Contribution of Imaging & Wireless Sensor Technologies to Refinement of Animal Experimentation

Didima de Groot PhD ERT
didima.degroot@tno.nl
Contents of Presentation

- Imaging *in vivo*

- Wireless sensor technology
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• Imaging *in vivo*
  – Regulatory context
    • Reproduction Toxicology
      – OECD Guideline Ext1GenReproToxStudy
      – Developmental Neurotoxicology

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• Imaging *in vivo*
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      » Neuropathology and Behaviour
      » MRI and PET

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      - Neuropathology
      - MRI
      - Behaviour
      - PET
  - Benefit for animals
- Wireless sensor technology
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• Imaging *in vivo*

• **Wireless sensor technology**
  – Rodent (rat): Mo-Chi Tracker: developmental & maternal toxicology
  – Non-rodent (minipig): Physiology Platform: safety pharmacology
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• *Wireless sensor technology*
  – Rodent (rat): Mo-Chi Tracker: developmental & maternal toxicology
  – Non-rodent (minipig): *Physiology Platform*: safety pharmacology
Part I
Imaging in vivo
The Extended One Generation Reprotox. study

• OECD: development of Extended One Generation Study protocol:
The Extended One Generation Reprotox. study

- OECD: development of Extended One Generation Study protocol:
  - Saves animals without giving in on safety for man
    - Exposure pre-mating, gestation, F1
    - Substitute the 2-generation protocol (reduces animals, costs and time)
    - Additional parameters for effects on the nervous and immune systems, and endocrine regulated processes → relative sensitivity
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  - Two-step procedure:
    - Ensure fast adoption of new guideline by including endpoints in already existing guidelines (no validation issues!)
    - Optimize / renew endpoints using (innovative) sensitive technologies that could improve animal reduction even further
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Comparison brain weight vs. MRI-volume Organotins PND21,61

N=10/group, per test-age

Brain weight after death

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>Wgbrain</th>
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<tr>
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* = significant difference

Control, DOTC, TBTOdev, TBTOjuv

Didima de Groot et al.
Organotins: $[^{18}\text{F}]$FDG brain microPET

Mean $[^{18}\text{F}]$FDG uptake PND 18,22,35,62

Brain functioning

TBTO: Glucose metabolism ↓

N=3-4

Different from control

Didima de Groot et al.
Organotins: $[^{18}F]$FDG brain microPET, **Motor act.**

### Mean $[^{18}F]$FDG uptake PND 18,22,35,62

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<thead>
<tr>
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<th>Control</th>
<th>DOTC</th>
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<th>TBTOjuv</th>
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<tr>
<td>SUV +/- sem</td>
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* Different from control

**Brain functioning**

**TBTO**: Glucose metabolism ↓

N=3-4

### Motor activity PND 13,17,21,61

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* Different from control

**Behavioral testing**

**TBTO**:  
- Motor activity ↓

**Others**:  
- Auditory startle response ↓
- FOB: neuromuscular ↓

N=10
Micro array gene expression profiling: summary

- **TBTO** has larger effect on biological processes in general than **DOTC** (more significant categories)
  
  - **TBTO** has effect on:
    - Development (specific for neuro)
    - Locomotory behaviour
    - Glucose metabolism (insulin signaling)
    - Cell death (apoptosis)
  
  - **DOTC** has effect on:
    - Also Development
    - Also Locomotory behaviour
    - Immune system development (immunological synapse)
In vivo imaging & Dev. Neurotox.

Conclusion

• better and more detailed information on DNT → more predictive to man → refinement (animal 3Rs)
• statistical power ↑ → fewer animals → reduction
• proposed: better and more efficient strategy to study potential toxicity through combined application of
  • Imaging → study dynamic processes over time
  • Gene expression → explain underlying processes at distinct test age

Prospects

• Working in an imaging network brings optimal solutions! → optimal information; best science → refinement
• Multi-modal imaging like MRI/PET for preclinical and clinical assessment
In vivo imaging & Dev. Neurotox.

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Part II
Wireless Sensor Technology
Holst Centre

founded by imec (B) & TNO (NL)

- Wireless communication
- Digital signal processing
- Energy harvesting
- Sensing and read-out

Ultralow power WBAN

Wireless Body Area Network
Holst Centre

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Ultralow power WBAN

Wireless Body Area Network

- Communication among sensor nodes around human’s body:
  - monitor vital body parameters and movements;
  - sensed with numerous sensor nodes: multiple signals
- Transmission of multiple signals to a home base station
- Forwarding signal to e.g. hospital via WLAN, cellular network or public switched telephone network
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- WBAN sensor nodes :a.o.EEG, ECG/RMV, EMG, EOG, skin temp/conduct
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Wireless Body Area Network
Holst Centre Wireless Sensor Technology & Animal welfare
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- Animal use in biomedical research is under intense societal debate
- In conflict with its mandatory use to study undesired effects of drugs.
- Legislation demands studies in rodent (rat, mouse) and non-rodent (dog, non-human primate) – outlined in regulatory test guidelines.

Didima de Groot et al.

11th BfR-Forum “Refinement”, 13-14 Dec. 2011, Berlin, Germany
Holst Centre Wireless Sensor Technology & Animal welfare

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- Collaboration (authorities, industry, academia, regulatory agencies) to accomplish refinement and reduction of animal use in (preclinical) safety testing → **minipig** as alternative non-rodent model [RETHINK]:

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  - → can improve human drug safety → contribute to 3Rs (Refinement)
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→ for superior predictivity and translation to man
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Goal: further refinement with Holst Centre wireless sensor technology in this area of (mandatory) safety evaluation studies.
ECG Necklace / HR / acceleration sensor nodes
Focus of Pilot study: animal (dis)comfort and quality/relevance of signals
**Focus of Pilot study**: animal (dis)comfort and quality/relevance of signals

- Location: Ellegaard Gottingen Minipigs Facilities, Dalmose, DK
- Subject: 6 Month old Minipigs
- Holst wireless technology: ECG Necklace/acceleration
**Focus of Pilot study:** animal (dis)comfort and quality/relevance of signals

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**Result Pilot study**
ECG Necklace / HR / acceleration sensor nodes in minipig

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- Location: Ellegaard Gottingen Minipigs Facilities, Dalmose, DK
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Result Pilot study
- Minipig accepted wearing of sensor without any problems
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Result Pilot study
- Minipig accepted wearing of sensor without any problems
- Signals of ECG and X,Y,Z acceleration and Heart rate OK
Wireless sensor technology:
- Integrative multimodal physiology platform
- Non-invasive, animal-friendly

Who benefits?
Who benefits?

- Wireless sensor technology:
  - Integrative multimodal physiology platform
  - Non-invasive, animal-friendly

- Animals (refinement, reduction)
- Society (debate on animal use)
- Animal right parties / Alternatives Centres
- Governmental / regulatory Authorities
- Contract Research Laboratories (CROs)
- Hard/software etc developers
- Industry (pharma, food, chemistry)
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**Wireless sensor technology:**
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3R push

Animals
Animals Right Parties
Society
Governmental authorities
Academia
Regulatory authorities
Industry / CROs
Breeders
Users
End-users

3R implementation
Conclusion

• Holst Centre wireless sensor technology can perfectly fit-in to contribute to the principles of animal 3Rs (Refinement \(\rightarrow\) Reduction):
  – Advanced health monitoring within reach:
    • non-invasive, animal-friendly
    • continuous, repeated monitoring
    • with multiple sensor nodes
    • addressing more organs simultaneously
  – More information can be obtained from fewer animals \(\rightarrow\) refinement
  – Decision making during drug development is stepping up
Prospects

- Development of an integrative multimodal system, for simultaneous assessments of physiological parameters indicative of the health of specific organs, and the individual as a whole → animal-friendly, information increasing, cost reducing

- Miniaturization for use on small animals

- Smart sensor node combinations with accompanying behaviour will allow definition of characteristic behaviours (locomotion, localization, body posture) which, in turn, may be indicative of e.g. anxiety, pain, depression etc. Hence, specific biomarkers of behaviour may be discovered
Wireless Sensor Technology

Mother-Child Tracker

Mo-Chi Tracker
# Dev.Tox.: Physical and Sensory Developmental Landmarks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test Period on postnatal day</th>
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<td>Anogenital distance</td>
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<tr>
<td>Surface righting</td>
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<tr>
<td>Pinna unfolding</td>
<td>2, 3, 4, 5, 6</td>
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<tr>
<td>Hair growth</td>
<td>4, 5, 6, 7, 8, 9, 10</td>
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<tr>
<td>Tooth eruption</td>
<td>9, 10, 11, 12, 13, 14, 15</td>
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<tr>
<td>Eye opening</td>
<td>14, 15, 16, 17, 18, 19, 20, 21</td>
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<tr>
<td>Air righting</td>
<td>14, 15, 16, 17, 18, 19, 20, 21</td>
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<tr>
<td>Auditory canal opening</td>
<td>10, 11, 12, 13, 14, 15</td>
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<tr>
<td>Auditory response</td>
<td>13, 14, 15</td>
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<tr>
<td>Pupil reflex</td>
<td>21, 22, 23, 24, 25</td>
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</tbody>
</table>
Automated ‘stressless’ monitoring of intact litter [dam+pups]: Mo-Chi Tracker
Automated ‘stressless’ monitoring of intact litter [dam+pups]: Mo-Chi Tracker

- TNO product idea Mo-Chi Tracker (Mother-Child tracker) for Dutch SBE
- Supported Ministry of Economic Affairs
- Principle of the product-idea is based on biological differences that *a priori* exist between adult mother animal and young immature pups with regard to e.g.: Size, weight, posture, voice (USVs), speed, metabolic rate etc.
- Measures pup development and communication mother-pup →
- allows to distinguish between maternal and developmental toxicity →
- over generations
Thank you for your attention!
With many thanks to
all our collaborators, colleagues, scientists and students

Especially
Animal Facilities, Behaviour-/Biotechnicians, Histotechnicians

MRI / PET imaging
N Jetten¹, V.J de Groot¹, M Berk¹,³, R Nederlof¹, CF Kuper¹
B Voet¹, M Bogaart¹, E. Uitvlugt², R Dierckx², L vd Horst¹, A Veltien³

Gene expression
Marijana Radonjic, Ros Stierum
With many thanks to
all our collaborators, colleagues, scientists and students

**Wireless sensor technology for animal wellbeing**
Supported and funded by the Holst Centre

**Mo-Chi Tracker**
Supported and funded by TNO SBIR program /Dutch Ministry of Economic Affairs
Back-up slides
Benefit on animal welfare

Neuropathology
Guideline ‘neuropathology’ survey: >160 rats sacrificed

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Low dose</th>
<th>Medium dose</th>
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>160
Proposed alternative ‘neuropathology’: animal reduction 50%

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Note: MRI was performed on days PND 61, with sacrifice of animals. MRI scans were analyzed for vehicle and high dose groups before neuropathology. Analysis included splitting brains into halves for neuropathology and microarray gene expression. Base group selection was based on available information.
Proposed alternative ‘neuropathology’: animal reduction 50%

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PND 21: MRI all animals; sacrifice 5 ♂, 5 ♀ after scanning

PND 61: MRI other 5 ♂, 5 ♀ (repeated measures!); sacrifice
Proposed alternative ‘neuropathology’: animal reduction 50%

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• Analysis MR scans of vehicle and high dose prior to neuropathology
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- Base group selection for further analysis on available information
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More information with <50% of animals
Benefit on animal welfare

Behaviour
Guideline ‘behaviour’: >80 rats repeatedly measured

**PND 13, 17, 21, 61: FOB, MA; PND 23 Startle Response**

<table>
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<tr>
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Limitations:
- Mild burden on animal welfare, but inter-individual variation high
- N=10 rats/group; time-consuming testing
- Tests developed for adult animals; test-age may not be optimal
- Interference one test with another
- Interpretation of changes: developmental delay? Persisting effects?
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Interpretation of changes: developmental delay? Persisting effects?
Proposed alternative ‘behaviour’: animal reduction 50%

PND 17, 21, 35, 61: [18F]FDG microPET + Motor Activity

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• Use [18F]FDG uptake = measure for glucose metabolism ↔ brain activity ↔ synaptic activity ↔ neuronal activity

Keep Motor Activity testing (link to conventional testing) but combine • with microPET testing before tracer dosing and during tracer distribution.

Benefit animal welfare: Better information with fewer animals and tests; brain activity of conscious rat is measured under anaesthesia.
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Wireless sensor technology & refinement

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