DEVELOPMENT OF NATIONAL CAPACITIES FOR THE IMPLEMENTATION OF ALTERNATIVE METHODS: A NEW CHALLENGE IN URUGUAY

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Institut Pasteur International Network (RIIP)
In 2009: NATIONAL LAW 18.611
PROCEDURES FOR THE USE OF ANIMALS IN EXPERIMENTATION, TEACHING & RESEARCH ACTIVITIES

NATIONAL COUNCIL OF ANIMAL EXPERIMENTATION

- Register Qualified Institutions
- Ethics Committee on the Use of Animals (each institution)
- National System of Personal Accreditations
### Institutional programs - “One health” concept

<table>
<thead>
<tr>
<th>Animal Health</th>
<th>Genomics</th>
<th>Human Health</th>
<th>Cancer Immunology</th>
<th>Biotechnology</th>
</tr>
</thead>
<tbody>
<tr>
<td>zoonotic diseases or microbiology.</td>
<td>including human and medical genomics, pathogen genomics.</td>
<td>INDICYO. Inflammatory &amp; metabolic diseases.</td>
<td>Cancer Immunology</td>
<td>PROTEMCA. Molecular, Cellular &amp; Animal Technology.</td>
</tr>
</tbody>
</table>
Program of Molecular, Cellular and Animal Technology

ProTeMCA: Founded in 2015

R + D + i

Final Product
(Protein, Test, Bioassay, Model)
1. Design & refinement of products of biotechnological interest

- Protein engineering
- Biosensors. Recombinant protein
- HTS, HCA, metabolic engineering, human and animal health

HTS: High-throughput screening
HCA: High content analysis
2. Development of new cell-based assays for HTS, HCA & toxicology studies
3. New animal models for toxicological and disease studies

- CRISPR/Cas9
- Lentivirus

Model
- Murine
- Ovine

Application
- Biomedicine,
- Agricultural production,
- Animal health,
- Bioreactors

Implementation of 3R
AIM

DEVELOPMENT OF NATIONAL CAPACITIES FOR THE IMPLEMENTATION OF ALTERNATIVE METHODS (2017)

- To implement a quality management system (GLP lab, documentation)
- To implement validated OECD protocols for toxicology allowing the REDUCTION or REPLACEMENT of experimental animals
- To train HR in the application and validation of Alternative Methods
- To deliver national and regional training courses on Alternative Methods
- To advice regulatory agencies on the uses of Alternative Methods
**In vitro skin sensitization test:**

OECD TG 442E: human Cell Line Activation Test (h-CLAT)

![Diagram of the OECD TG 442E test procedure]

- **THP-1 cells** + test chemical
- Treated cells + α-CD54 & α-CD68
- Flow Cytometry Analysis

**Symbols:**
- THP-1 cells
- Test chemical
- Surface markers
- α-CD86
- α-CD54
IMPLEMENTATION OF OECD TG 442E

Examples of two substances used as positive and negative skin sensitization controls.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>CV75</th>
<th>Flow cytometry analysis</th>
<th>Relative Fluorescence Intensity (RFI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactic Acid</strong></td>
<td>CV75 739 µg/mL</td>
<td>α-CD54-FITC</td>
<td>α-CD86-PE</td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DNOCB</strong></td>
<td>CV75 6 µg/mL</td>
<td>Counts</td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td></td>
<td>Vehicle control</td>
<td></td>
</tr>
</tbody>
</table>

- **CD86**<br>Non-skin sensitizer<br><br>CD86: <150%<br>CD54: <200%

- **CD86**<br>Skin sensitizer<br><br>CD86: >150%<br>CD54: >200%
**In vivo Acute Oral Toxicity:**

Up and Down Procedure. Two strains of soil bacteria were used

- Evaluation of parameters for animal welfare score. If \( \geq 3 \) animals die, conduct the Main Test
- Gross necropsy performed in every animal, microscopic examination of organs if evidence of gross pathology
- If \( \geq 3 \) animals survive, LD50 is greater than 2000 mg/kg
Annual training course
Ethic Use of Experimental Animals

February 2017
Hands on training course: XTREME-Bruker *in vivo* technology
October 2017
International course: Cell and Animal Models for Drug Discovery
Métodos Alternativos al uso de Animales de Experimentación: Evaluación in vitro de irritación (TG 439) y sensibilización cutánea (TG 442E).

Sensibilización dermatológica ensayos Test OECD TG442E.
Quality management system is implemented

Two OECD validated tests are implemented:
- OECD TG 442E (hCLAT)
- OECD Test 425

Different training courses were delivered

Uruguayan Regulatory Agencies & Companies start to discuss on the implementation of alternative methods
To implement new OECD protocols for toxicological studies

<table>
<thead>
<tr>
<th>SKIN</th>
<th>OCULAR</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In chemico, in vitro</strong></td>
<td><strong>In vitro</strong></td>
<td><strong>In vivo</strong></td>
</tr>
<tr>
<td>OECD TG 442C</td>
<td>OECD TG 437</td>
<td>OECD TG 402</td>
</tr>
<tr>
<td>(Direct Peptide Reactivity Assay)</td>
<td>(Bovine cornea opacity &amp; permeability)</td>
<td>(Acute Dermal Toxicity)</td>
</tr>
<tr>
<td>OECD TG 442D</td>
<td>OECD TG 460</td>
<td>OECD TG 403/436</td>
</tr>
<tr>
<td>(KeratinoSens™)</td>
<td>(Fluorescein Leakage, MDCK)</td>
<td>(Acute Inhalation Toxicity)</td>
</tr>
<tr>
<td>OECD TG 442E</td>
<td></td>
<td></td>
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<tr>
<td>(U-SENS™)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OECD TG 439</td>
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<tr>
<td>(Reconstructed Human Epidermis)</td>
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PERSPECTIVES

To introduce silicone animal models for training courses

To install In-Vivo XTREME II system (Bruker)

To develop new cell-based assays

To become center of reference in Uruguay

To establish collaborations outside Uruguay
THANK YOU

Cecilia Abreu
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Sergio Pantano
Marcelo Comini

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