Incorporating Alternative Methods Into Medical Device Safety Testing Strategies

Recently Available *In Vitro* Models

Presented by: James McKim, Ph.D., DABT
Chief Science Officer for CeeTox, Inc.
Medical Devices Cover a Wide Range of Products

Wireless Implantable Medical Devices

- Deep Brain Neurostimulators
- Cochlear Implants
- Gastric Stimulators
- Cardiac Defibrillators/Pacemakers
- Foot Drop Implants
- Insulin Pumps
New Devices: Artificial Trachea Grown From Patient’s Stem Cells

A large number of medical device safety studies are negative

There are alternative methods that are being used now

New alternative methods are becoming available
### ISO/FDA Test Chart

<table>
<thead>
<tr>
<th>Device Categories</th>
<th>Initial Evaluation</th>
<th>Supplemental Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact Duration</td>
<td>Cytotoxicity</td>
</tr>
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<td>Body Contact</td>
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<td>Skin</td>
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**A** = Limited exposure 5 24 hours  
**B** = Prolonged exposure (24 hours - 30 days)  
**C** = Permanent contact (> 30 days)

• FDA and ISO evaluation tests  
0 = Additional tests for FDA

**Cytotoxicity**  
Acute LD50/systemic toxicity  
Genotoxicity  
Hemocompatibility  
Sensitization  
Irritation
New Models For Predicting Systemic Toxicity are Available

Acute oral LD50

Subchronic toxicity

Important considerations for medical devices
Extracts (polar and non-polar)
Mixtures
Active chemicals in low concentration
Exposure/time
Concentration-response
Development of device controls
What is Systemic Toxicity and What do We Want From Alternative Methods?

- **Toxicity that occurs after a chemical is absorbed into general circulation**
  - **Acute systemic toxicity**
    Single dose, and short exposure time
    Intrinsic toxicity of a chemical, **LD<sub>50***, organ effects
  - **Subacute systemic toxicity**
    Repeated-dose study, typically 14 day
    Information on toxicity following repeated exposure
    Helps establish doses for subchronic studies
  - **Subchronic systemic toxicity**
    Repeated-dose, typically 28 and 90 days
    Organ specific effects
    Establish NOAEL and LOAEL
    Regulatory implications FDA and EPA
  - **Chronic systemic toxicity**
The Goal is to Predict Human Systemic Toxicity

- Most value = Subchronic
- What data are required for risk assessment
  - NOAEL, LOAEL
  - RfD, Benchmark dose

Realistically

Ideally
Focus on Cell Biology and Physical Chemical Properties Improves the Model

Biochemical Function
- Membrane Integrity
- Mitochondrial Function
- Cell Proliferation
- Redox-State
- Oxidative Stress
- Apoptosis

Physical-Chemical Properties
- Solubility
- Partition coefficients
- Pka
- Protein binding
- Metabolic stability
- Metabolic activation
- Transporter interaction

Pharmacology
- CNS receptors
- Cardiovascular receptors and ion channels

Pharmacokinetic Parameters
- Clearance
- Bioavailability
- Volume of distribution
Multiple Endpoints Are Essential For Correct Interpretation of *In Vitro* Data

**FIG. A:** Exposure Concentration (µM)
Development of an Acute Toxicity Model: A Collaboration With LOREAL Paris

**Prediction (validation, global set)**

<table>
<thead>
<tr>
<th></th>
<th>&lt;5</th>
<th>5-50</th>
<th>50-300</th>
<th>300-2000</th>
<th>≥2000</th>
<th>Total</th>
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<tbody>
<tr>
<td>GHS1(&lt;5)</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>7</td>
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<tr>
<td>GHS2(5-50)</td>
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<td>7</td>
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<td>GHS3(50-300)</td>
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<td>15</td>
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<td>2</td>
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<tr>
<td>GHS4(300-2000)</td>
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<td>3</td>
<td>18</td>
<td>2</td>
<td>25</td>
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<tr>
<td>GHS5(≥2000)</td>
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<td>1</td>
<td>0</td>
<td>4</td>
<td>14</td>
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<tr>
<td><strong>Total</strong></td>
<td>3</td>
<td>18</td>
<td>25</td>
<td>29</td>
<td>18</td>
<td>93</td>
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**Optimised Model, 93 RMs: 70% correct predictions (SOT 2012)**
Merging Thresholds Significantly Improved Predictive Power

<table>
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<th>Total</th>
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<td>&lt;300</td>
<td>300-2000</td>
<td>≥2000</td>
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<tr>
<td>GHS1-2-3(&lt;300)</td>
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<td>5</td>
<td>1</td>
<td>46</td>
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<td>GHS4(300-2000)</td>
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<td>18</td>
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<td>28</td>
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<td>GHS5(≥2000)</td>
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<td>2</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>25</td>
<td>19</td>
<td>93</td>
</tr>
</tbody>
</table>

*Optimised Model, 93 RM: 77% correct predictions (SOT 2012)*
Incorporation of Physical-Chemical Properties and Receptor Binding Improved Predictive Power
Manuscript is Being Prepared

Working on improving efficiency for screening
Alternative Methods for Predicting Skin Sensitization

NAMSA/CeeTox Collaborative Project
Most In Vitro Approaches Focus on Chemical Reactivity, Keratinocytes, or Dendritic Cells as the Test System

**Induction Phase**

- **Chemical**
- **Keratinocytes**
- **Langerhans Cells**

**Peptide binding**

- **Keratinocytes**
  - Identification of unifying events
  - Characteristic of chemical sensitizers
  - Biochemical, molecular, chemical

- **Dendritic cells**
  - Identification of unifying events
  - Characteristic of chemical sensitizers
  - Biochemical, molecular, chemical

**HaCaT 3D Skin Models**

**Dendritic cells from blood**

**Cell lines derived from blood**

Required for Immune response
Alternative Methods Currently in The Validation Process

- **Direct peptide reactivity assay (DPRA)**
  - Synthetic peptides with cysteine or lysine

- **MUSST**
  - Myeloid U-937 Skin Sensitization Test
  - Monitors expression of CD86 by flow cytometry

- **hCLAT**
  - Human cell line activation test (THP-1)
  - CD86 and CD54 by flow cytometry

- **KeratinoSens**
  - HaCaT cells transfected with a luciferase reporter construct under the control of ARE

- **SenCeeTox® (In Vitro Sensitization Assay)**
  - Combines cell viability, Nrf2/ARE gene expression, and direct reactivity
  - Uses both HaCaT and 3D skin models
  - Express dose as mass per unit area of skin
  - Provides potency category (non-weak, moderate, strong/extreme)
New Method (SenCeeTox®) is Based on Known Steps in the Sensitization Process

- Dose (mass/unit area)
- Penetration into skin (exposure)
- Chemical binding to protein (haptenization)
- Activation of essential signaling pathways in skin epidermis (signaling in Epidermis)
- Recruitment of Langerhans cells
- Transport to local lymph node
- Proliferation of T-cells
The *In Vitro* Sensitization Method (IVSA) Incorporates Key Events Occurring in the Epidermis

**SenCeeTox®**

- **Solubility**
- **Chemical Reactivity**
  - Direct and Indirect
- **Cytotoxicity**
- **Gene expression**
- **Human Cell Models**
  - HaCaT cells (high solubility)
  - Human 3D Skin Models (low solubility)

**IVSA**
Reconstructed Human Skin For Determining Chemical Sensitization Models Penetration and Early Activation of Key Signaling Pathways

Critical Factors
Chemical size
Partition coefficient
Solubility
Penetration
Finished product testing

Direct Application to dendritic cell models may increase false positive responses
Multiple Signaling Pathways are Required In Order to Identify Potency Categories

Eleven Genes Monitor Three Separate But Related Signaling Pathways

- **Nrf2/ARE Genes**
  - NADPH Quinone oxido reductase
  - Aldoketoreductase
  - Thioredoxin
  - Interleukin 8
  - Aldehyde dehydrogenase
  - Hemeoxygenase 1
  - Glutamate cysteine ligase catalytic subunit C

- **AhR/XRE**
  - CYP1A1

- **Nrf1/MRE**
  - Metallothionein 1
  - Metallothionein 2
Learning to Interpret Gene Expression Data by Building a Training Set of 39 Chemicals

Non
- Glycerol
- Benzoic acid
- 1-Butanol
- SDS
- Lactic acid
- Limonene
- Vanillin
- Salicylic acid

Weak
- Hydroxcitronellal
- Phenyl benzoate
- Benzyl cinnamate
- Eugenol
- Citral
- trans-2-hexanal
- Diethyl maleate
- Diethyl sulfate

Moderate
- Phenylacetaldehyde
- Perillaldehyde
- 2-hydroxy-ethyl-acrylate
- Isoeugenol
- Phthalic anhydride
- 2-aminophenol
- 1,4-phenylenediamine
- Propyl Gallate

Strong
- 1-chloro-2,4-dinitrobenzene
- 5-chloro-2-methyl-4-isothiozolin-3-one

Extreme
- Diphenylcyclopropenone
- p-Benzoxquinone

Proprietary to CeeTox, Inc.
Chemical Reactivity is a Key Characteristic of Chemical Sensitizers

GSH Levels of Glycerol and p-Benzquinone

Non-Sensitizer

Extreme Sensitizer

Compounds and Concentrations

% GSH Compared to Control
Multiple Methods Can Improve Predictive Power

Direct Peptide Reactivity Assay

Chemicals Tested

- Blank
- LA (Lactic Acid)
- BA (Benzoic Acid)
- pBQ (p-Benzoquinone)
- HyEtAcrylate

Peptide Remaining (μM)

Multiple Genes Are Required In Order to Accurately Predict Potency Categories
**Blinded Study With 58 Test Compounds Showed Good Predictivity**

<table>
<thead>
<tr>
<th>+ In vivo</th>
<th>- In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P+</strong></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3</td>
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<tr>
<td></td>
<td><strong>28</strong></td>
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<tr>
<td><strong>P-</strong></td>
<td></td>
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<tr>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td><strong>39</strong></td>
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</table>

**Totals**

<p>| | |</p>
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<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>31</strong></td>
<td><strong>36</strong></td>
</tr>
</tbody>
</table>

**Sensitivity** = 81 % How good the assay is at predicting a sensitizer  
**Specificity** = 92 % How good the assay is at predicting a non-sensitizer
Allergic Contact Dermatitis (Sensitization) Requires Activation of Key Signaling Pathways

McKim JM et al. (2012) Cutan Ocular Tox, Early On-line, Informa Press
Evaluation of Down Stream Effector Molecules Implicates TNF and CCL5

Induction of signaling genes by sensitizers at 100 µM

- p38 MAPK
- TNF
- CCL27
- CCL5
- CCL17

-fold induction

p-benzoquinone
2-aminophenol
4-vinylpyridine
2,3-butanedione
eugenol
benzoic Acid
glycerol
Reconstructed Human Skin Provides a Viable Model for Testing Medical Device Extracts

Figure 2 from Bolmarcich, 2006
Reactivity Assay Comparing Positive Control Response With Different Vehicles
Nrf2/ARE Mediated Gene Expression Following Exposure to Extracts from Dental Cement Without 2-HEMA
Nrf2/ARE Mediated Gene Expression Following Exposure to Extracts from Dental Cement Containing 2-HEMA

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Induction</th>
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<tbody>
<tr>
<td>NQO1</td>
<td>0.9%SC, DMSO</td>
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<tr>
<td>AKR1C2</td>
<td>0.9%SC, DMSO</td>
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<td>IL8</td>
<td>0.9%SC, DMSO</td>
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<tr>
<td>CYP1A1</td>
<td>0.9%SC, DMSO</td>
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<tr>
<td>ALDH3A</td>
<td>0.9%SC, DMSO</td>
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<tr>
<td>HMOX1</td>
<td>0.9%SC, DMSO</td>
</tr>
<tr>
<td>GCLC</td>
<td>0.9%SC, DMSO</td>
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</tbody>
</table>

Gene Response Following Exposure to Extract From Cement With 2-HEMA

Legend: 50%, 75%, 100%
Summary of *In Vitro* Versus *In Vivo* Testing

<table>
<thead>
<tr>
<th>Device &amp; Extract Vehicle</th>
<th>2-HEMA (µg/g)</th>
<th>IVSA</th>
<th>GPMT</th>
<th>LLNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental Cement + 2-HEMA Saline</td>
<td>1200</td>
<td>Moderate</td>
<td>NS</td>
<td>NS (SI=1.2)</td>
</tr>
<tr>
<td>Dental Cement + 2-HEMA Sesame Oil</td>
<td>480</td>
<td>Non</td>
<td>NS</td>
<td>NT</td>
</tr>
<tr>
<td>Dental Cement + 2-HEMA DMSO</td>
<td>1300</td>
<td>Moderate</td>
<td>NT</td>
<td>NS (SI=0.8)</td>
</tr>
<tr>
<td>Dental Cement (-) 2-HEMA Saline</td>
<td>&lt;5.2</td>
<td>Non</td>
<td>NS</td>
<td>NS (SI=0.8)</td>
</tr>
<tr>
<td>Dental Cement (-) 2-HEMA Sesame Oil</td>
<td>&lt;5.2</td>
<td>Non</td>
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<tr>
<td>Dental Cement (-) 2-HEMA DMSO</td>
<td>&lt;5.2</td>
<td>Non</td>
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<td>NS (SI=0.7)</td>
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NS = Non-Sensitizer
SI = Stimulation Index (≥3.0 is a sensitizer)
NT = Not Tested

Application of a New *In Vitro* Sensitization Assay for the Evaluation of Medical Device Extracts

J.M. McKim, Jr., D.J. Keller, J.R. Gorski, and L.H. Moilanen

1CeeTox Inc., Kalamazoo, MI, 2NAMSA, Northwood, OH and 3M Medical Department, 3M Center, St. Paul, MN
An *in vitro* method for detecting chemical sensitization using human reconstructed skin models and its applicability to cosmetic, pharmaceutical, and medical device safety testing

James M. McKim Jr¹, Donald J. Keller III¹, and Joel R. Gorski²

¹CeeTox, Inc., Kalamazoo, MI, USA and ²Namsa, Northwood, OH, USA
Evaluation of Skin Irritation
Skin Irritation: EPI-200-SIT OECD TG439 SOP V7

- Skin irritation:
  - MTT viability assay (n=3)
  - IL-1α release (n=3)
  - Histology (n=2)

- Criteria for In vitro Interpretation

<table>
<thead>
<tr>
<th>In Vitro Results</th>
<th>In Vivo Prediction</th>
</tr>
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<tbody>
<tr>
<td>Mean tissue viability ≤ 50%</td>
<td>Irritant (I), (R38 or GHS category 2)¹</td>
</tr>
<tr>
<td>Mean tissue viability &gt; 50%</td>
<td>Non-irritant (NI)</td>
</tr>
</tbody>
</table>

¹Irritant prediction according to the European Union (EU) or Globally Harmonized System of Classification and Labeling of Chemicals (GHS) classification.

Note: Endpoints in yellow above are not required, but may add interpretive value.
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<td>Circulating Blood</td>
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**Summary and Conclusions**

- **Cytotoxicity**
- Acute LD50/systemic toxicity
- **Genotoxicity**
- Hemocompatibility
- Sensitization
- Irritation

**Medical Device Challenges**

- Sensitivity
- Cell or tissue model selected
- Controls
- Exposure of cells
- Exposure time
- Solubility

---

A: Limited exposure (5-24 hours)  B: Prolonged exposure (24 hours - 30 days)  C: Permanent contact (> 30 days)

* FDA and ISO evaluation tests  0 = Additional tests for FDA
Thank You