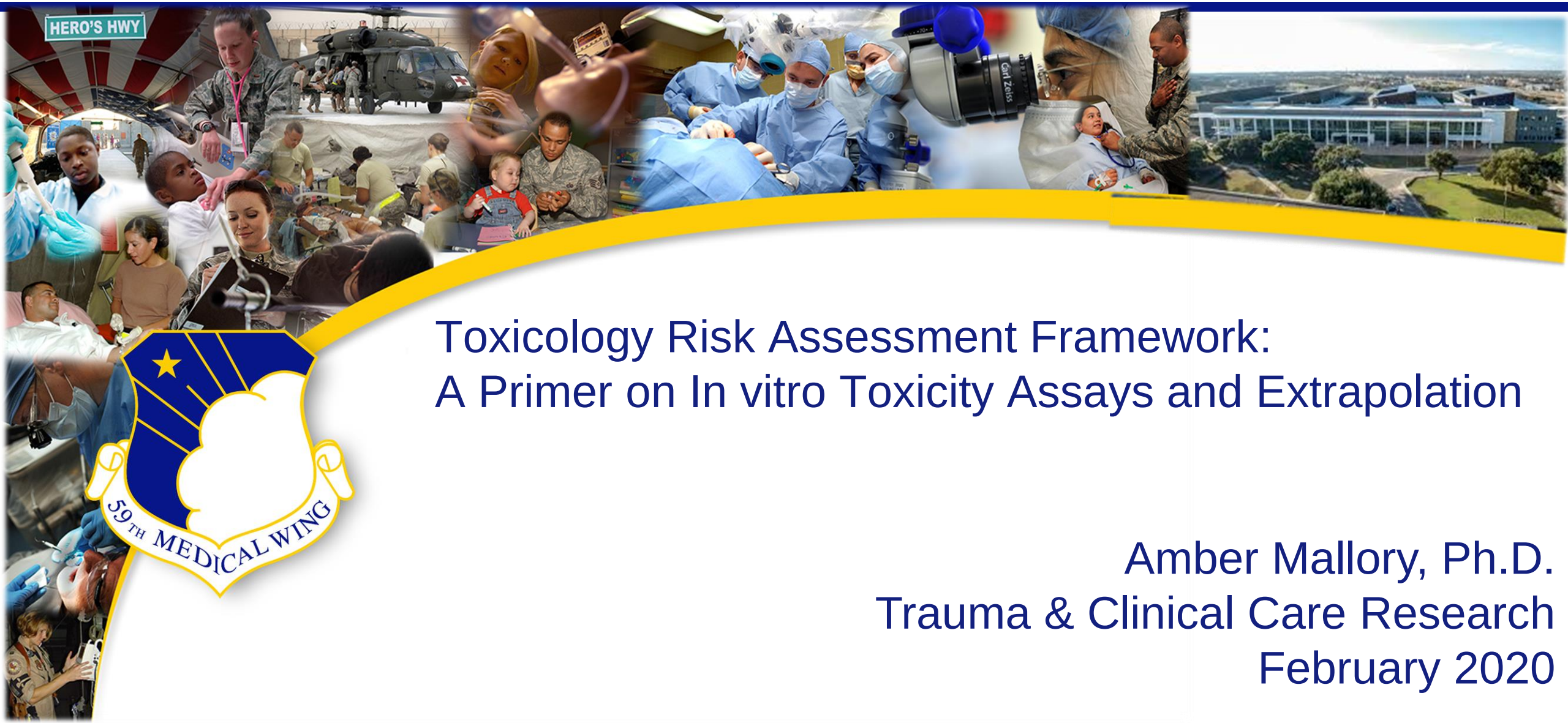
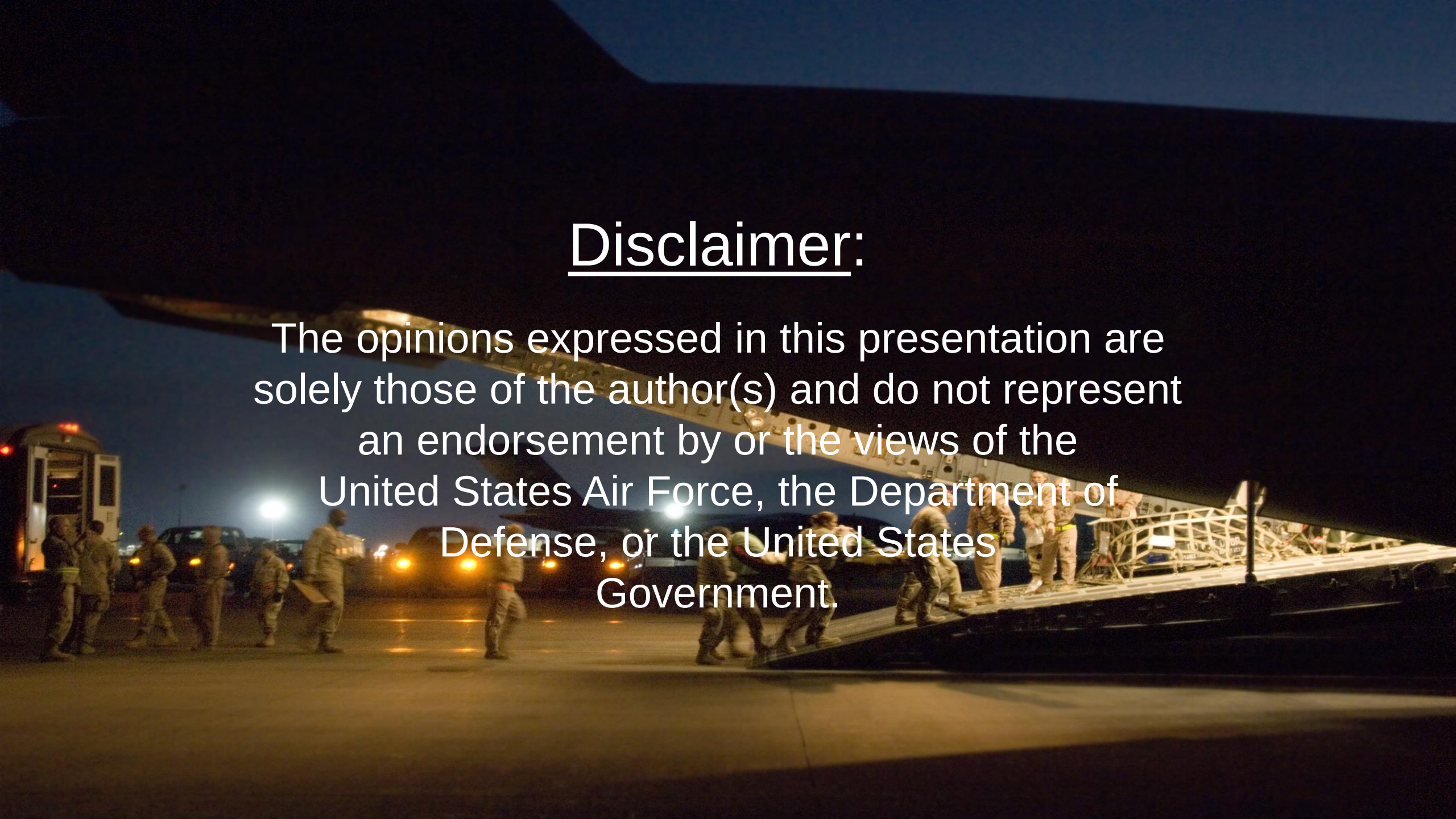
59th Medical Wing



Toxicology Risk Assessment Framework:
A Primer on In vitro Toxicity Assays and Extrapolation

Amber Mallory, Ph.D.
Trauma & Clinical Care Research
February 2020



Disclaimer:

The opinions expressed in this presentation are solely those of the author(s) and do not represent an endorsement by or the views of the United States Air Force, the Department of Defense, or the United States Government.

OUTLINE

- 59th Medical Wing Overview
  - Defense Health Agency and Military Services
  - Science and Technology Office
    - Research Areas and Capabilities
    - Joint Austere Medicine
    - Clinical Resuscitation, Emergency Science, Triage & Toxicology Program
- Intro to Toxicology
  - Brief History
  - Basic Concepts
  - Branches of Toxicology
- Predictive Toxicology
  - Research Strategies
  - Models
  - Proposed screening scheme

Military Medicine: A Shifting Landscape

World Events, Global Economy, Tech Innovations, NDAA, DHA, Service Policies, Mission Needs…

Growing Military Researchers
- Live (clinically-relevant) Simulations/Models of advanced LSI, surgical techniques
- Aligning research requirements for faculty, staff, resident scholarly activities
- Robusting STEM/Research Education

Changing Battlespace
- Advance POI and ERC of critically-ill or injured patient clinical outcomes, survival, and health/Return to Duty
- Readiness and clinical competence in en route care providers
- Disaster medicine best practice, prep & response (research & training)
- Directed Energy (diagnose & treat)

In-Garrison Care Advancements
- Warfighter, family, beneficiaries
- Expertise for conducting & integrating advances in medical research & tech
- Evidence Based Practice, Quality & Patient Safety

Translating Military Relevant Clinical Research
- Real World cost of healthcare, Personnel
- Resource Impacts/constraints: Energy, Water
- Quality Process Validation for Medical Devices and optimizing CPGs
- Monitor and measure parameters in patients
- Novel treatments to prevent wound infections and other post-injury complications

Military Health System
- **OASD(HA) - Responsible for effective execution of the DoD medical mission, providing and maintaining readiness for medical services and support to members of the Military Services; their families… and others entitled to or eligible for DoD medical care and benefits, including those under TRICARE.**

Defense Health Agency
- **Vision: A Combat Support Agency, leads the Military Health System integrated system of readiness and health to deliver the Quadruple Aim—increased readiness, better health, better care, and lower cost.**
- **Mission: Unified and Ready.**

AF/SG
- **Vision: Air Force Warrior Medics… Mission-Focused, Excellence Driven.**
- **Mission: Ensure medically fit forces, provide expeditionary medics and deliver Trusted Care to all we serve.**

AETC Commander
- **"*Help us invent the future …*one idea at a time!"**

59 MDW Commander
- **Vision: Exemplary Care, Global Response.**
- **Mission: Developing Warrior Medics Through Patient-Centered Care.**

Critical National and Local Resource

- Strategic Asset
  - "Home of Military Medicine"—SA Chamber of Commerce assessed $4B direct, $1B indirect economic impact
- 12 MTFs/$1.2B Budget /12,000 staff/250,000 beneficiaries
- 37 GME programs--600 residents, 22 GHSE programs--78 residents
- Contingency/Humanitarian response -- Teams on call 24/7 and ~150 Service Members deployed
- Significant Medical Innovation, Research, Education, Training
- Brook Army Medical Center
  - DoD's most productive inpatient facility
  - DoD's only CONUS Level 1 Trauma Center
  - DoD's only Bone Marrow Transplant Unit
  - DoD's only Burn Center
  - Center for the Intrepid
- Wilford Hall Ambulatory Surgical Center and Clinics
  - DoD's largest outpatient facility
  - DoD's largest Blood Donor Center
- DoD's largest centralized appointment/referral management system

Patients First, Partners Always

https://www.health.mil/Military-Health-Topics/Access-Cost-Quality-and-Safety/Access-to-Healthcare/Multi-Service-Markets/San-Antonio-Military-Health-System

59th Medical Wing Science & Technology

Vision: ***Grow Medical Leaders, Drive Innovations in Patient Care and Readiness***

Mission: ***Conduct clinical studies and translational research and apply knowledge gained to enhance performance, protect the force, and advance medical care and capabilities across the global health system***

Readiness, Healthcare, Education, Training, and Research

59th MEDICAL WING

DEFENSE HEALTH AGENCY · DEPARTMENT OF DEFENSE · PRO CURA MILITIS

SAMHS
San Antonio Military Health System

Chief Scientist
Science and Technology
Providing operational capability through…

Warrior Medics • Mission Ready • Patient Focused

- ***Lead & Support Research***
- ***Advance Modernization Efforts***
- ***Foster and Build Collaborations***
- ***Address End User Needs***
- ***Ensure Scientific Excellence and Programmatic Relevance***
- ***Wing, SAUSHEC, AFMS, LAF, ASD/OSD, Joint Cmts / S&T, Adv Dev liaisons***

https://www.59mdw.af.mil/Units/Chief-Scientist-ST/

JBSA Main Office: (210) 292-2097

Predominant Research Areas

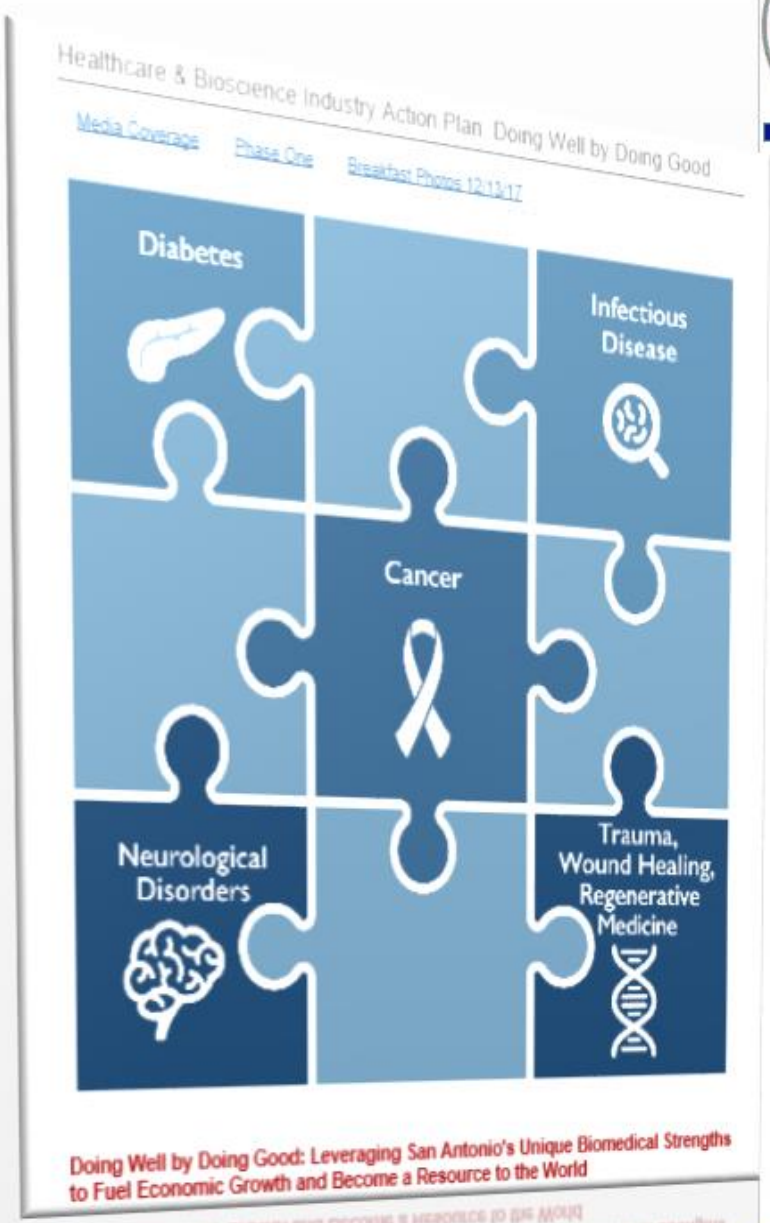
- Trauma Critical Care-En Route Care
  - Trauma, Hemostasis & Resuscitation/Point of Injury Care
  - Immunomodulation of Trauma
  - Regenerative & Restorative Medicine
  - Neuroradiology
- Infectious Diseases
  - Preventative Measures, Rapid Detection & Surveillance
  - Vector-borne and Zoonotic Diseases
- Omics
  - Disease Genetics, Genomics, Proteomics, and Bioinformatics
  - Precision Medicine
- Healthcare Research & Applications
  - Apps and Virtual Healthcare, Simulation Training
- New Focus Areas/Partnerships—Research Enablers
  - Autonomous Systems, AI, Sensors, Synthetic Biology

Major Programs/Capabilities

- **Clinical Investigations and Research Support (CIRS)**
  - Clinical Investigations Program, Readiness and Certification Training
- **Nursing Research / Center of Clinical Inquiry**
  - Chief Nurses consultation/24 MTFs; Research & Evidence Based Practice
- **Dental Education, Research and Consultation**
  - USAF Post Graduate Dental School and Clinics, JBSA-Lackland
  - Dental Research and Consultation Service, JBSA-Fort Sam Houston
- **Integrated Clinical Medicine and Center for Molecular Detection**
  - Rapid Pathogen Detection/Analysis, Trainee Healthcare, Precision Medicine Research
- **En Route Care Research Center**
  - Co-located at USA Institute of Surgical Research (USAISR); AE-ERC research in Collaboration with other Organizations
- **Clinical Resuscitation, Emergency Science & Toxicology Program**
  - Animal research conducted at CIRS, JBSA-Lackland
  - Emergency Medicine Residents participate to fulfill graduation requirements
- **Trauma and Regenerative Medicine Research**
  - Clinical Investigations, JBSA-Lackland; USAISR/BHT & Tri-Service Research Laboratory (TSRL), JBSA Fort Sam Houston

San Antonio Military Medical Research

Strong DoD-Federal-Academia-Industry Synergy



San Antonio Military Medical Center

Audie L. Murphy VA Memorial Hospital

AF Post-Graduate Dental School & Clinic

Center for the Intrepid

Battlefield Health & Trauma Research Institute

Tri-Service Research Laboratory NAMRU/SA, 711th HPW

Wilford Hall Ambulatory Surgical Center

Center for Molecular Detection

Clinical Research Support

UT Health Science Center San Antonio

UT San Antonio

BioMed SA

"San Antonio. City of Science and Health."

Theater of Operations ↔ Garrison Care

Joint Austere Medicine Research Portfolio

Role 1
Point of Injury

Role 2
Mobile Field
Surgical
Team/EMEDS

Role 3
EMEDS +25
AF Theater
Hospital

Role 4
OCONUS
Definitive Care

Role 5
US-based MTF
Full Range/
Definitive Care

Post-Acute Care
Dept of Veteran
Affairs

VA Health

Joint Integrative Clinical Medicine Research Portfolio

Performing Research and Providing Deliverables to Address Joint Medical Priorities across the Continuum of Care

Joint Austere Medicine (JAM)

Mission:

Investigate innovative mechanisms to increase clinician skills and practices to utilize early detection methods, latest treatment regimens, and recovery from communicable illness and non-battlefield injuries to improve return to duty turnaround times.

Objectives:

- **Provide access to and understanding of recent surgical devices, and techniques to improve patient care and enhance cost-savings and return to fight**
- **Conduct research to identify the most efficacious learning architecture to improve clinical readiness and ensure skills proficiency and sustainment**
- **Investigate resuscitation, stabilization, triage, and treatment modalities and their applicability in austere environments**
- **Support better health initiatives and improve return to duty rates for non-battlefield related injuries occurring in austere training and deployed environments**
- **Test, evaluate, and realize progressive and autonomous approaches to assist clinicians with patient care in austere environments**

JAM Portfolio Research Timeline

Near-Term (1+ yr)

- Material strength and integrity performance of medical devices after repeated disinfectant exposure;
- Initial stability results of common medications stored at extreme temperatures;
- Partner in early screening and development of potential therapies for use in austere environments
- Strengthen ties with DoD agencies to provide clinical subject matter expertise on size, weight, ruggedization requirements at the early stages of technology and device development for water sterilization and pathogen identification;

Mid-Term (3-7 yrs)

- Standardize practice and procedures of federally-regulated disinfectants for use in austere conditions;
- Conduct clinician end-user test, evaluation, and interpretation of biomonitoring data to inform utility of tech/apps as an aid to operational decision making
- Conduct preclinical research investigating impacts of diet, hydration and fatigue after food or water-borne illness;
- Participate in clinical trials and human subjects research for treatment of non-battlefield injuries occurring in multi-domain environments, including off label use of therapeutics;

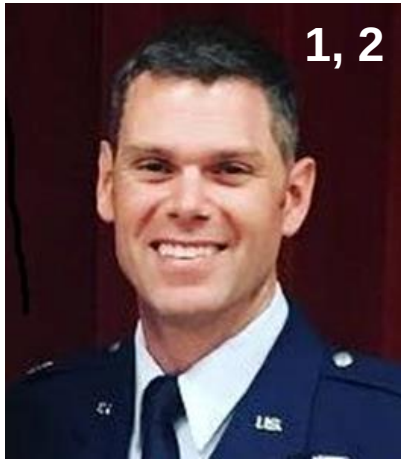
Far-Term (7+ yrs)

- Fully implement recognized methods and technologies to minimize cold chain storage requirements of medications and increase shelf-life of therapeutics;
- Complete integration of biomonitors and biosensors into warfighter readiness training modules for health and performance prediction;
- Clinician utilization, confidence, and acceptance of predictive indicators to adequately and reliable enable warfighter return to mission in austere settings

JAM Programs & Medical Directors

Programs

1. En Route Care Research Center (ECRC)
2. Clinical Resuscitation, Emergency Science, Triage & Toxicology (CREST^2)
3. Frontline Illness, Exposure, & Recovery Care Efforts (FIERCE)
4. Surgical & Technological Advancements for Traumatic Injuries in Combat (STATIC)
5. Improvements in Neurological, Sensory, & Perceptible Research (INSPR)



Lt Col Joseph Maddry, MD

MAJ Steven Schauer, DO

Lt Col Valerie Sams, MD

Col Erik Weitzel, MD

Joint Austere Medicine

Program	Vision	Military Relevance	Translation
En Route Care Research Center (ECRC)	Improve casualty care and survival during evacuation and transport maneuvers.	AFMS 2015 ICL AFMS 2017 ICL 2016 AFAE RDD 2018 Nat. Def. Strat	Better patient care & outcomes; improved cost efficiency for patient evac
Clinical Resuscitation, Emergency Science, Triage & Toxicology (CREST^2)	Research novel learning and clinician training platforms to improve triage and resuscitation strategies for trauma and critical care patients in resource-limited environments.	AFMS 2015 ICL AFMS 2017 ICL 2018 AFEM RDD DoD ICD 2018 Nat. Def. Strat	Recognized & implemented patient care training, guidelines for better care & improved readiness
Frontline Illness, Exposure, & Recovery Care Efforts (FIERCE)	Investigate innovative mechanisms to increase clinician skills and practices to utilize early detection methods, latest treatment regimens, and recovery from communicable illness and non-battlefield injuries to improve return to duty turnaround times.	AFMS 2017 ICL 2016 AFEM RDD DoD ICD 2018 Nat. Def. Strat	Portable & reliable austere diagnostic tools; disinfection SOPs & CPGs; reduced costs & improved return to duty rates
Surgical & Technological Advancements for Traumatic Injury In Combat (STATIC)	Establish clinical practice, training, and methods for integration of smart, cyber, and autonomous strategies using therapeutic and technological interventions for patients experiencing pain, cardiopulmonary, and multi-organ failure.	AFMS 2015 ICL AFMS 2017 ICL 2018 AFEM RDD DoD ICD 2018 Nat. Def. Strat	Manpower multiplier; better medical resource utilization, CPGs, improved patient care & outcomes; increased skill proficiency & sustainment
Improvements in Neurological, Sensory, & Perceptible Research (INSPR)	Enhance clinical skill competency and proficiency assessment and sustainment strategies for diagnosis and acute treatment of trauma to the head, neck, spine, central and peripheral nervous system, and sensory system.	AFMS 2017 ICL 2017 CDEE RDD DoD ICD 2018 Nat. Def. Strat	Dedicated CPGs & SOPs for neuro and sensory injuries DE/non-DE; process implementation→ better response time & care

CREST²

Current projects:

Hydroxocobalamin for Neuroprotection and Survival in a Hemorrhagic Swine Model Traumatic Brain Ischemia POI and ERC Care

Evaluation of Ox66 as an Oxygen Carrier for Treatment of Hemorrhagic Shock

Improving Survival and Lowering Resuscitation Fluid Requirements at Point of Injury and En Route Care by Hanging the Type or Concentration of Free Fatty Acid Bound to Albumin

Comparison of Different Resuscitation Fluids in Volume-Controlled and Uncontrolled Hemorrhage Models over Time in Swine (Sus Scrofa)

A novel shape memory polymer (SMP) foam for hemorrhage control

A novel Compensatory Reserve Measurement (CRM) for resuscitation after hemorrhage

Intranasal Ketamine as an Adjunct to Fentanyl for the Prehospital Treatment of Acute Traumatic Pain

1st assist and non-compressible truncal hemorrhage intervention training for emergency physicians and physician assistants

Comparison of standard left lateral thoracotomy vs. modified bilateral “clam shell” thoracotomy by emergency physicians

National Emergency Airway Registry (PROJECT NEAR)

EMSTruShoC LowDose, HighFrequency, OnSite training to Improve Trauma Field Care

An assessment of the novel, disposable, iView video laryngoscope for far-forward endotracheal intubation

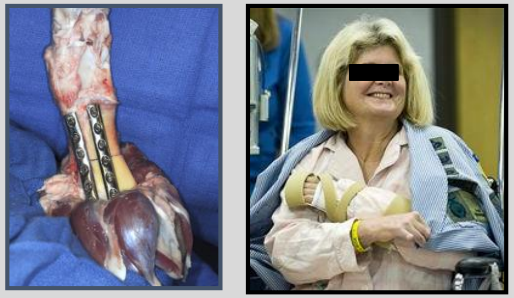
Development of Prototype Soft Epidermal Biosystems with Advanced Sensing Capabilities for Warfighters in Triage Settings

A multi-phase assessment of battlefield airway management devices used by medics

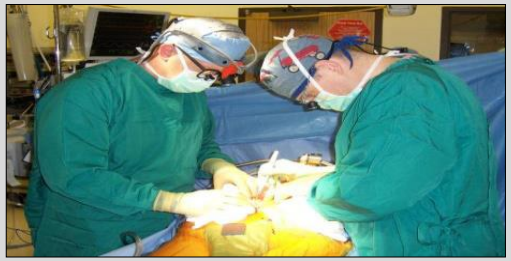
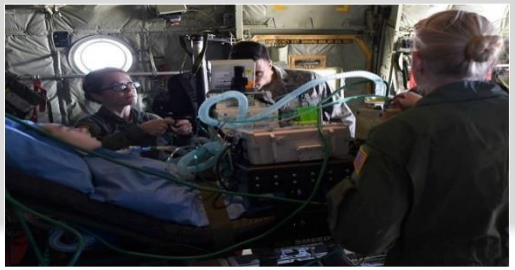
Attenuation of the Red Blood Cell Storage Lesion to Allow Extended Use of Previously Cryopreserved pRBC Units in Austere Environments

vitamin B-12

59MDW/ST Summary

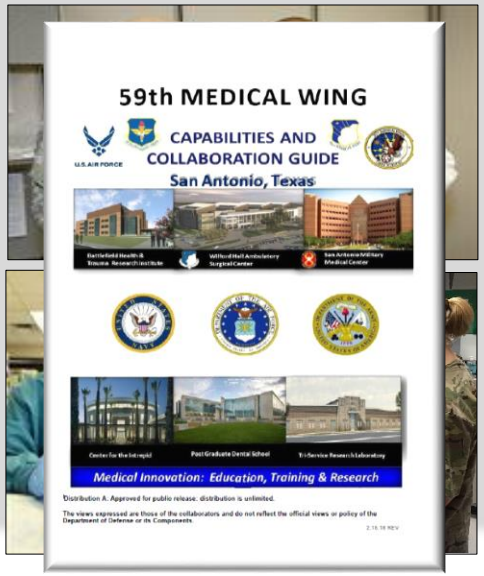




- Lead AF Clinical Research Platform; Largest DoD GME Platform
- Joint DHP Integrated – Programs and Partners (i.e., Across JPCs; USAISR, NAMRU-SA, Others)
- GME/GHSE-RDT&E Synergized, USU-affiliated Programs; largest AF CIF & Lead Translational Research Platform
- Military Readiness, Joint Force and Medical Care Requirements-aligned
- Lead AF eIRB site / implementation, Preparing for “eIACUC”
- First and Only DoD HRPP Accredited Program (AAHRPP)
- Long-standing AAALAC Accreditation with Merit
- Broad & Deep System Capabilities, Clinical Competencies/Collaborations



Grow Medical Leaders, Drive Innovations in Patient Care and Readiness

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Evolution of Toxicology

Brugmansia
(Angel's trumpet)

Peyote

- Shamans- Thousand of years extracting plants for hunting, healing, and use against enemies
  - Tubocurarine and curare on spears and arrow tips; acts as paralytic, adding asphyxiation to the arrow or dart wound
  - Strychnine (*Nux vomica*---nightshade)
- Moses Maimonides- 1198 "The Treatise on Poisons and Their Antidotes"*
  - Rabies, snake bites, & scorpion stings and how to treat associated injuries
    - Tourniquets
    - Herbal remedies for toxin
    - Hot & cold toxins correlating to hemolysis and neurotoxins, respectively

Texas
Coral Snake

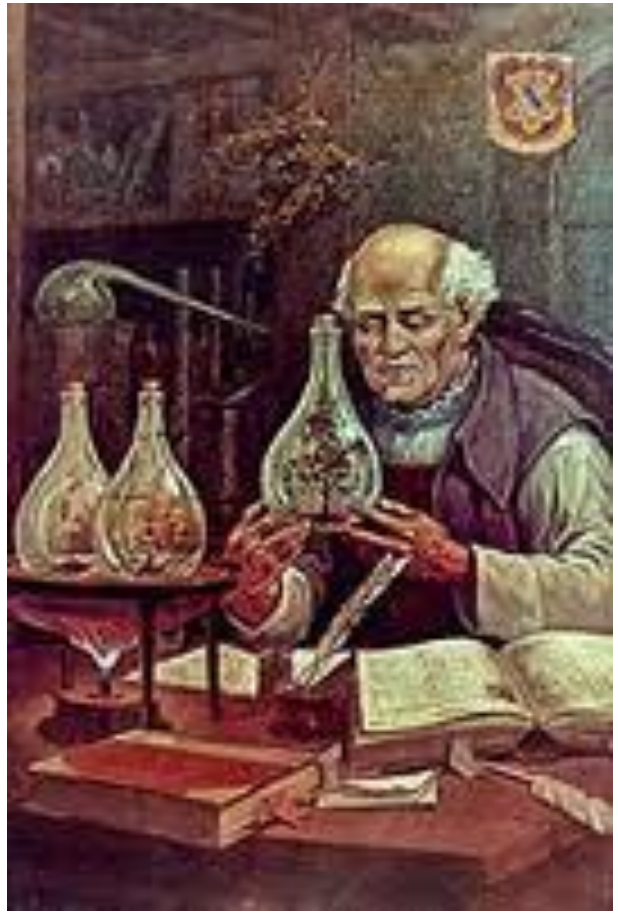
Green Viper

*Tox Sci 59, pg 196-197 (2011)

Evolution of Toxicology

- Paracelsus*
  - 16th century- Swiss physician
  - Identified role of chemistry in medicine
  - Conceptualized dose-response relationship

    "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy.“ – Paracelsus
  - Organ of toxicity
  - Animal experimentation to understand chemical exposure adverse effects and benefits

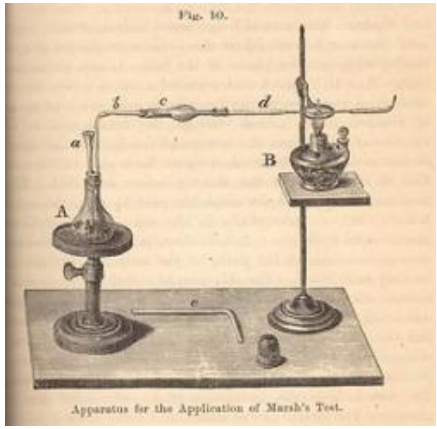


http://www.mysticmissal.org/paracelsus.htm

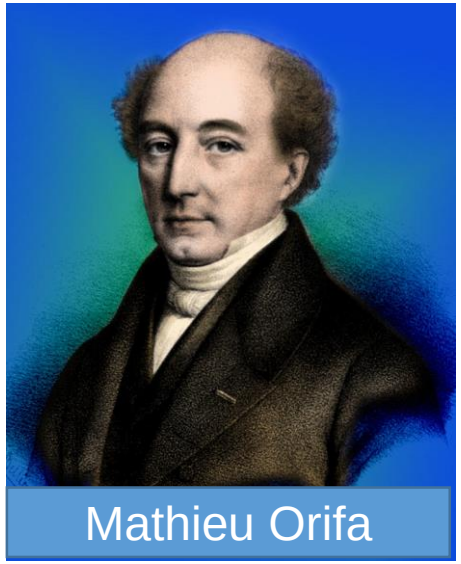
*Tox Sci, **53**(1), pg 2-4, (2000)

Evolution of Toxicology

- "Science of Poison"
  - Mathieu Orfila- began using chemical analyses of blood in forensic medicine in early to mid 1800's
  - Magendie-introduced concept of absorption and distribution of compounds in the body
- Modern Toxicology
  - Interest in the field increased after deaths from ether, chloroform and carbonic acids administered by physicians
  - Study of neurotoxicity associated with bootlegging and impurities in alcohol
  - Metal toxicity
  - Organophosphates-cholinesterase inhibition (post WWII)

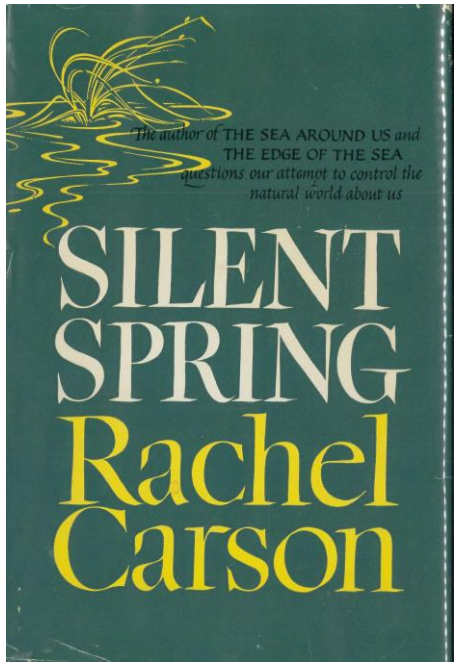


Marsh test to detect arsenic poisoning



"Traite Des Poisons" 1814

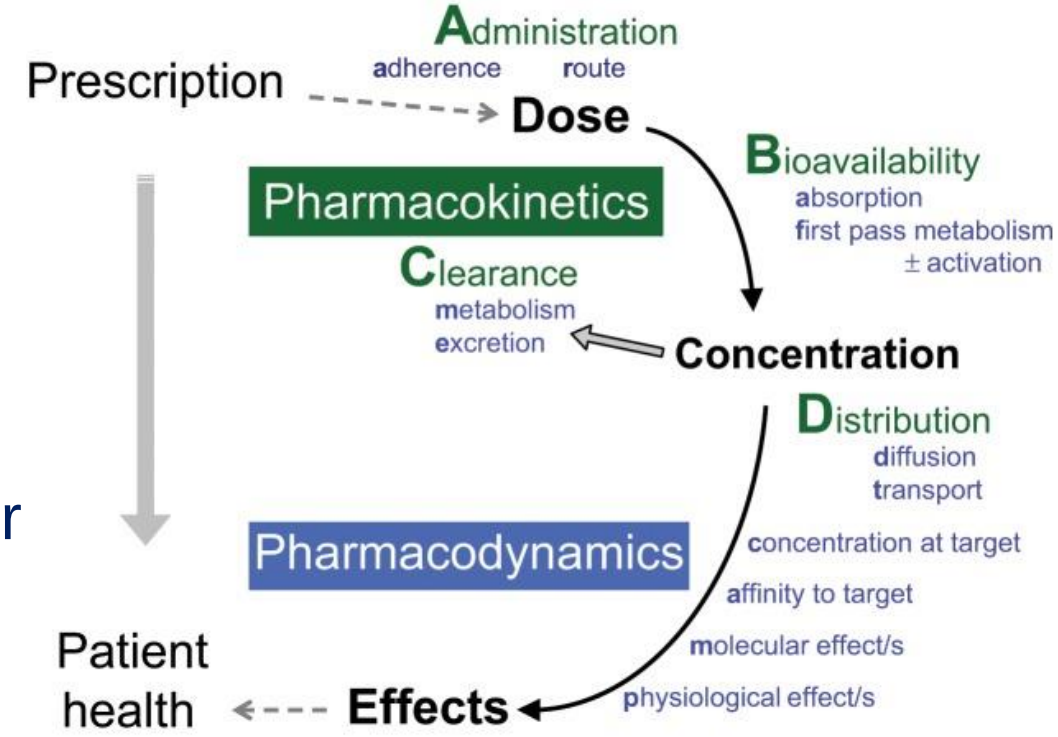
Evolution of Toxicology



- Quantification
  - Paper chromatography-developed in 1948; chemical separation
  - Identification of biomarkers
    - Blood and urine
    - Chemical metabolite quantification
- Regulation
  - 1938- FDA requires new drugs show safety before selling; 1966- FDA requests report on drug effectiveness performed by NAS/NRC and implements recommendations in 1968
  - 1982/3- Tamper resistant packaging req'd as result of cyanide placed in drug capsules
  - 1991- Common Rule for human subject protection
  - 1990's- Guidelines for gender differences, demographic rule, and pediatric rule

Concepts of Toxicology

1. Incidence of injury is proportional to the dose or some function of the dose
   - Exposure dose and Target dose
   - Mechanism of exposure
     - Ingested, inhaled, or injected
2. Toxicological threshold
   - Detox rate, excretion, and/or injury repair is less than the rate of exposure, absorption, or injury
     - Pharmacokinetics: Absorption, distribution, metabolism, excretion) → Administration, bioavailability, clearance, distribution
     - Population-based toxicology



Ther Adv Drug Saf v.4(1); 2013 Feb; PMC4110820

Concepts of Toxicology (cont.)

3. Bioavailability
   - Drugs-fraction of administered drug entering the systemic circulation
     - Oral administration- dependent on absorption and first pass metabolism
     - Rate- determines max concentration, but doesn't affect steady state or maintenance dosing regime
   - Environment
4. Clearance
   - Used to calculate steady state concentration and (with bioavailability) repeat dosing
   - Metabolism

Concepts of Toxicology (cont.)

5. Hazard

- Chemical and physical properties
- Metabolism and disposition
- Acute toxicity
- Repeated short-term exposure
- Long term (> 1/2 lifetime) exposure

Examples of LD50

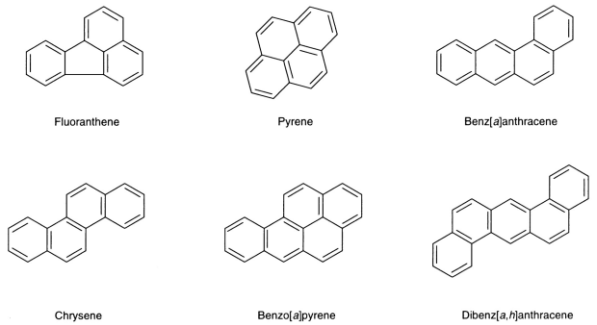
1) Oral Nicotine- 50 mg/kg in rats
2) Tetrahydrocannabinol-3000 mg/kg in dogs and NHP
3) Acetyl fentanyl- 9.3 mg/kg in mice

6. Terms

Term	Definition
LD50	Dose of chemical that produces death in 50% of population
NOAEL	No observable adverse effect level
LOAEL	Lowest observed adverse effect level
Cmax	Maximum concentration of a drug in the body after dosing
Bioavailability	Systemically available fraction of test article

Sub-disciplines of Toxicology

- Medical Toxicology
- Ecotoxicology
- Immunotoxicology
- Regulatory Toxicology
- Nanotoxicology
- Food Toxicology
- Forensic Toxicology
- Toxicogenomics
- Industrial Toxicology
- Molecular Toxicology
- Developmental Toxicology



Agency for Toxic Substances and Disease Registry

2019 Rank	Substance Name	Total Points	CAS RN
1	ARSENIC	1676	7440-38-2
2	LEAD	1531	7439-92-1
3	MERCURY	1458	7439-97-6
4	VINYL CHLORIDE	1356	75-01-4
5	POLYCHLORINATED BIPHENYLS	1345	1336-36-3
6	BENZENE	1327	71-43-2
7	CADMIUM	1318	7440-43-9
8	BENZO(A)PYRENE	1307	50-32-8
9	POLYCYCLIC AROMATIC HYDROCARBONS	1278	130498-29-2
10	BENZO(B)FLUORANTHENE	1253	205-99-2
11	CHLOROFORM	1201	67-66-3
12	AROCLOR 1260	1191	11096-82-5
13	DDT, P,P'-	1181	50-29-3
14	AROCLOR 1254	1172	11097-69-1
15	DIBENZO(A,H)ANTHRACENE	1160	53-70-3
16	TRICHLOROETHYLENE	1155	79-01-6
17	CHROMIUM, HEXAVALENT	1149	18540-29-9
18	DIELDRIN	1143	60-57-1
19	PHOSPHORUS, WHITE	1141	7723-14-0
20	HEXACHLOROBUTADIENE	1127	87-68-3
21	DDE, P,P'-	1126	72-55-9
22	CHLORDANE	1125	57-74-9
23	AROCLOR 1242	1125	53469-21-9
24	COAL TAR CREOSOTE	1124	8001-58-9
25	ALDRIN	1115	309-00-2

2019 Rank	Substance Name	Total Points	CAS RN
26	DDD, P,P'-	1113	72-54-8
27	AROCLOR 1248	1106	12672-29-6
28	HEPTACHLOR	1101	76-44-8
29	AROCLOR	1101	12767-79-2
30	BENZIDINE	1092	92-87-5
31	ACROLEIN	1090	107-02-8
32	TOXAPHENE	1089	8001-35-2
33	TETRACHLOROETHYLENE	1077	127-18-4
34	HEXACHLOROCYCLOHEXANE, GAMMA-	1076	58-89-9
35	CYANIDE	1069	57-12-5
36	HEXACHLOROCYCLOHEXANE, BETA-	1054	319-85-7
37	DISULFOTON	1048	298-04-4
38	BENZO(A)ANTHRACENE	1048	56-55-3
39	1,2-DIBROMOETHANE	1043	106-93-4
40	ENDRIN	1038	72-20-8
41	DIAZINON	1038	333-41-5
42	HEXACHLOROCYCLOHEXANE, DELTA-	1035	319-86-8
43	BERYLLIUM	1030	7440-41-7
44	ENDOSULFAN	1029	115-29-7
45	AROCLOR 1221	1028	11104-28-2
46	1,2-DIBROMO-3-CHLOROPROPANE	1027	96-12-8
47	HEPTACHLOR EPOXIDE	1021	1024-57-3
48	ENDOSULFAN, ALPHA	1019	959-98-8
49	CIS-CHLORDANE	1017	5103-71-9
50	CARBON TETRACHLORIDE	1013	56-23-5

2019 Rank	Substance Name	Total Points	CAS RN
51	AROCLOR 1016	1012	12674-11-2
52	COBALT	1011	7440-48-4
53	DDT, O,P'-	1009	789-02-6
54	METHOXYCHLOR	1007	72-43-5
55	PENTACHLOROPHENOL	1007	87-86-5
56	ENDOSULFAN SULFATE	1004	1031-07-8
57	DI-N-BUTYL PHTHALATE	993	84-74-2
58	NICKEL	993	7440-02-0
59	ENDRIN KETONE	993	53494-70-5
60	DIBROMOCHLOROPROPANE	984	67708-83-2
61	BENZO(K)FLUORANTHENE	974	207-08-9
62	TRANS-CHLORDANE	969	5103-74-2
63	ENDOSULFAN, BETA	968	33213-65-9
64	CHLORPYRIFOS	965	2921-88-2
65	XYLENES, TOTAL	962	1330-20-7
66	CHROMIUM(VI) TRIOXIDE	961	1333-82-0
67	AROCLOR 1232	959	11141-16-5
68	ENDRIN ALDEHYDE	959	7421-93-4
69	METHANE	952	74-82-8
70	3,3'-DICHLOROBENZIDINE	941	91-94-1
71	2-HEXANONE	940	591-78-6
72	2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN	940	1746-01-6
73	BENZOFLUORANTHENE	937	56832-73-6
74	TOLUENE	914	108-88-3
75	ZINC	913	7440-66-6

OUTLINE

- 59th Medical Wing Overview
  - Defense Health Agency and Military Services
  - Science and Technology Office
    - Research Areas and Capabilities
    - Joint Austere Medicine
    - Clinical Resuscitation, Emergency Science, Triage & Toxicology Program

- Intro to Toxicology
  - Brief History
  - Basic Concepts
  - Branches of Toxicology

- Predictive Toxicology
  - Research Strategies
  - Models
  - Proposed screening scheme

Predictive Toxicology

WHAT are we trying to predict?

- Biological disposition from physical-chemical constants?
- Altered cell or organ system function from reaction with macro-molecules?
- Irreversible consequences of reversible changes?
- Implications of selected measurable variables to overall health and survival of the test organisms?
- Effects in individuals of one species from tests conducted in another?
- Incidence in large populations from tests on small samples?

RISK ASSESSMENT framework→ What is reasonable? What is SAFE?….

Environmental Health Perspectives Vol. 32, pp. 261-266, 1979

In silico Strategies

- Goal to reduce, refine, and replace animals
- Understanding relationship between chemical structure and biological responses
  - Quantitative Structure-Activity Relationship (QSAR); adequate for small molecules but not as useful for biologics

    - TOPKAT- Toxicology Predictions by Komputer-Assisted Technology
    - CASE-Computer-Assisted Structure Evaluations
      - MultiCASE, CASETOX, MultiCASE Expert Systems
        - CASETOX has non-modifiable training set
        - MultiCASE user can modify or add own module

In silico Challenges

- Example- Developmental toxicology
  - Despite availability of comprehensive teratogen catalogs, SAR predictions for dev. tox. have been largely unsuccessful
  - Why?
    - Model must predict toxicity for every organ in embryo (and consider developmental stages)
    - Development is dynamic
      - Cell migration, differentiation, apoptosis cues, gradients
  - Outlook- statistically based approaches may be more useful and successful rather than using a rule-based approach

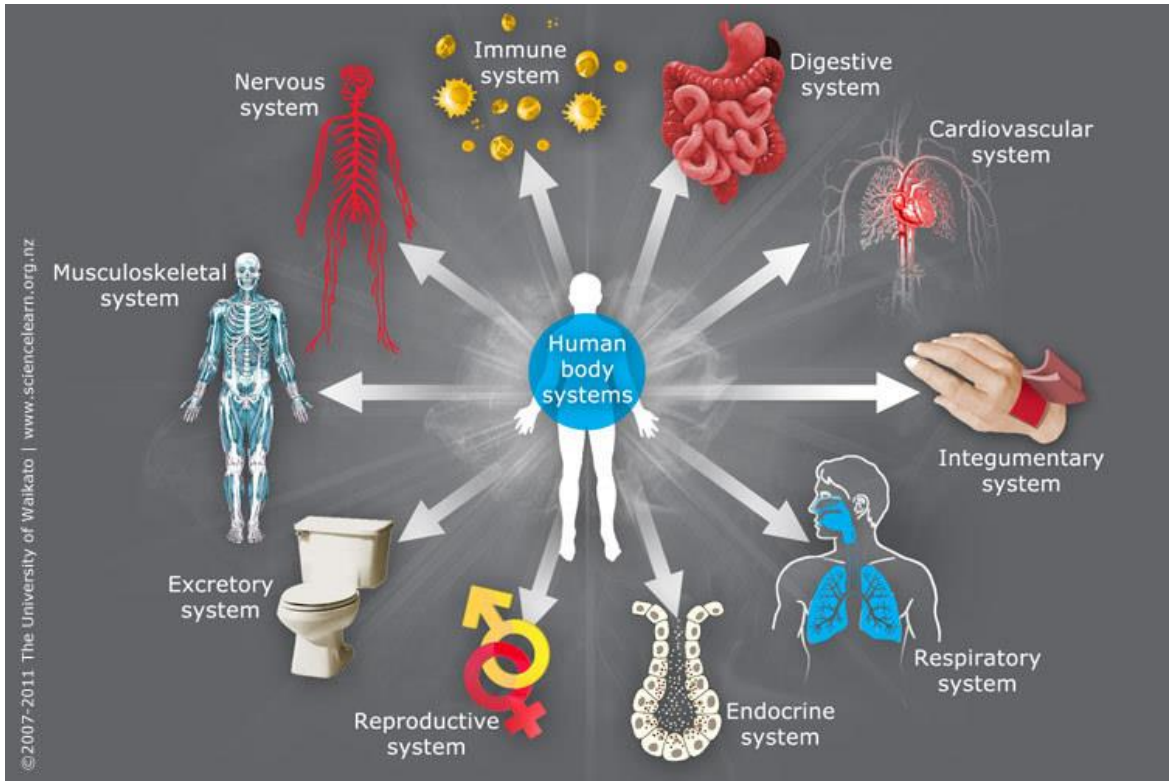
In silico Challenges

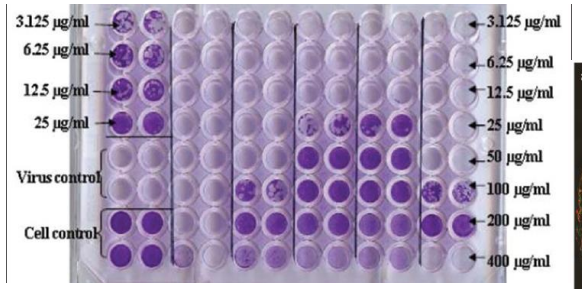
- Inconsistency in reporting unit of measure
  - Lack of experimental information
  - Variability in assay timepoints
  - Multiple cell lines
  - Data processing and normalization
  - Not reproducible
  - Different assays
  - Inappropriate or data misinterpretation
  - Silos and non-interdisciplinary communication
  - Proprietary and intellectual property not reported

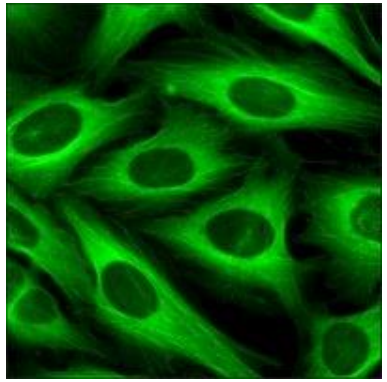
In silico Tools

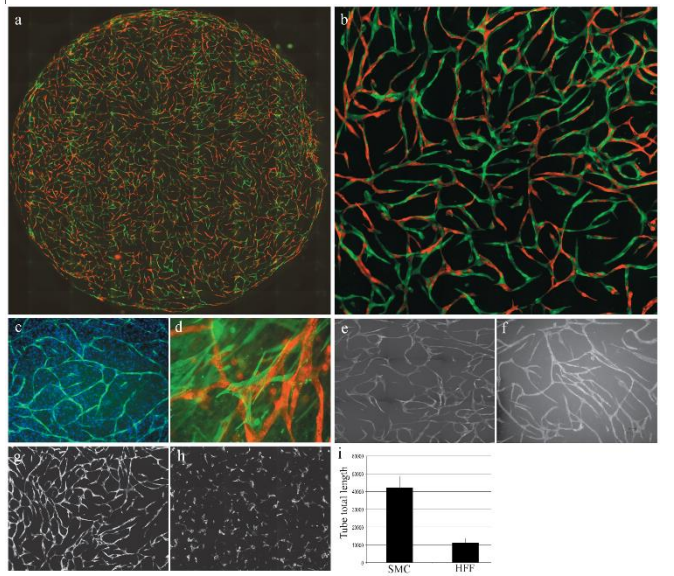
- Computational predictions alone are only acceptable for assessing genotoxic impurities in small molecule drugs
  - Due to quantity limitations; insufficient amounts for experimental testing
  - Requires 2 prediction systems
    - Expert rule based
    - Statistical analyses of structural mutagenicity relationship
- More common and acceptable (for now) in silico is used:
  - Prioritization of newly identified, never evaluated molecules
  - In combination with wet chemistry and in vitro testing

Bench Models

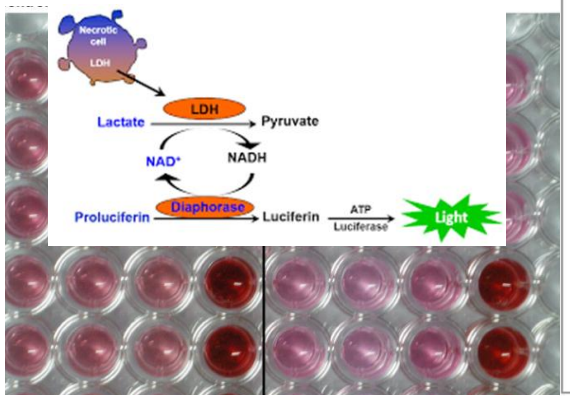


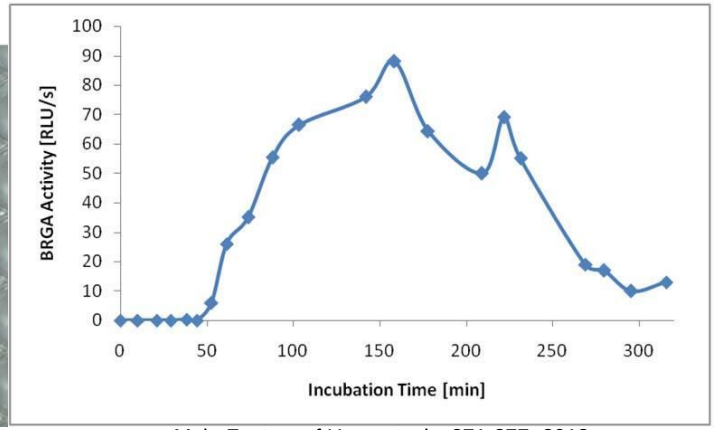






doi: https://doi.org/10.1371/journal.pone.0005798.g002



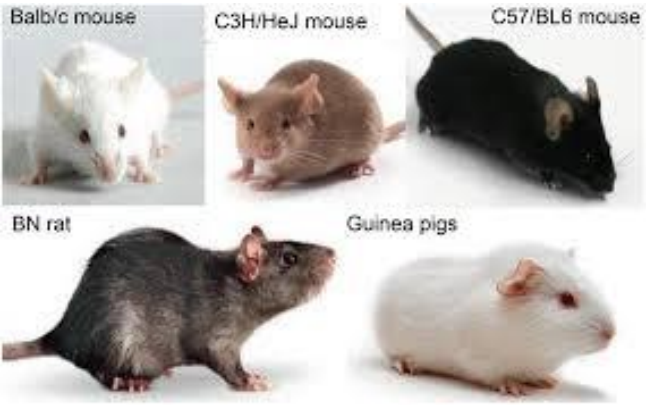


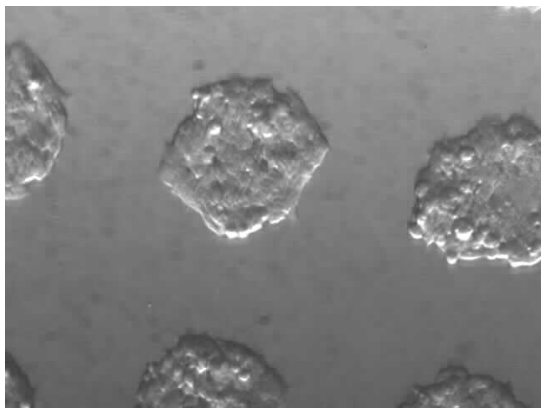
Main Factors of Hemostasis. 271-277. 2013

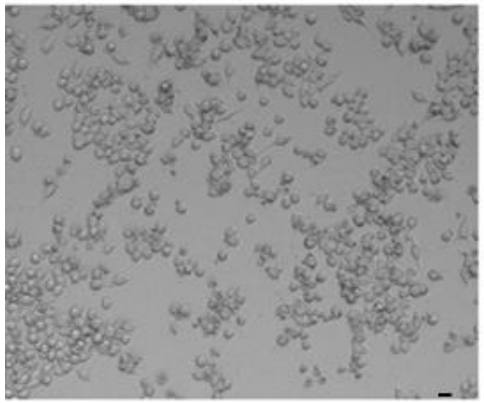
Bench Models

- Dependent on System being studied
  - In general, confidence is better with increasing complexity of test system
  - Other considerations- metabolism (cyp450), genotoxicity

In vivo > ex vivo slices > aggregates > co cultured primary cells > primary cells > human cell lines > other cell lines







Approaches to Assessing Toxicity

- Cardiotoxicity
  - Functional assays
    - Isolated rabbit heart
    - Purkinje fibers
    - Ventricular isolates
  - In vitro
    - IC50
    - Cloned hERG channel systems
    - Ikr channel (cardiac action potential repolarization)
    - K current; Na channel and L type Ca
  - In silico
    - hERG K+ channel affinity QSAR
    - Drug/receptor binding structural req's (consider overlap)
    - 1/2/3-D models of ventricular repolarization
    - Comparative molecular field analyses vs grid-independent descriptors 3D QSAR- consider molecule lipophilicty and flexibility
  - In vivo
    - Telemetry in dogs and NHP

- Hepatotoxicity
  - Functional assays
    - ALT-alanine aminotransferases
    - AST-aspartate aminotransferase
    - Urea
  - In vitro
    - Covalent protein (glutathione) adducts
    - Oxidative stress
    - Mitochondrial damage
    - Cholestasis
    - Reactive metabolites
    - Cell viability (human hepatocytes)
    - Liver on a chip
  - In silico
    - Drug induced liver injury using QSAR trained from 1254 compounds (Int J Mol Sci. 2019 Apr; 20(8): 1897.)
    - Proposed hepatocyte imaging assays readouts fro multiple factors
  - In vivo
    - Mice-acetaminophen vs rat
    - Concanavalin A-based mouse model for hepatitis

- Neurotoxicity
  - Functional assays
    - Electrophysiology
    - Signal transduction
    - Alterations in inhibitory and/or excitation circuitry
  - In vitro
    - Cell types (neurons, oligodendrocytes, astrocytes, microglia)
    - 3D models/co-culture
    - Brain slices
    - Neuron outgrowth
    - Acetocholinesterase activity
  - In silico
    - QSAR approaches on blood brain barrier
    - Linear regression on organic solvent vs polarizability, sum of + atom charges, sum of proton acceptor and dipole moment (Biomedical Chemistry: Research and Methods Vol 1 No 3 (2018): Special Issue)
  - In vivo
    - Rodent assessments for motor function, behavior, and neuropath
    - Zebrafish
    - C. elegans

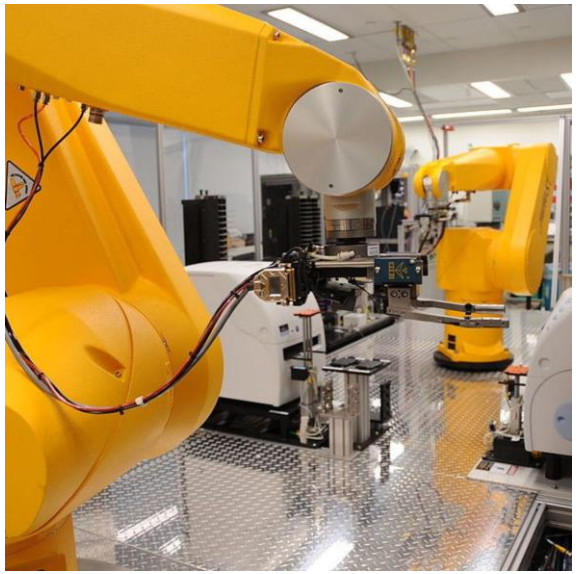
Hazard Assessment

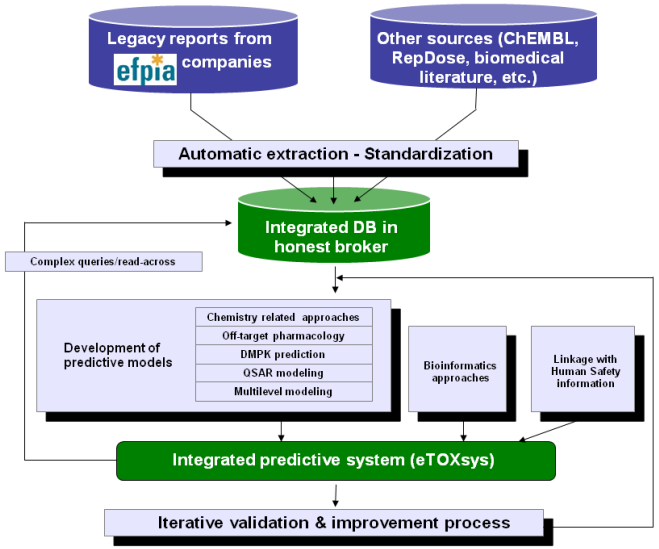
- Risk assessment- process to identify hazards and risk factors that may cause harm
  - Identify ways to eliminate hazard and control risk
- Current efforts are advanced and well-organized in Europe
  - Toxicity data collection is common for chemicals classified in the European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation
  - Creation of eTOX to leverage preclin data for small molecules and share them
    - Contains 1900 chemical structures
    - 8000 in vivo data

Basic Clin Pharmacol Toxicol. 2018 Sep;123 Suppl 5:29-36. doi: 10.1111/bcpt.12956. Epub 2018 Feb 6.

Databases (non-exhaustive)

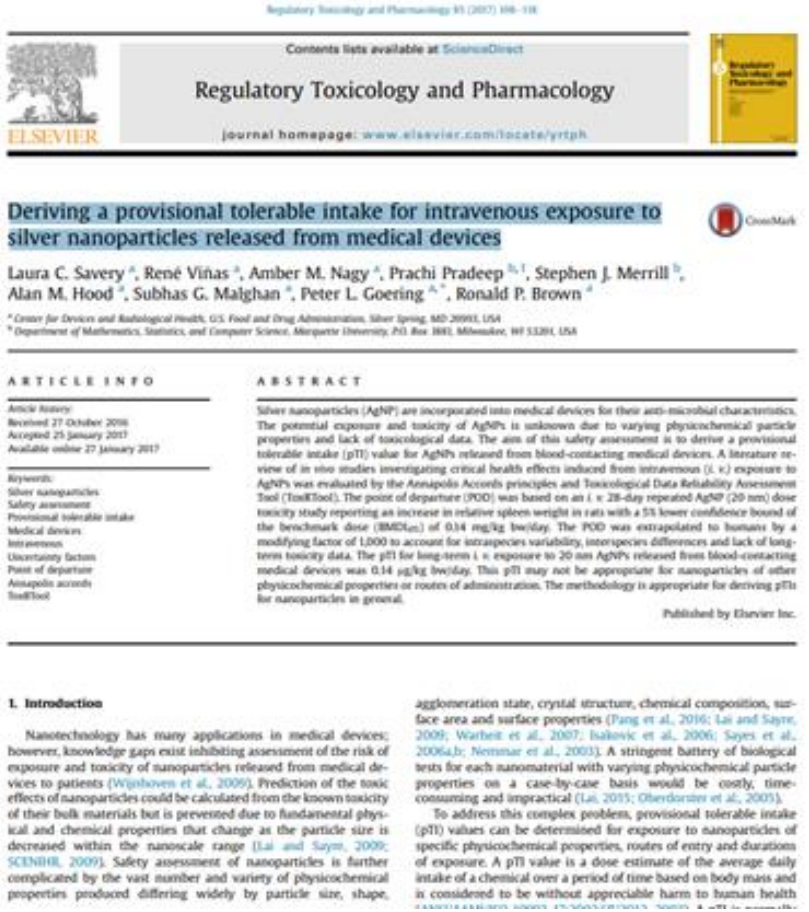
- TOXNET (Retired)- ToxLine in PubMed; PubChem
- EURL (pesticides/residue)
- ToxRefDB stores data related to ToxCast
  - HTP screening assays (US-EPA)
- IUCLID
  - International Uniform ChemicaL Information Database
- ECHA-European chemical property search
- eTOX
- ChEMBL





Data Extrapolation

- Nanoparticles
- Silver is well characterized; nano-silver differences?
    - Ion elution→oxidative stress, inflammation



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Deriving a provisional tolerable intake for intravenous exposure to silver nanoparticles released from medical devices

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Point of departure
Annapolis accords
ToxRTool

ABSTRACT

Silver nanoparticles (AgNP) are incorporated into medical devices for their anti-microbial characteristics. The potential exposure and toxicity of AgNPs is unknown due to varying physicochemical particle properties and lack of toxicological data. The aim of this safety assessment is to derive a provisional tolerable intake (pTI) value for AgNPs released from blood-contacting medical devices. A literature review of in vivo studies investigating critical health effects induced from intravenous (i. v.) exposure to AgNPs was evaluated by the Annapolis Accords principles and Toxicological Data Reliability Assessment Tool (ToxRTool). The point of departure (POD) was based on an i. v. 28-day repeated AgNP (20 nm) dose toxicity study reporting an increase in relative spleen weight in rats with a 5% lower confidence bound of the benchmark dose ($BMDL_{05}$) of 0.14 mg/kg bw/day. The POD was extrapolated to humans by a modifying factor of 1,000 to account for intraspecies variability, interspecies differences and lack of long-term toxicity data. The pTI for long-term i. v. exposure to 20 nm AgNPs released from blood-contacting medical devices was 0.14 μg/kg bw/day. This pTI may not be appropriate for nanoparticles of other physicochemical properties or routes of administration. The methodology is appropriate for deriving pTIs for nanoparticles in general.

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1. Introduction

Nanotechnology has many applications in medical devices; however, knowledge gaps exist inhibiting assessment of the risk of exposure and toxicity of nanoparticles released from medical devices to patients (Wijnhoven et al., 2009). Prediction of the toxic effects of nanoparticles could be calculated from the known toxicity of their bulk materials but is prevented due to fundamental physical and chemical properties that change as the particle size is decreased within the nanoscale range (Lai and Sayre, 2009; SCENIHR, 2009). Safety assessment of nanoparticles is further complicated by the vast number and variety of physicochemical properties produced differing widely by particle size, shape, agglomeration state, crystal structure, chemical composition, surface area and surface properties (Pang et al., 2016; Lai and Sayre, 2009; Warheit et al., 2007; Isakovic et al., 2006; Sayes et al., 2006a,b; Nemmar et al., 2003). A stringent battery of biological tests for each nanomaterial with varying physicochemical particle properties on a case-by-case basis would be costly, time-consuming and impractical (Lai, 2015; Oberdorster et al., 2005).

To address this complex problem, provisional tolerable intake (pTI) values can be determined for exposure to nanoparticles of specific physicochemical properties, routes of entry and durations of exposure. A pTI value is a dose estimate of the average daily intake of a chemical over a period of time based on body mass and is considered to be without appreciable harm to human health

Approach

Question:

Can a provisional tolerable intake (pTI) level for IV AgNP exposure be derived based off existing literature?

Variables:

- Toxicity data is limited
- AgNP formulations are not uniform

Methods:

- Literature Evaluation using Annapolis Accords
- Data quality assessed with Toxicological Data Reliability Assessment Tool (ToxRTool)

Annapolis Accords: Risk Assessment

- ✓ Risk Assessment should be complete
- ✓ All relevant information should be used in risk assessment
- ✓ Elimination of risks should be based on clear definitions
  - ✓ Quantitative & Qualitiatuve estimates based on definitation of hazard, types, and amount of exposure, variability of response, and effects over time
- ✓ Assessments should clearly communicate sources, assumptions, limitations, and uncertainty about the scientific data
- ✓ Risk considerations should be clearly communicated
  - ✓ Seriousness of hazards should include quantitative estimates and consideration of qualitative factors
  - ✓ Use by scientists, public, and policy makers

Criteria for Selecting Tox Studies

Table 1
Criteria for selection of toxicological studies based on Annapolis Accords principles.[a]

Principle	Criteria for Inclusion in Derivation of a Provisional Tolerable Intake Value
Rigor	– Evaluated for proper conduct and analysis. – Greater weight given to more rigorous studies. – Studies with poor methods discounted.
Power	– Statistical power of the experimental design examined for ability to detect effects of a given magnitude. – For example, some "negative" studies may misinterpret a low level of response as a lack of response.
Corroboration	– Determine if effects are replicated in similar studies or under varied conditions to predict if effects would be seen under conditions of human exposure as well. – Conversely, lack of corroboration of effects provides a basis for doubting either the validity of single experimental results or their applicability to other species or conditions of exposure.
Universality	– Effect seen in multiple species by various routes of exposure gives confidence that the effect may apply to humans. – If an effect is restricted to a certain species, strain, or route of administration, there is less confidence in the ability to generalize the response to other species or routes.
Proximity	– When effects exist in a species taxonomically-related to humans or at exposure doses similar to those expected in humans, such results weigh more heavily than effects found in taxonomically less related species by less relevant routes or at markedly different doses.
Relevance	– Knowledge of the underlying biologic basis for toxicity in animals can assist in determining whether similar metabolism, mechanisms of damage and repair and molecular targets of toxic action occur in humans. Accordingly, confidence in applicability to humans can increase or decrease.
Cohesion	– Extent to which all data are consistent and subject to a single, biologically plausible explanation increases the weight-of-evidence when comparing situations where inconsistencies require ad hoc explanations and exceptions to general patterns.

[a] Principles outlined as in (Gray et al., 2008).

Provisional Tolerable Intake

- Dose estimate of the average daily intake of a chemical over a period of time based on body mass and is considered to be without harm to human health*
- Milligram per kilogram of body weight per day (mg/kg bw/day)
- Context of this derivation was to identify an allowable level for a leachable chemical in a medical device

*ANSI/AAMI/ISO 10993-17:2002/(R)2012, 2003

pTI Methods

- Literature search of in vivo studies investigating health effects from AgNP exposure via intravenous dosing
  - Inclusion criteria;
    - Admin route similar to exposure of blood contacting med dev.
    - Relevence to tox effects/human
    - Biomarkers/end effects measure considered to be adverse
    - Data has rigor, power, corroboration, relevance, universality
- Identified Point of Departure (POD; represented as benchmark dose or NOAEL)
- Extrapolated to humans by incorporating modifying (uncertainty) factor
  - Modifying factors account for uncertainty in the datasets
    - Chronic exposure data
    - Intraspecies variation
    - Intraspecies differences
  - Built off previous safety assessment for di(2-ethylhexyl)phthalate for PVC med. Devices
  - Occupational Exposure limit for AgNP (NIOSH)

*ANSI/AAMI/ISO 10993-17:2002/(R)2012, 2003

Calculations of pTI

- Point of Departure
  - 2 studies* (28-day repeat exp of 20 nm AgNP in rats)
  - Reported BMD05 (lower limit for 95% CI) for critical organ weight, hematology, bold chem, & immune parameters; BMD05 was determined by fitting dose-response curve over entire dose range
  - Most critical effect was increase in spleen weight a dose of 0.14 mg/kg bw/day

- Uncertainty factors (UF)
  - UF1: Inter-individual; default typically 10
  - UF2: Interspecies; default typically 10
  - UF3: Quality and relevance of the data; ranges from 1-100
    - Assigned 10 due to no chronic studies, and possibility that most sensitive health effect endpoint may not have been evaluated
  - Calculated MF by multiplying 3 uncertainty factors; MF= 1000

$$pTI = POD/MF = \frac{\textbf{0.14 mg/kg bw/day}}{\textbf{1000}}$$

0.14 µg/kg bw/day

*http://dx.doi.org/10.1016/j.biomaterials.2013.06.048
http://dx.doi.org/10.1186/1743-8977-11-21

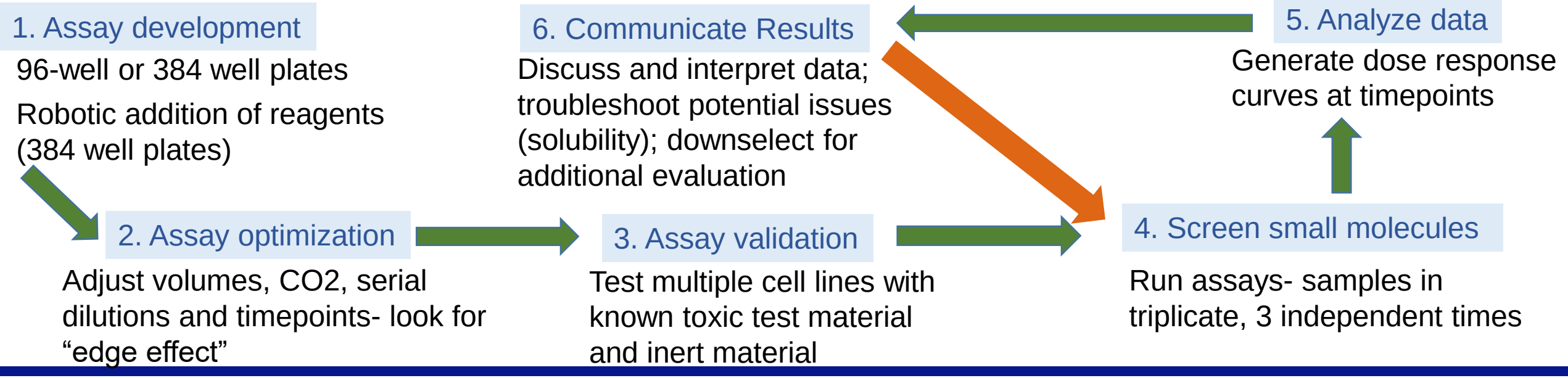
Limitations & Conclusions

- Sensitive health effects may not have been accounted for
  - Reproductive toxicity- other studies have variable reporting; accounted for by assigning UF3 of 10

- AgNP dose that patients are exposed to is needed to complete safety assessment
- Provisional TI limit was derived
- Value in line with pTI for oral exposure after accounting for bioavailability

Proposed AMD Tox Approach

- High throughput screening using commonly accepted in vitro assays
  - Extracellular lactate dehydrogenase activity as measurement of cell viability
  - Tetrazolium reduction (WST-1/MTT)- measurement of mitochondrial activity
  - GFP cell line signal quantification



Proposed AMD Tox Approach

- High throughput screening using commonly accepted in vitro assays
  - Adaptation to 384 well plate
  - Autonomous reagent addition
  - Real time data collection
- Initial screening with kidney, liver, skin, and lung cell lines
- Down select for additional assays with primary cells

Questions

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