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

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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)
• **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

• **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
**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.
The ADMET Center of Excellence is supported by DTRA-JSTO. It provides data to guide the design of compounds with candidate quality attributes, which are required to support the nomination of a clinical candidate.

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

The ADMET Center is supported by DTRA-JSTO.
• **Overview:** Addressing Proactive and Responsive Toxicology Assessments

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

- **Characteristics of an Organ**
  - 3Dimensional
  - Multicellular
  - Physiological fluid
  - Dynamic environment

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

- **Goal:** Rapidly ID chemicals that would require further toxicity testing

- **Insight into required studies for OEL**

- **No observable toxicity**

- **A safe work environment for our Airmen is determined**

**Tier 2: Mechanistic Toxicity Studies**

- **3D Dynamic Multi-Organ Exposures**
  - Reinervated Perfusion Plate: Dynamic Circulation and Perfusion of Culture Medium Within a Multi-welled Plate

- **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
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
- mRNA 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

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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

**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.
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)
**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

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

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

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

ADME (in vitro) Assay validation of predictions

In vitro 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

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