In vitro approaches to modern toxicology

ToxPath21
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OUTLINE:

JRC

Context

In vitro models

Examples from hepatotoxicity

Pathologists' roles
The Joint Research Centre (JRC)

As the science and knowledge service of the Commission our mission is to support EU policies with independent evidence throughout the whole policy cycle.

~ 3000 staff
Almost 75% are scientists.
Headquarters in Brussels.
Research facilities located in 5 Member States.
Chemical Safety & Alternative Methods Unit

Projects

VALIDATE new *in vitro* methods

DISSEMINATE knowledge of alternative approaches
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Pathologists' roles
Approaches in toxicology are changing
Why.....

....alternatives to current approaches
TOXICITY TESTING IN THE 21ST CENTURY
A VISION AND A STRATEGY
If we understand HOW chemicals cause adverse outcomes........
Integrated approaches to testing and assessment (IATAs)
Toxicologic Pathology

A discipline that applies the professional practice of **pathology**, the study of diseases, to **toxicology**, the study of the effects of chemicals and other agents on humans, animals, and the environment.
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Pathologists' roles
Alternative animal models

Drosophila melanogaster

Caenorhabditis elegans

Zebrafish

Medaka
In vitro cell culture models

- **Cell types:**
  - Primary cells
  - Cell lines
  - Stem cells

- **Cell culture systems**
  - Monoculture
  - Co-culture
  - 2D
  - 3D
  - Organs-on-a-chip
Primary cells

Immortalized cell lines
Stem cells

Embryonic stem cells

Somatic or adult stem cells

Locations of Somatic Stem Cells in the body:
- Brain
- Blood vessels
- Teeth
- Skin
- Liver
- Heart
- Bone marrow
-Peripheral blood
-Gut
-Skeletal muscle
Stem cells

Human induced pluripotent stem cells (hiPSCs)

Chemical screening
Monoculture

Cryopreserved human hepatocytes

differentiated HepaRGs & biliary cells

Co-culture
Co-culture

hiPSC derived neuronal and glial cells, differentiated for 21 days

NF200, Tau, MAP2 are neuronal markers
GFAP is a glia (astrocyte) marker
Two and three dimensional culture systems
hiPSC-derived Neuronal Stem Cells

2D vs 3D
Sandwich cultures

Primary rat hepatocyte monolayer

Primary rat hepatocyte sandwich culture

24hrs after plating

72hrs after plating, bile canaliculi

courtesy of Ian Cotgreave, Swetox
Other models

**Precision-Cut Tissue Slices (PCTS)**


**Organotypic culture system**

(Organoids)

Organs-on-Chips
lung

(Huh et al., 2010 Science.25;328(5986):1662-8)
Human-on-a-chip

The human-on-a-chip concept
(Huh et al., 2010 Science.25;328(5986):1662-8)
Assay automation
high throughput screening (HTS) and high content imaging (HCI)
Multiplexing of 4 stains

HepaRGs exposed to Valinomycin 24h

Blue – Hoechst nuclei
DeadGreen Membrane permeability
Red- MitoHealth
depth red - LipidTOX
merged
Tox Testing in the 21st century is already here......

ToxCast

Robotic platforms

1536 well plates

Integrated computer modeling
Sophisticated models cannot substitute understanding
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Pathologists' roles
PHH and HepaRG spheroids repeated exposure to Chlorpromazine for 8 days

(Hendriks et al, 2016, Scientific Reports 6, 35434)
Identified key events (dark green)
- bile accumulation
- oxidative stress
- inflammation
- activation of nuclear receptors.

# Systems Information

<table>
<thead>
<tr>
<th>Molecular effects</th>
<th>Cellular effects</th>
<th>Tissue/organ effects</th>
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<tbody>
<tr>
<td></td>
<td>Adaptive response</td>
<td>Adverse effects</td>
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<tr>
<td>Nuclear factor activation (FXR, PXR, CAR)</td>
<td>↑ FXR</td>
<td>BA accumulation</td>
</tr>
<tr>
<td>Transporter inhibition</td>
<td>↑ CYP 450</td>
<td>Inflammation</td>
</tr>
<tr>
<td>BA synthesis/metabolism</td>
<td>↓ CYP 7A1</td>
<td>Cytotoxicity</td>
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<tr>
<th>Biomarkers/targets for therapy</th>
<th>Symptomatic/palliative therapy</th>
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<tr>
<td>Cholangitis</td>
<td>ALT, AST, γ-GT, S-Bili elevation</td>
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<tr>
<td>NASH</td>
<td>Fibrosis</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Cirrhosis</td>
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<tr>
<td>Cholestasis</td>
<td>Liver failure</td>
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Liver fibrosis

(Barr et al, 2015, Radiology, 276:845-861)
Liver fibrosis

HepaRG and primary human HSCs cultured as 3D spheroids

cross-linked collagen

Single (40µM)

Allyl Alcohol day 21

Repeated (200µM)

collagen1

αSMA

(Leite et al, 2016, Biomaterials 78:1-10)
AOP to Liver Fibrosis

**CHEMICAL**
- Protein Alkylation
- Cell injury
- KC activation
- TGF-β1 expression
- HSC activation
- ECM alteration
- Liver Finosis

**VIRUS**
- HCV envelope glycoproteins E1 and E2 binding to cell membrane

Inflammation
Oxidative stress

**Health**
- molecular/cellular studies

**Disease**
- clinical studies
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Pathologists' roles
Holistic background and understanding
Thank you for your attention!

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