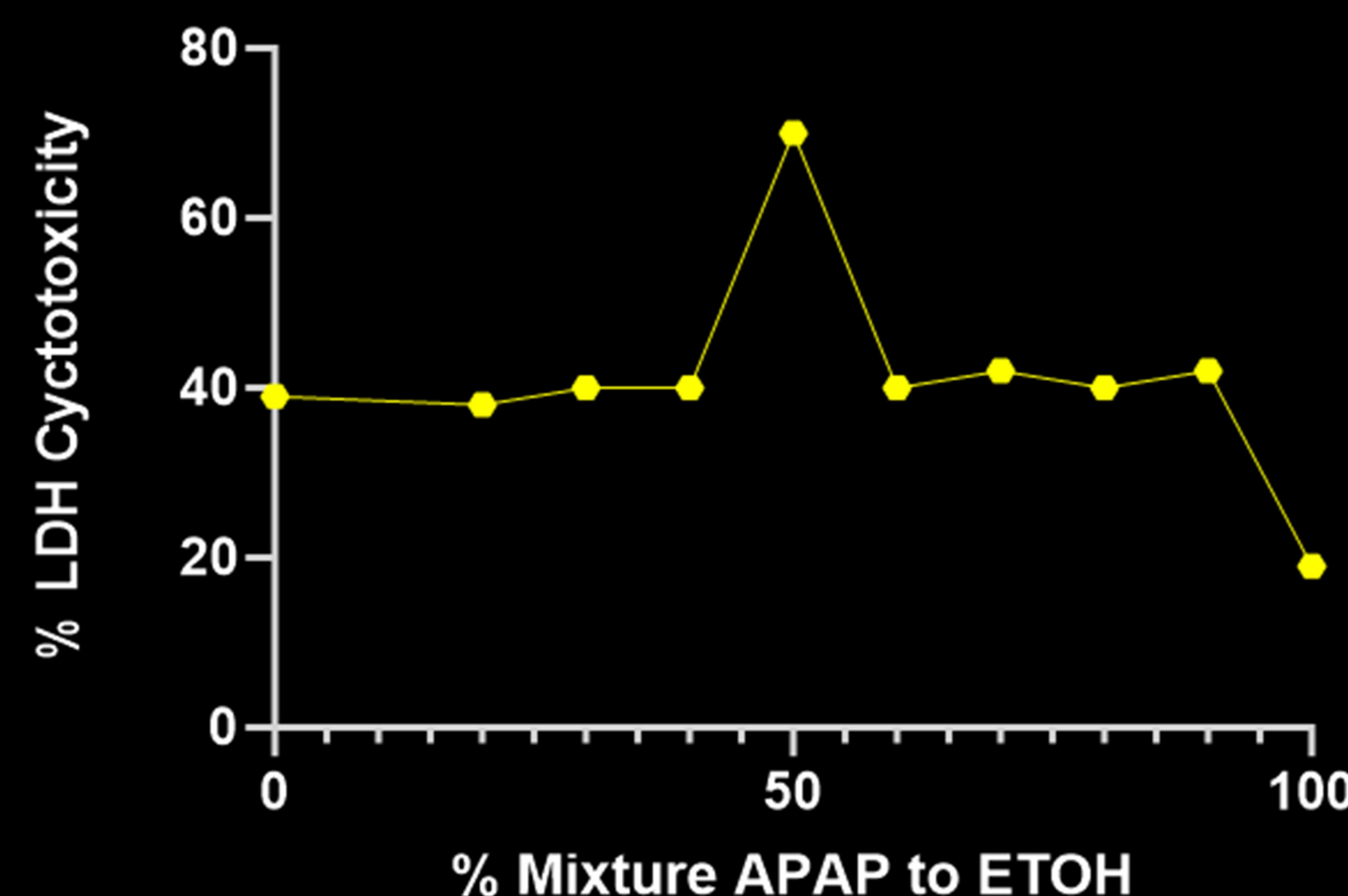


Mixtures

Dose-Response Cytotoxicity with Acetaminophen and Ethanol



Combination of Acetaminophen and Ethanol



Building the Isobole

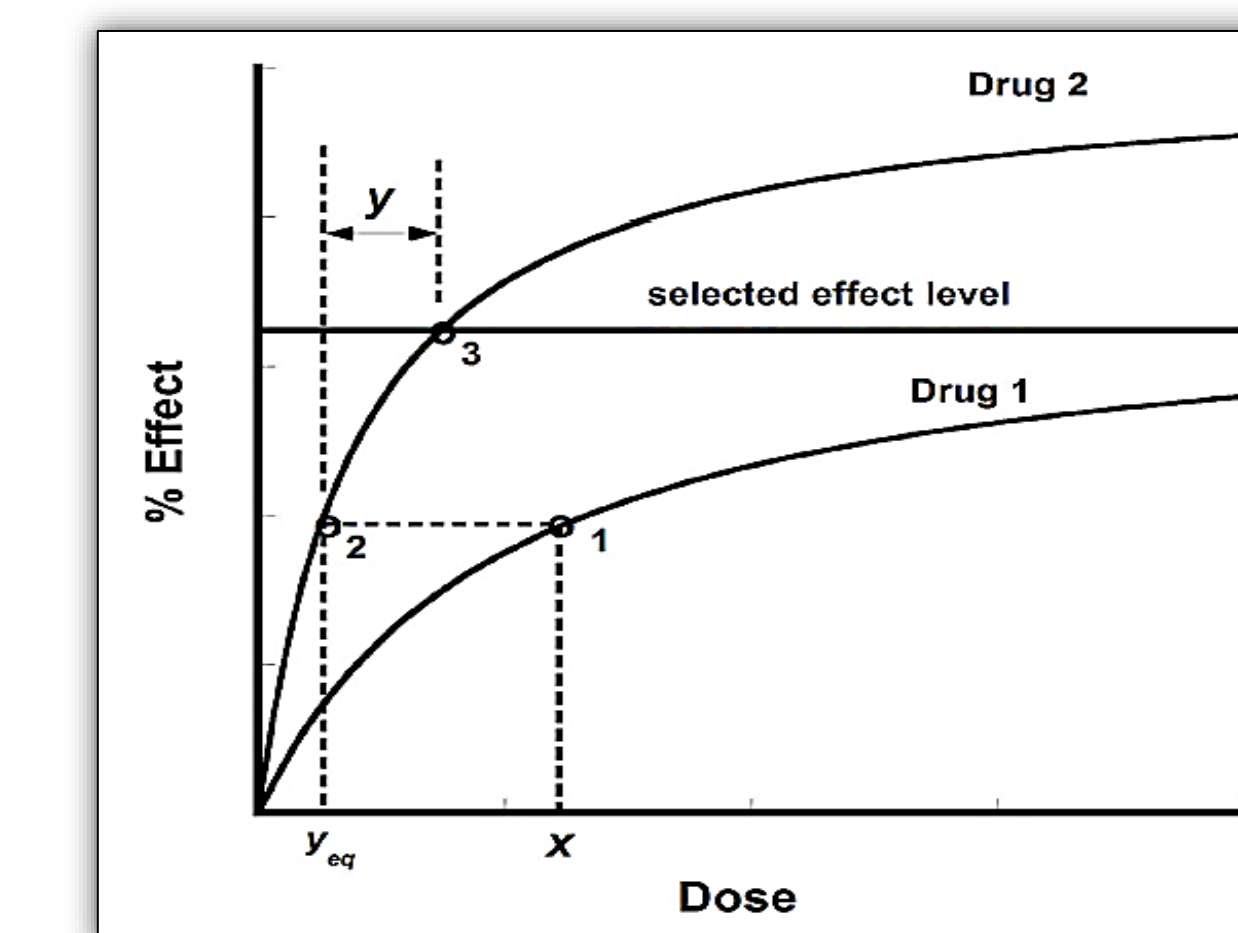
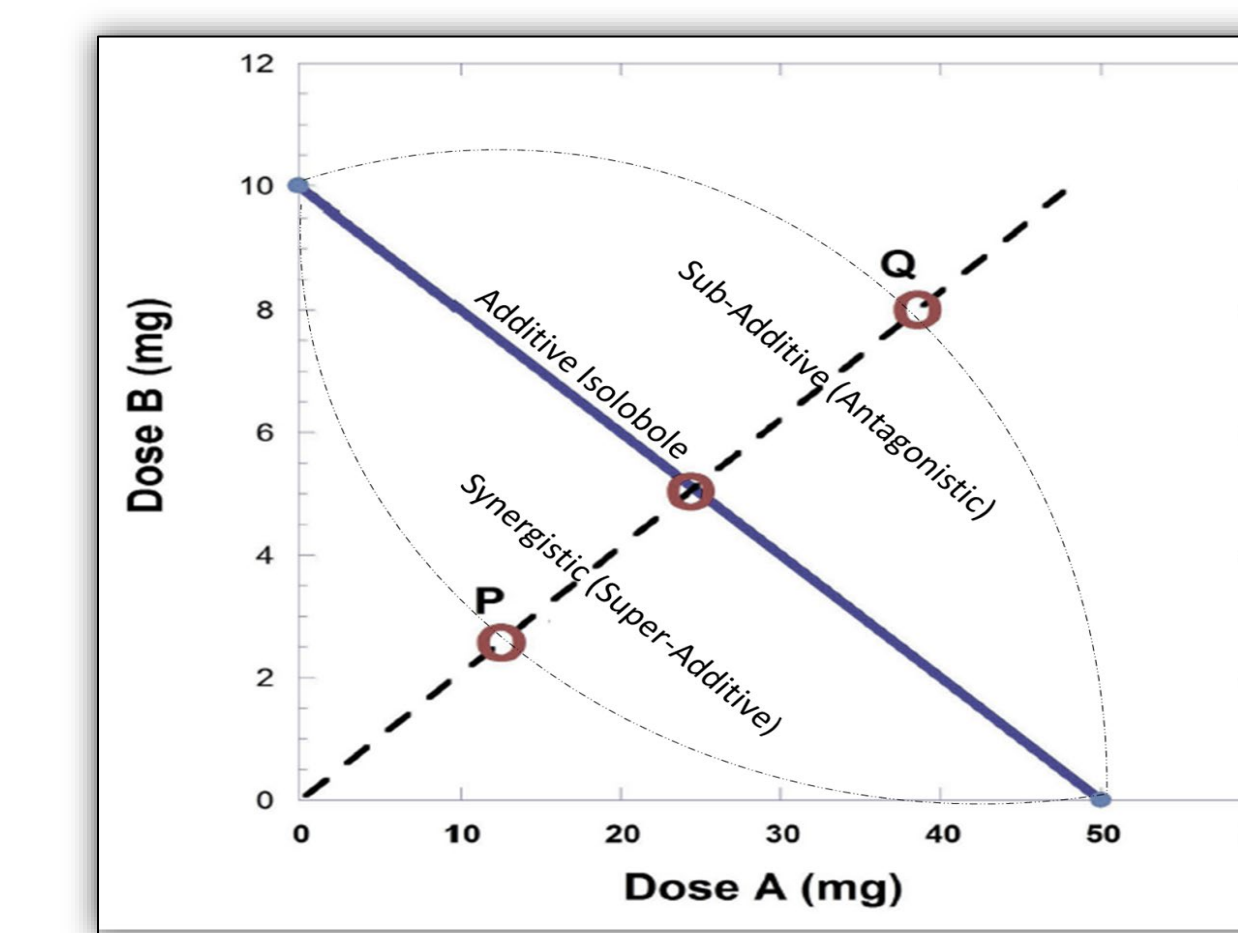
Equation 1: Generating Mixture Dose to Yield 50% Response

$$\frac{b}{B_{50}} + \frac{a}{A_{50}} = 1$$

Equation 2: Plotting the Dose-Effect Curve

$$E = E_{\max} D / (D + C)$$

Isobologram



Conclusion/Next Steps

At 1.4 mg/mL acetaminophen and 14 mg/mL ethanol (both produce 13% hepatotoxicity individually) combined showed synergistic effects at 50% combination with 70% cytotoxicity. An isobole will be built to exhibit the characterization of these chemical combinations and determine at what concentrations at 50% mixture will there an additive effect. Additionally, amoxicillin clavulanate will continue to be investigated as it has been tested with acetaminophen and exhibited hepatocellular toxicity.

References :

Tallarida R.J. Drug Combinations: Tests and Analysis with Isoboles. Curr Protoc Pharmacol. 2016 Mar 18;72:9.19.1-9.19.19. doi: 10.1002/0471141755.ph0919s72. PMID: 26995550; PMCID: PMC4839184

Acknowledgements: I would like to thank DTRA-CBS, Sara Peacock & Kristen Fulbright, for their support in funding this through project funding number CB10705.



DEVCOM CBC @ DTRA CBD S&T Conference

Scan the QR Code to view all of CBC's
2024 DTRA CBD S&T Conference materials
<https://cbc.devcom.army.mil/cbdst-conference/>