An Update from the Department of Defense for the Interagency Coordinating Committee on the Validation of Alternative Methods

Emily Reinke, Ph.D., D.A.B.T
Biologist, Health Effects Division
Toxicology Directorate
U.S. Army Public Health Center

Objective

- Provide an annual update on the Department's activities related to alternative methods for toxicology testing for the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Public Forum

DoD Activities that Support ICCVAM

- Participating in ICCVAM Acute Toxicity Working Group; In Vitro In Vivo Extrapolation (IVIVE) Working Group; Ocular and Dermal Irritation Working Group; Read Across Working Group; Reproductive and Developmental Working Group; Skin Sensitization Working Group

- Tri-Services Toxicology Consortium (TSTC)
  - Representatives from relevant DoD organizations
  - Share knowledge and ideas, collaborate on projects, and implement best practices

- One Health Initiative
  - Facilitates communication and collaboration across disciplines where the health of humans, animals, and the environment intersect

- Alternative animal models
  - Relative replacements of one species for another – i.e. - selecting species with lower neurophysiological development, when feasible

Upcoming Activities

- Emerging Toxicological Approaches in Rapid Chemical Hazard Assessment: Technical Interchange and Roadmap Development Workshop

DOD Programs that Support ICCVAM

- U.S. Army Corp of Engineers: Engineer Research and Development Center
- U.S. Army: Edgewood Chemical Biological Center (ECBC)
- U.S. Army Medical Institute for Chemical Defense (USAMRICD)
- U.S. Army Public Health Center (APHC)
- U.S. Air Force: Air Force Research Laboratory (AFRL)
- U.S. Air Force: School of Aerospace Medicine
- U.S. Navy: Naval Medical Research Unit (NAMRU)
- Defense Advanced Research Projects Agency (DARPA)
- Defense Threat Reduction Agency (DTRA)

U.S. Army Corps of Engineers:
Engineer Research and Development Center (ERDC)

- **Toxicity Computational Modeling Efforts**
  - Digital Automated Molecular Screening Library (DAMSL)
    Molecular docking for de novo prediction of molecular initiating events in adverse outcome pathway
  - Deep Learning Quantitative Structure Activity Relationship (QSAR) Models
    PPAR-gamma (human); Estrogen receptor (human); Others in development
  - Autoencoder Predicting Estrogenic Chemical Substances (APECS)
    Burgoon (2017) Computational Toxicology 2: 45-49
  - Frequent Itemset Mining Prediction for Aquatic Toxicology Predictions
    Burgoon (2016) Bulletin Environmental Contam Toxicology 96: 779-83

- **Synthetic Biology- Developing focused support for the Environmental Impact Assessment of Synthetic Biology**

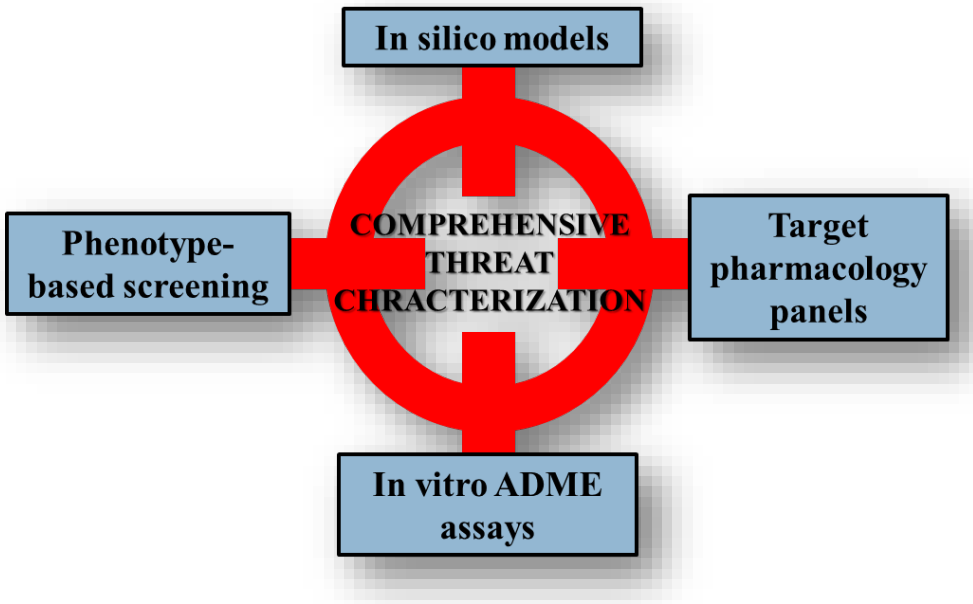
- **IVIVE**
  - Development of a iPSC liver hepatocyte model to develop oral RfDs based on specific endpoints in liver cells (steatosis)
  - Proof of concept for further work

US Army: Edgewood Chemical Biological Center (ECBC) Predictive Toxicology

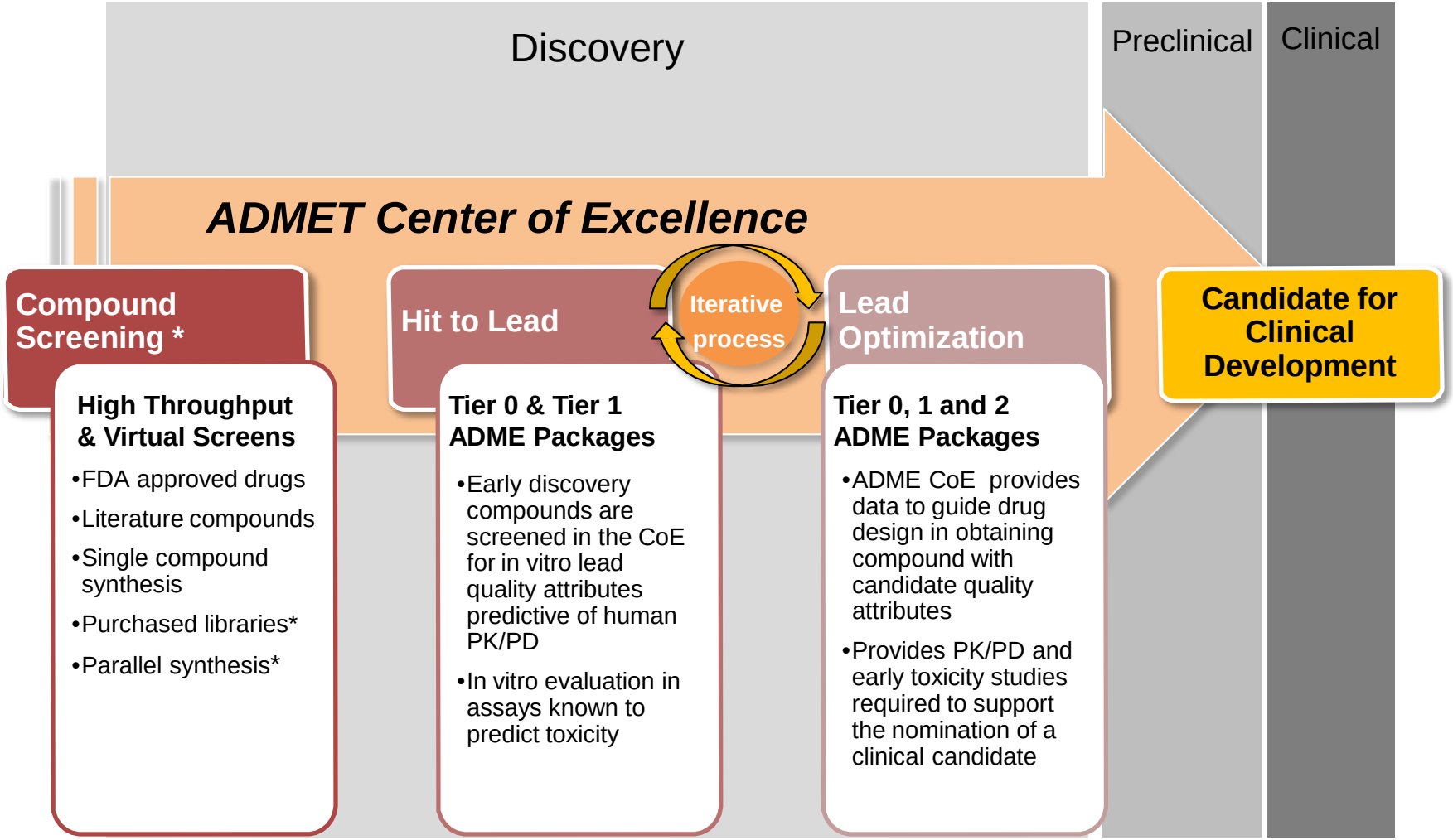
Objective: To develop a predictive screening toolbox for threat agent compounds and to rapidly characterize high priority COIs using next-generation toxicity screening methods.

Description of Effort:
Establish computational and high-throughput approaches to characterize human relevant toxicity of identified threat agents. These methods will include:

1) ***In silico* models** for receptor target prediction and ADME properties,
2) **Phenotype-based threat agent screening** in zebrafish to determine cardiotoxicity and behavioral profiling (e.g. phenomics),
3) **Target pharmacology panels** against G-protein coupled receptors and ligand/voltage gated ion channels
4) ***In vitro* ADME assays** to predict protein binding, metabolic stability and blood-brain barrier permeability.



USAMRICD: Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Center of Excellence



* Includes High Throughput Screening, Virtual Screens, rational drug design and/or pharmacology screens

The ADMET Center is supported by DTRA-JSTO

U.S. Air Force: Air Force Research Laboratory (AFRL)

- **Overview:** Addressing Proactive and Responsive Toxicology Assessments

Goal: To develop enhanced in vitro models that correlate with in vivo findings and have increased predictive power for toxicity assessments

To transform in vitro cultures

To be more representative of an organ system

LIVER

CHARACTERISTICS OF AN ORGAN
- 3-Dimensional
- Multi-cellular
- Physiological fluids
- Dynamic environment

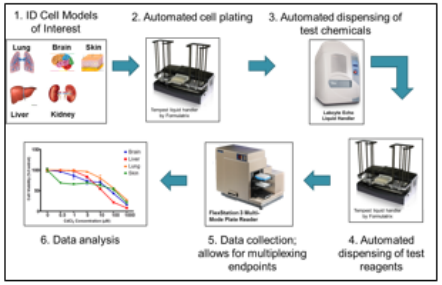
Which will lead to augmented in vitro applicability:

In silico modeling

Increased Correlation

Development of New Technologies for AF missions

Tier 1: AF Specific In Vitro Screening to Rapidly Assess Toxicity



ENDPOINTS

Cell viability, cytotoxicity, apoptosis, oxidative stress, mitochondrial membrane potential, DNA damage, cell metabolism

GOAL: Rapidly ID chemicals that would require further toxicity testing

Dose-dependent toxicity in target organ models

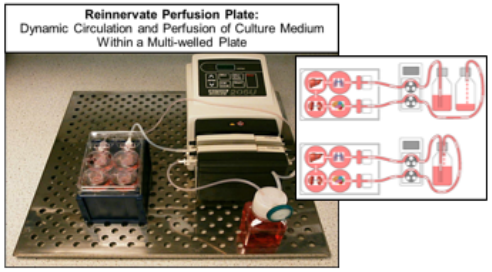
Insight into required studies for OEL

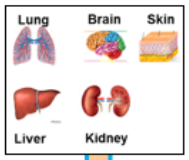
No observable toxicity

Tier 2: Mechanistic Toxicity Studies

3D Dynamic Multi-Organ Exposures

Reinnervate Perfusion Plate: Dynamic Circulation and Perfusion of Culture Medium Within a Multi-welled Plate





Use specific dosages/in vitro models from Tier 1

Endpoints: Physiological Changes, Gene Expression

GOAL: ID chemicals and select dosages for Tier 3 Assessments

Tier 3: In Vivo Studies

Acute & Chronic In vivo Studies

Select Dose Response for ADME Toxicity Evaluation

GOAL: Determine Occupational Exposure Limits & Safety Precautions

A safe work environment for our Airmen is determined

U.S. Air Force: School of Aerospace Medicine

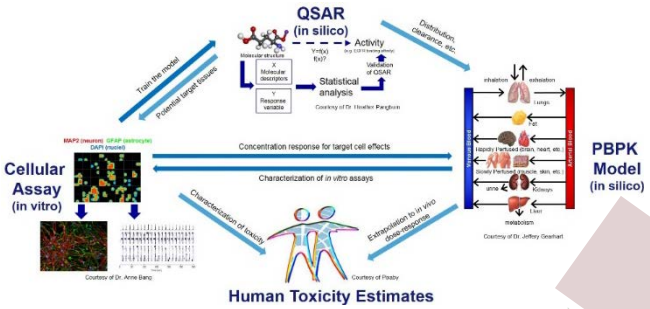
Biological Systems Modeling
- Cockpit exposure reconstruction via Physiologically Based Pharmacokinetic (PBPK) Models
- Predicting neuronal targets of AF compounds using Quantitative Structure Activity Relationship (QSAR) Modeling
- Incorporating operational features of flight into PBPK
- Application of High Throughput ToxicoKinetic (HTTK) for rapid operational response

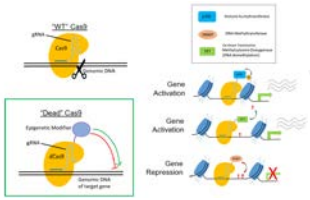
In Vitro Data Production
- Human induced pluripotent stem cell neuronal, cardiomyocyte and hepatocyte models with phenotypic and physiological measures
- Personalized approach/genetic influence on toxin susceptibility
- Versatile analytics pipeline, for data management, feature selection/scoring, and genotype-phenotype analysis

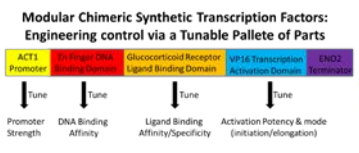
In Vivo Verification
- Assessment of Respiratory and Cognitive Effects from Acute Low Level Exposures to Environmental Chemicals Under Operational Features of Flight

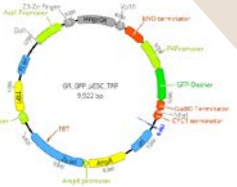
Epigenetics
- miRNA response exposure classifier
- Epigenetic markers of AF specific exposures
- Synthetic biology tools to edit epigenetic changes

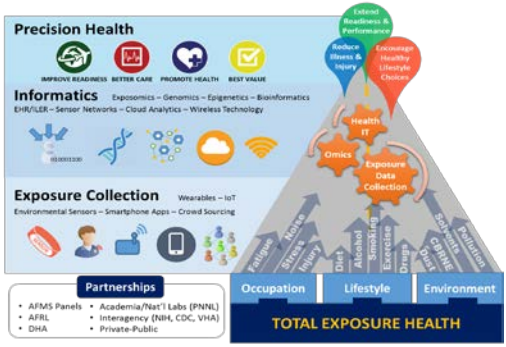
Synthetic Biology
- Synthetic biology tools to edit epigenetic changes
- Applying synthetic biology tools to modulate stress











DARPA: Rapid Threat Assessment (RTA) Program

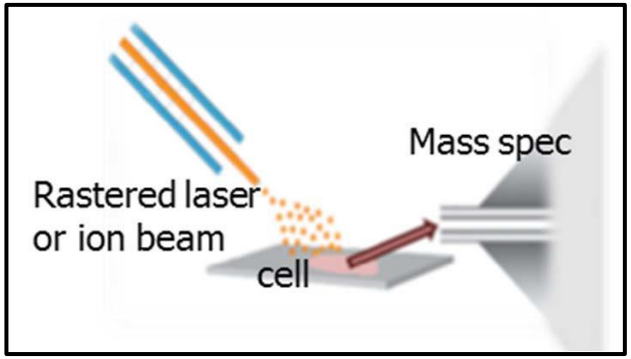
Problem: It takes many years to figure out how threats or drugs work

RTA Goal: In 30 days, figure out how a chemical, threat agent, drug, or biologic exerts its effects on biological systems

Potential Impact to ICCVAM: Significant decrease in time to understand mechanism of action, decrease in need for animal studies throughout process

Inspiration: New rapid mass spec imaging method

Mass Spectrometry Imaging



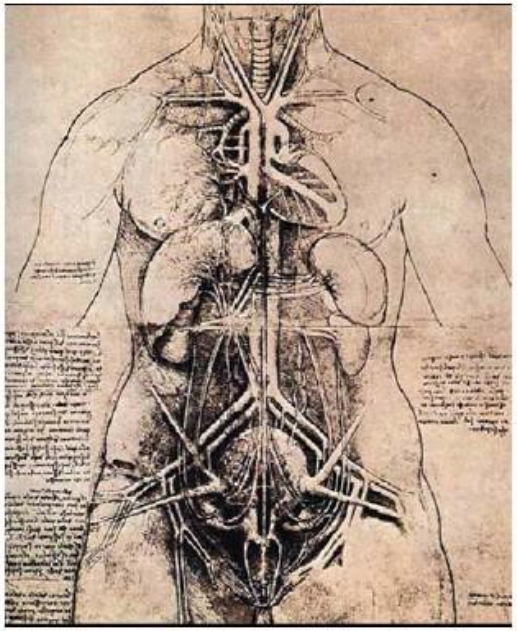
Status:

- Five year program, approx. 12 months remaining
- Three main performers – GWU, UC Boulder, Vanderbilt University
  - Proof of Concept Demonstration: Detected and identified the canonical mechanism of action of Bendamustine, a nitrogen mustard used as a chemotherapeutic, in 30 days.

DARPA Microphysiological Systems Program

Develop an *in vitro* platform that uses human tissues to evaluate the efficacy and toxicity of medical countermeasures.

In other words, build a human-on-a-chip:



- All ten human physiological systems will be functionally represented by human tissue constructs:
  - Circulatory
  - Endocrine
  - Gastrointestinal
  - Immune
  - Integumentary
  - Musculoskeletal
  - Nervous
  - Reproductive
  - Respiratory
  - Urinary
- Tissue viability for at least 4 weeks.
- Commercialization plan.

Status: Five year program, in middle of 5th year

MIT-Completing platform development with 10 interacting organ systems (lung, gut, liver, pancreas, kidney, muscle, heart, brain, endometrium, skin)

Harvard/Wyss-Completing development of organ chips (lung alveolus, lung airway, heart, kidney proximal tubule, kidney glomerulus, gut, liver, blood brain barrier, bone marrow placenta)

DTRA: Ex vivo Countermeasure Evaluation and Licensure (XCEL) Program

XCEL: Development of Integrated-multi-organs-on-a-chip platforms to revolutionize assessment and evaluation of threat agents and medical countermeasures for chemical and biological defense and beyond

ATHENA – Los Alamos National Laboratory	ECHO – Wake Forest Institute of Regenerative Medicine
Liver and Cardiac Organoids (working on lung and kidney)	Liver and Cardiac Organoids
Modular microfluidics	Functional Assessment (Reactivity) and long-term viability
Universal Media Development	Bioprinting – augments function and controls spatial distribution
Ion Mobility – Mass Spectroscopy analysis of analytes/metabolites	Modular microfluidic system with rejuvenating in-line sensors

DTRA: Computational Rapid Identification & Scientific Threat AnaLysis (CRISTAL)

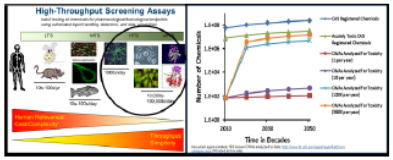
Objective: Provide a systematic capability that uses predictive characterization tools to augment and refine toxicology assessments in order to provide a more rapid hazard operational assessment and estimate of human toxicity on compounds of interest.

Limited laboratory validation of physicochemical property prediction

Molecular structure and Physicochemical properties
- Structure-activity relationship (SAR)
- Quantitative structure-activity relationship (QSAR)
- Quantitative Structure-property relationship (QSPR)

ADME (*in vitro*) Assay validation of predictions

ADME Predictions based on QSAR modeling
- Mechanism of action prediction
- Identify potential nodes of convergence with known chemicals



***In vivo* toxicity validation, select**

Acute toxicity predictions, 1 animal model
- Dermal
- Inhalational
- Neurotoxicity
- Cytotoxicity

FY21: 10 compounds per year, ~$7B
Current: 1 compound per year, ~$7B
Fewer animals: 1 model versus 3

JSTO shall develop integrated computational and in vitro predictive models to assist in identifying those current and emerging chemical biochemical materials that have the potential as CB threats of concern to the force. (FY15-19 PIP)

Summary

- In partnership with other Federal agencies, academia, and industry, the Department of Defense remains committed to refine, replace, and reduce reliance on animal models when scientifically valid

- Dtic.Belvoir.pm.list.animalreg@mail.mil