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

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TNO innovation for life

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

- **Wireless sensor technology**

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    - Benefit for animals
- **Wireless sensor technology**

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  - Rodent (rat): Mo-Chi Tracker: developmental & maternal toxicology
  - Non-rodent (minipig): Physiology Platform: safety pharmacology

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  - Non-rodent (minipig): **Physiology Platform**: safety pharmacology

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Part I

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Imaging *in vivo*

The Extended One Generation Reprotox. study

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The Extended One Generation Reprotox. study

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  - 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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Regulatory Dev.NeuroTox. testing (rat)

Exposure

Dose groups:
≥ 3; Control

pregnant rat (F0)

offspring (F1)

80 ♀ + 20 ♂ 800-1000 pups

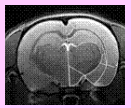
N=10/sex/group

Guideline endpoints (neuro) development

Development
Behavior

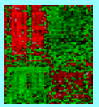
Neuropathology

Magn. Resonance
structural
brain imaging



[18F]FDG PET
functional
brain imaging

microarray gene expression:
development



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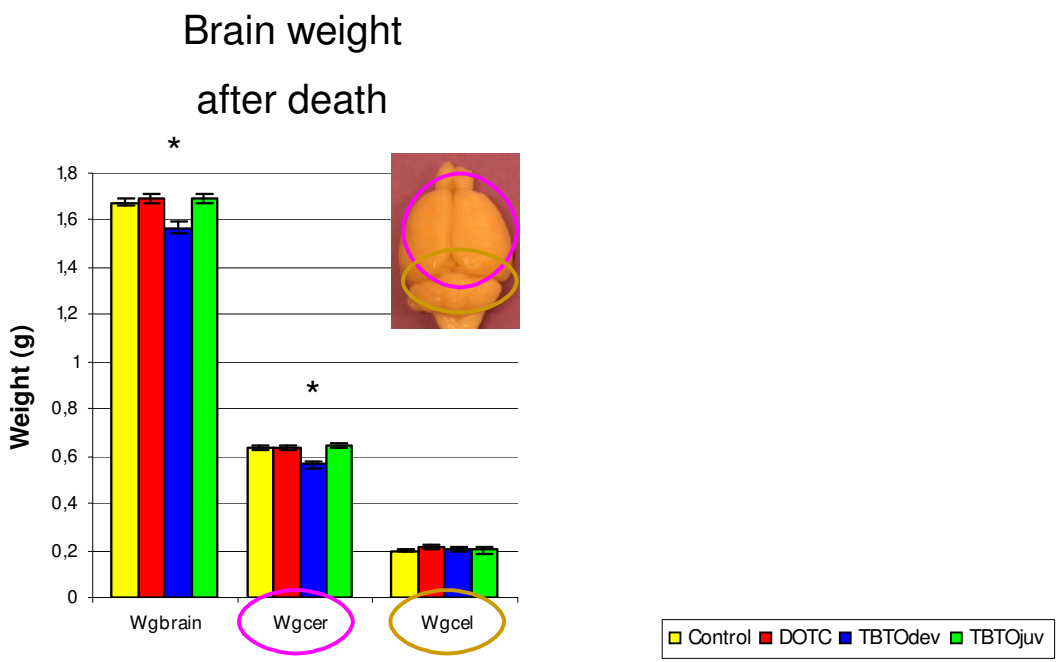
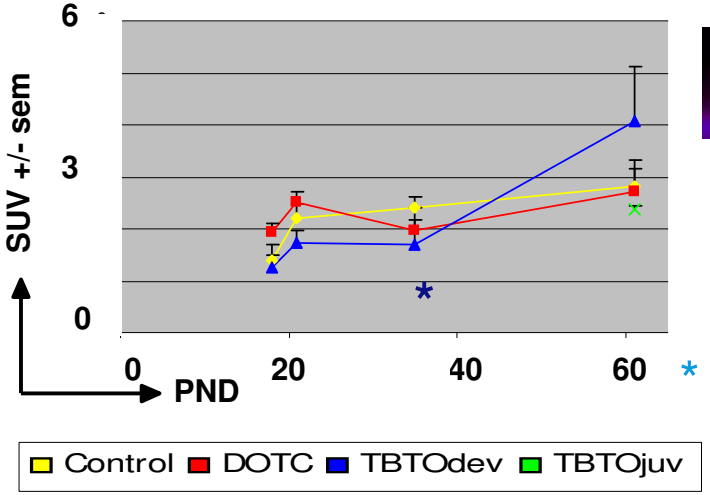
Comparison brain weight vs. MRI-volume Organotins PND21,61

N=10/group, **per test-age**

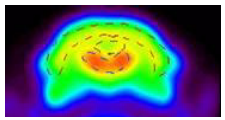


Organotins : [^{18}F]FDG brain microPET

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



Brain functioning



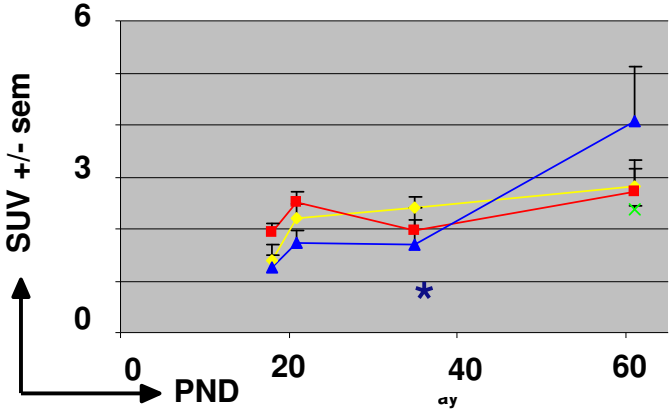
TBTO: Glucose metabolism ↓

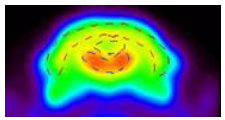
N=3-4

* Different from control

Organotins : [^{18}F]FDG brain microPET, Motor act.

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





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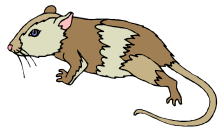
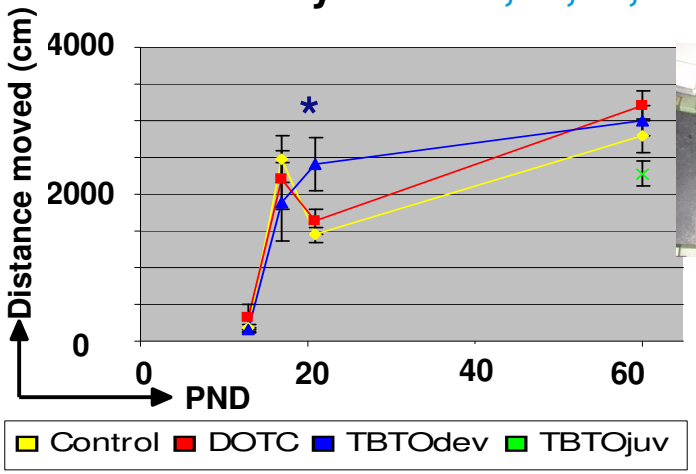


Motor activity PND 13,17,21,61



N=10

* Different from control

Behavioral testing

TBTO:
- Motor activity ↓

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

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Micro array gene expression profiling: summary

N=5

- **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

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Part II

TNO innovation for life

Wireless Sensor Technology

Holst Centre

founded by imec (B) & TNO (NL)

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

→ **Ultralow power WBAN**

Wireless Body Area Network

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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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Holst Centre Wireless Sensor Technology & Animal welfare

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Holst Centre Wireless Sensor Technology & Animal welfare

- 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.

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  – → can improve human drug safety → contribute to 3Rs (**Refinement**)

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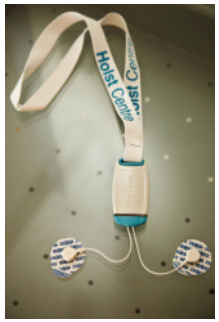
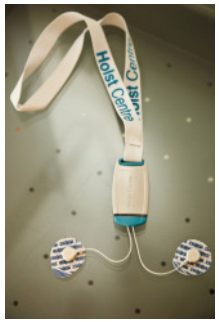
- **Goal**: further refinement with Holst Centre wireless sensor technology in this area of (mandatory) safety evaluation studies.

ECG Necklace / HR / acceleration sensor nodes



ECG Necklace / HR / acceleration sensor nodes in minipig



Focus of Pilot study: animal (dis)confort and quality/relevance of signals

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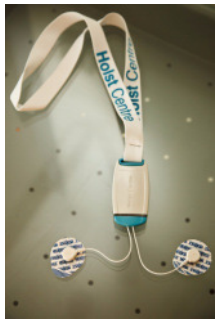

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- Holst wireless technology: ECG Necklace/acceleration

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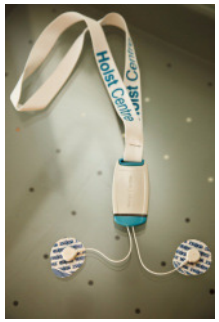

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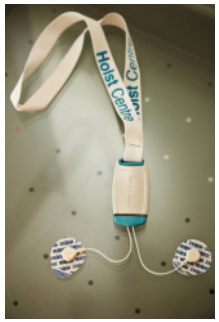

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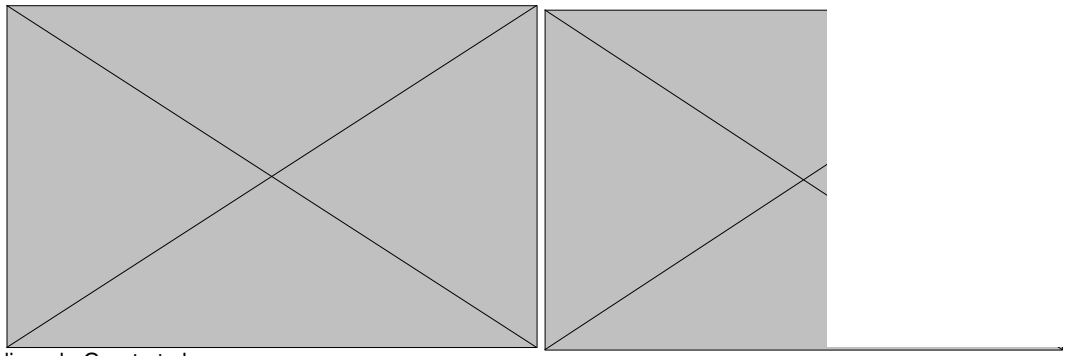

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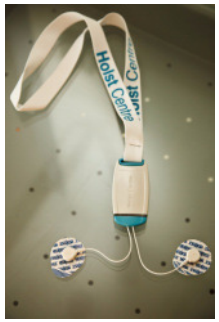

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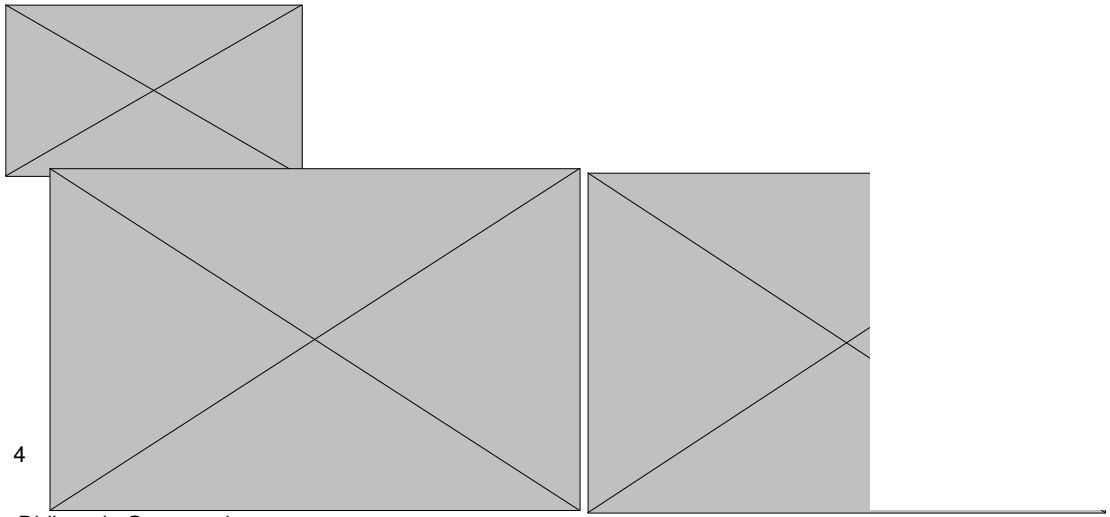

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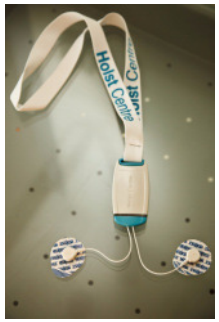

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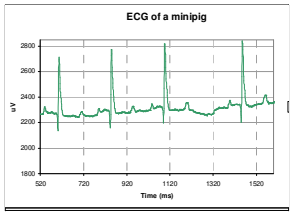

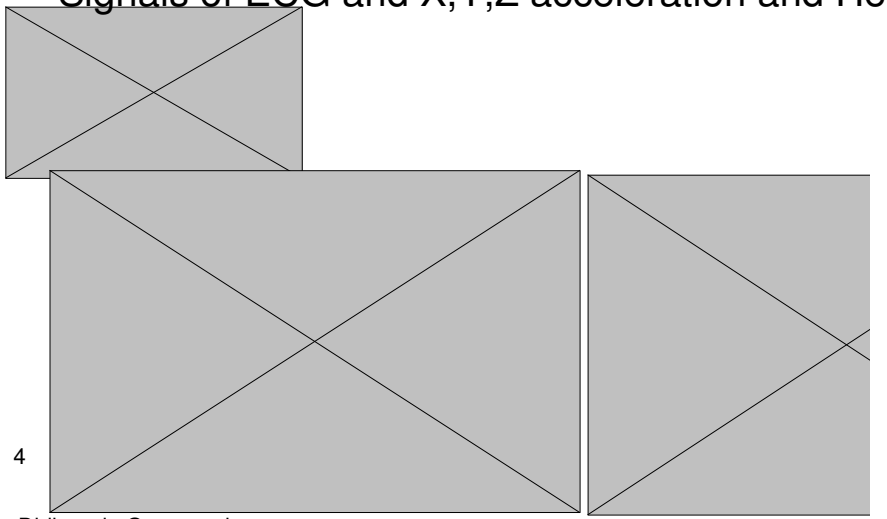

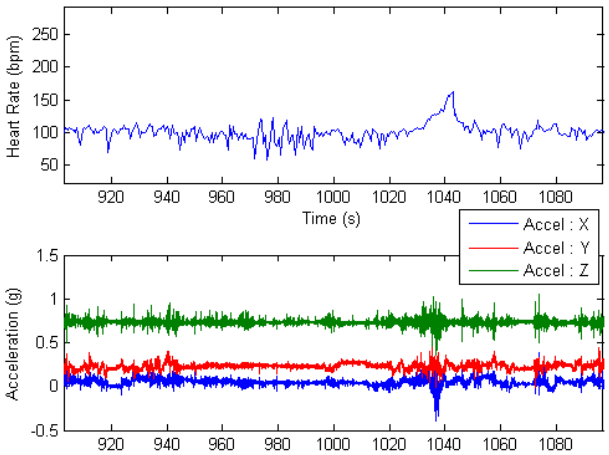

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  - **Integrative multimodal physiology platform**
  - **Non-invasive, animal-friendly**

Who benefits?

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- Animals (refinement, reduction)
- Society (debate on animal use)
- Animal right parties / Alternatives Centres
- Governmental / regulatory Authorities
- Contract Research Laboratories (CROs)
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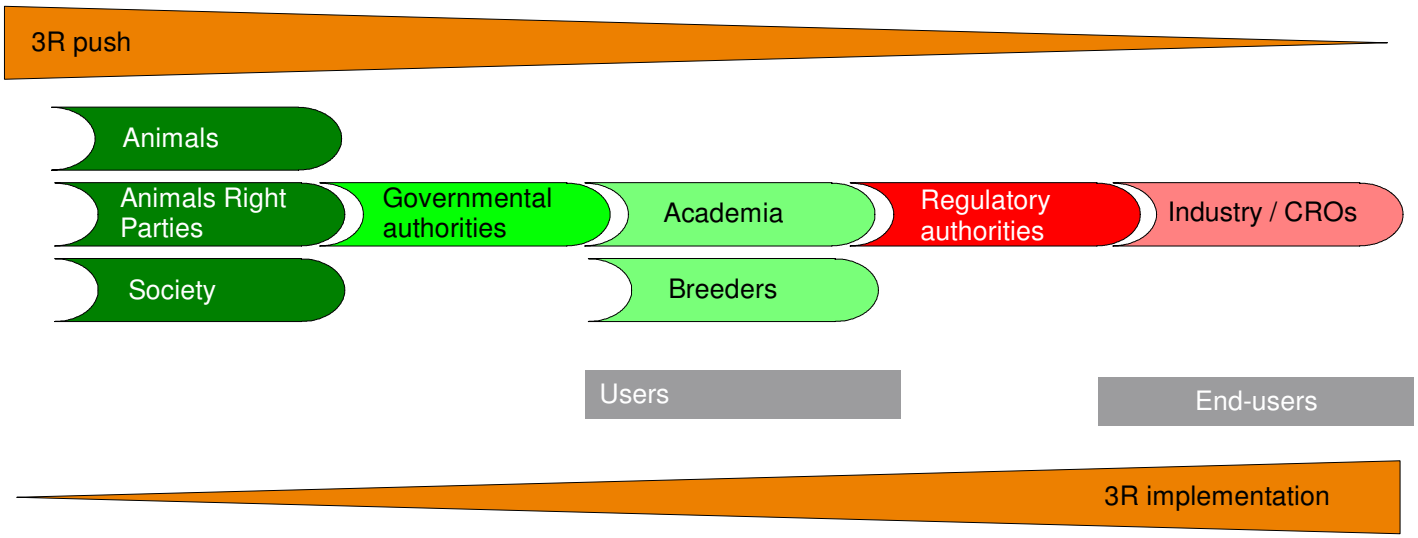

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Conclusion

- Holst Centre wireless sensor technology can perfectly fit-in to contribute to the principles of animal 3Rs (Refinement → 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 → **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

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Wireless Sensor Technology

Mother-Child Tracker
Mo-Chi Tracker

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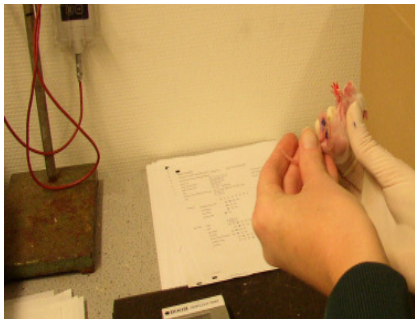
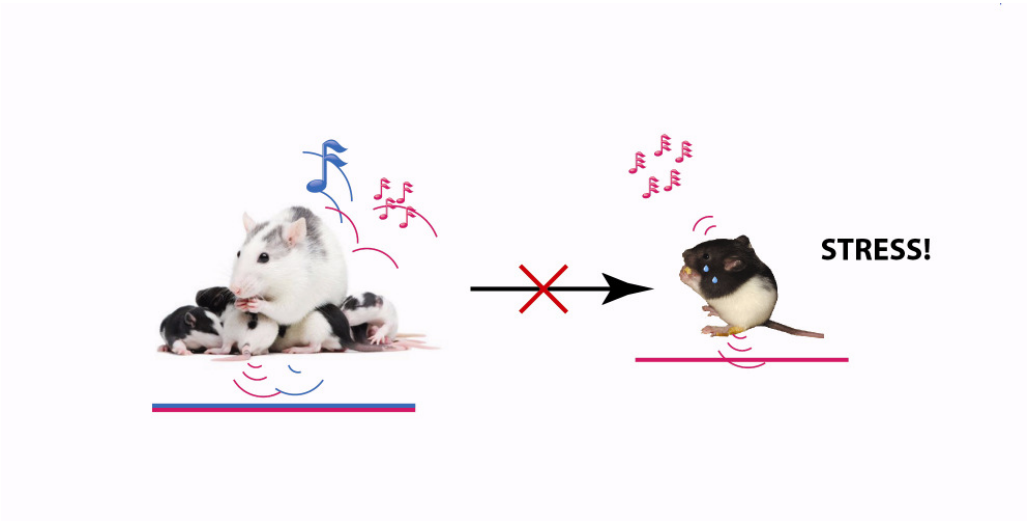
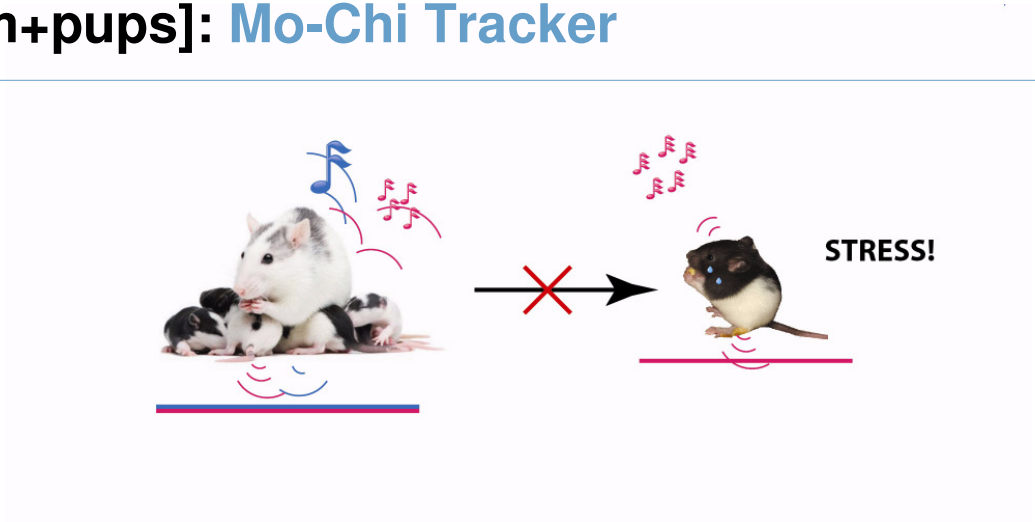
Dev.Tox.: Physical and Sensory Developmental Landmarks

Parameter	Test Period on postnatal day
Anogenital distance	1
Surface righting	2, 3, 4, 5, 6, 7
Pinna unfolding	2, 3, 4, 5, 6
Hair growth	4, 5, 6, 7, 8, 9, 10
Tooth eruption	9, 10, 11, 12, 13, 14, 15
Eye opening	14, 15, 16, 17, 18, 19, 20, 21
Air righting	14, 15, 16, 17, 18, 19, 20, 21
Auditory canal opening	10, 11, 12, 13, 14, 15
Auditory response	13, 14, 15
Pupil reflex	21, 22, 23, 24, 25




STRESS!

Automated *'stressless'* monitoring of intact litter [dam+pups]: Mo-Chi Tracker



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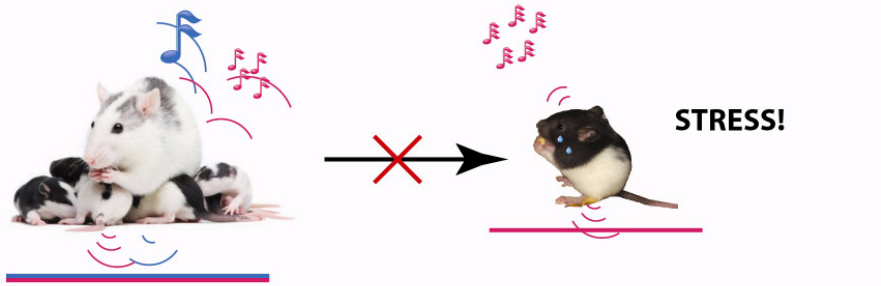

- TNO product idea **Mo-Chi Tracker** (Mother-Child tracker) for Dutch SBE
- Supported Ministry of Economic Affairs
- **Principle** of the product-idea is based on biological **differences** that *a priori* exist between adult mother animal and young immature pups with regard to e.g.: Size, weight, posture,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

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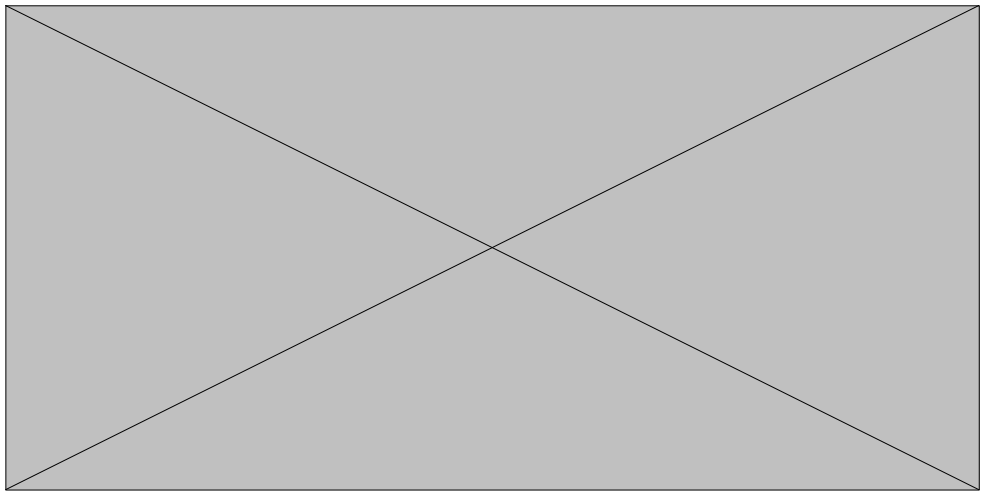
Thank you for your attention!



1. Didima de Groot *et al* | 1. TNO
2. Erik de Vries *et al* | 2. Groningen UMC / PET
3. Arend Heerschap *et al* | 3. Nijmegen Radboud UMC/ MR(S)I

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Especially

Animal Facilicities, Behaviour-/Biotechnicians, Histotechnicians

MRI / PET imaging

N Jetten[1], V.J de Groot[1], M Berk[1,3], R Nederlof[1], CF Kuper[1]

B Voet[1], M Bogaart[1], E. Uitvlugt[2], R Dierckx[2], L vd Horst[1], A Veltien[3]

Gene expression

Marijana Radonjic, Ros Stierum

1. Didima de Groot *et al* | 1. TNO
2. Frank Bouwens *et al* | 2. Imec/Holst: Sensor technology
3. R Bulthuis, B Bierman | 3. Metris, Produxi: Mo-Chi tracker

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Wireless sensor technology for animal welbeing

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Mo-Chi Tracker

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TNO innovation for life

Back-up slides

Benefit on animal welfare

Neuropathology

Guideline ‘neuropathology’ survey: >160 rats sacrificed

PND 21

Vehicle		Low dose		Medium dose		High Dose		TOTAL
10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	80

+

PND 61

Vehicle		Low dose		Medium dose		High Dose		TOTAL
10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	80

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>160

Proposed alternative ‘neuropathology’: animal reduction 50%

Vehicle		Low dose		Medium dose		High Dose		TOTAL
10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	80

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Vehicle		Low dose		Medium dose		High Dose		TOTAL
10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	80

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

→ PND 61: MRI other **5** ♂**, 5** ♀(repeated measures!); sacrifice

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10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	80

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- Analysis MR scans of **vehicle** and **high dose** prior to neuropathology

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- Split brain into 2 halves: neuropathology + microarray gene expression
- 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 repeately measured

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

Vehicle		Low dose		Medium dose		High Dose		TOTAL
10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	80

Guideline ‘behaviour’: >80 rats repeately measured

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

Vehicle		Low dose		Medium dose		High Dose		TOTAL
10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	80

Limitations:

- Mild burden on animal wellfare, **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

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10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	80

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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

Vehicle		Low dose		Medium dose		High Dose		TOTAL
4 ♂	4 ♀	4 ♂	4 ♀	4 ♂	4 ♀	4 ♂	4 ♀	32

Proposed alternative 'behaviour': animal reduction 50%

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

Vehicle		Low dose		Medium dose		High Dose		TOTAL
4 ♂	4 ♀	4 ♂	4 ♀	4 ♂	4 ♀	4 ♂	4 ♀	32

- Include juvenile age, e.g. PND 35
- Use [18F]FDG uptake = measure for glucose metabolism ↔ brain activty ↔ synaptic activity ↔ neuronal activity
- Keep Motor Activity testing (link to conventional testing) but combine with microPET testing *(before tracer dosing and during tracer distribution).*

Proposed alternative 'behaviour': animal reduction 50%

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

Vehicle		Low dose		Medium dose		High Dose		TOTAL
4 ♂	4 ♀	4 ♂	4 ♀	4 ♂	4 ♀	4 ♂	4 ♀	32

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

Benefit animal wellfare: Better information with fewer animals and tests; brain activity of concious rat is measured under anaesthesia

Wireless sensor technology & refinement