# How to link test system to the prediction of developmental neurotoxicity (DNT)

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***In Vitro Toxicology and Biomedicine***
***Doerenkamp-Zbinden Chair ,***
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# New methods should not ‚copy' this approach

OECD test guideline TG 426 defines animal DNT testing

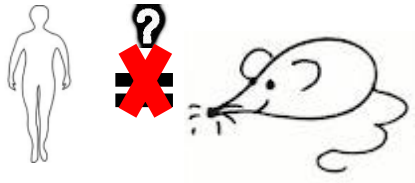
OECD TG426 (DNT)

Rodent 


Readouts:
Pathology
Function (sensory, motor, behavioural, ...)

**Main feature:**
Endpoint description of relative changes
(e.g. brain size, spontaneous motor behaviour, etc..)

**Problem:**
What do the relative changes mean?
Are the changes relevant to human development?
Concordance between species: 60%

Human 

# Some key questions for moving ahead: what can we learn from the past and from other big fields?

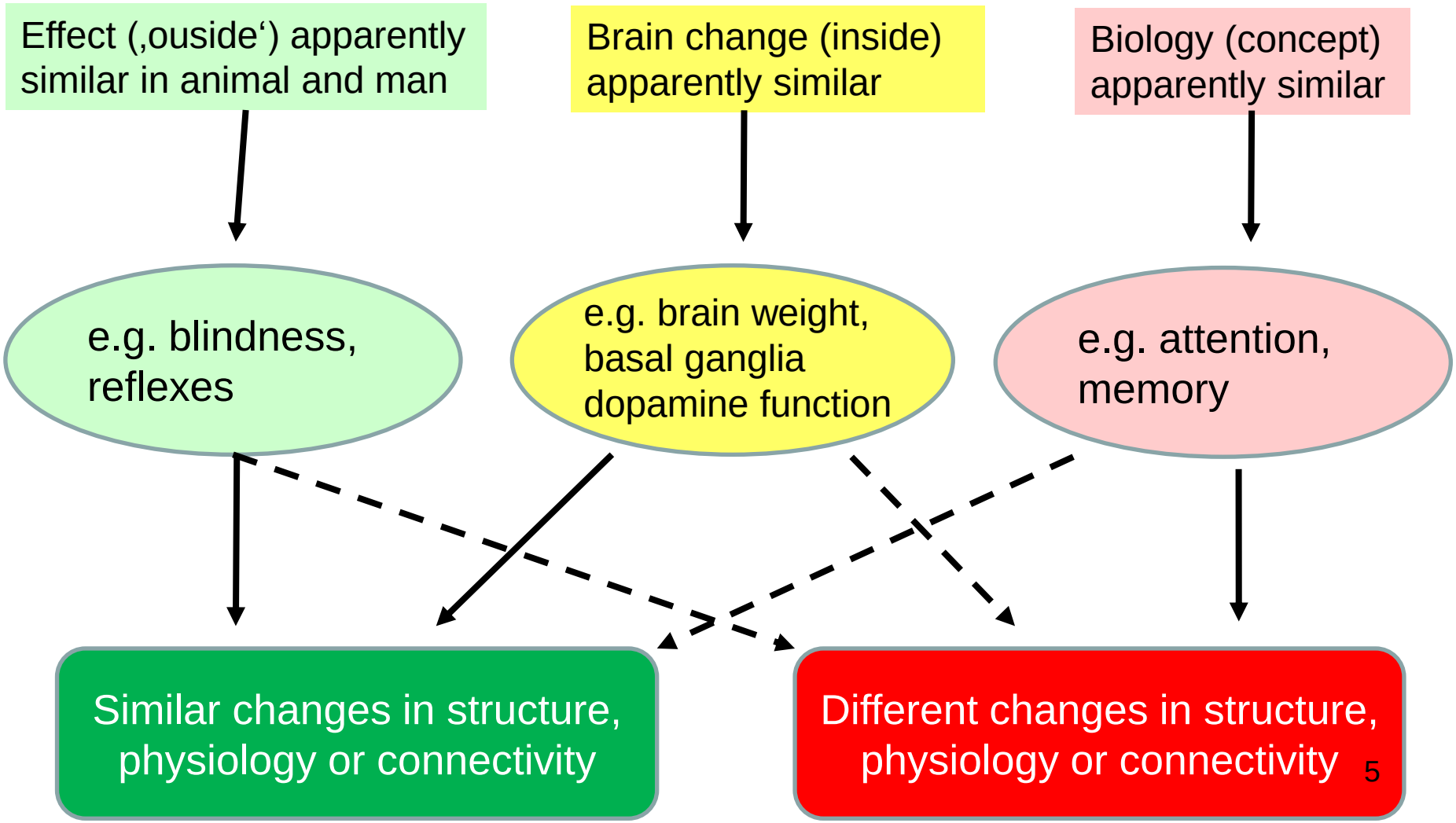
1. What are the **assumptions** underlying the use of **animal models**?

2. Can we make similar **assumptions for in vitro models**?

3. What can we **learn from psychiatry**?
(categories of models)

4. What can we **learn from biologics** drug production?
(switch from endpoint control to process control)

# Animals yield **three** categories of data



# Conclusion I

1. Current models (animals) mainly model altered states (fixed endpoints)

2. Most endpoints relevant to man cannot be measured in animals (language disturbance, attention span, IQ, etc...)

3. **Assumption to make animal models work**:
there are changes of brain structure or organisation that are conserved;
external signs for such endogenous changes are used as surrogate endpoint

# Illustrations

## Different phenotype, same brain changes...:

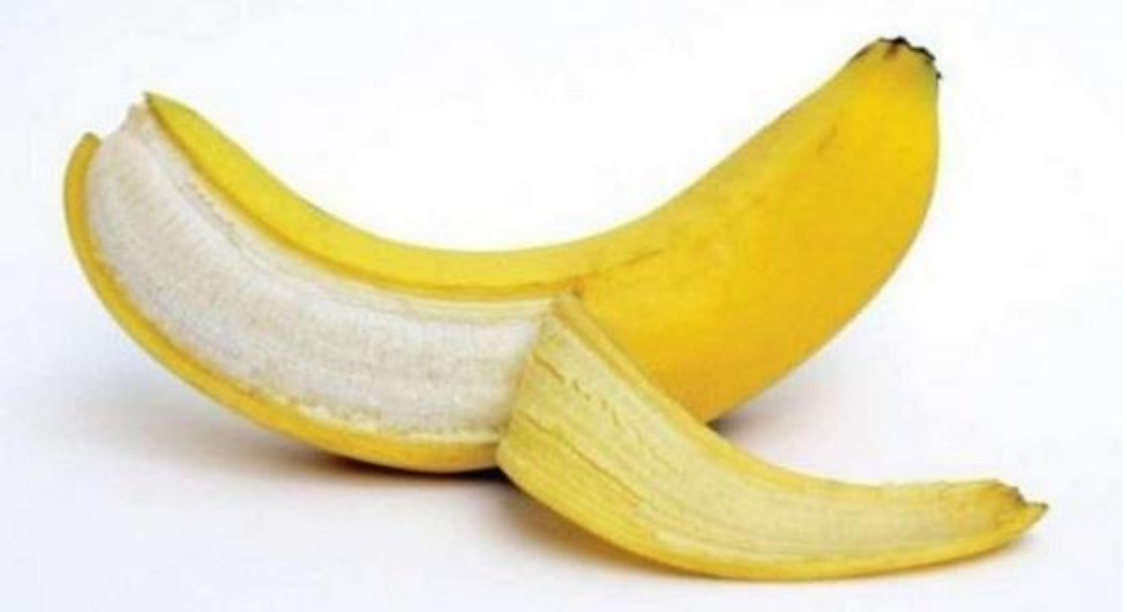
Augmented basal ganglia dopamine release (amphetamine):
Hyperactivity in **animals**
Psychosis in **humans**

## Same phenotype (outside), different brain changes...:

Altered brain weight in **animals** due to loss of neurons
Altered brain weight in **humans** due to reduced gliogenesis and neurite growth

Blindness in **animals** due to toxicity to retinal ganglia cells
Blindness in **humans** due to reduced blood supply to optic nerve

# Shoud we look at wrapping (outside) or at content (endogenous change)?



# Can we learn from psychiatry/neurology research?

(Research area > 1000-fold larger than DNT research)

## Disease:

Disease symptoms can look very similar on the outside
(→ **phenotype, exophenotype**),
although they have, e.g. in genetic diseases, entirely different causes and internal changes
(→ **endophenotype**)

## Models:

Models can from the outside look very similar to the disease
(→ **face validity**),
Models can refer to a similar internal/mechanistic working
(→ **construct validity**)

# Conclusion (II) from brain sciences:

The biological changes ‚**inside the brain**‘ are
an anchoring point to define a disease:
They are called **endophenotypes**.

Models with construct validity refer
to **comparable endophenotypes**, and allow comparisons
between disease model and human disease

**In toxicology:**
predictive models reflect human-relevant
**toxicity endophenotypes**.
The exophenotypes (reduced verbal memory,
diminished executive functions, social anxiety etc.)
are hard to model.

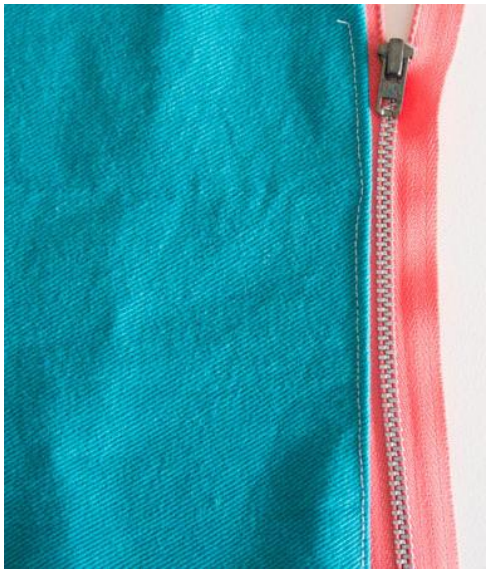
# Conclusion (II) from brain sciences:

**In toxicology:**
predictive models reflect human-relevant
**toxicity endophenotypes**.

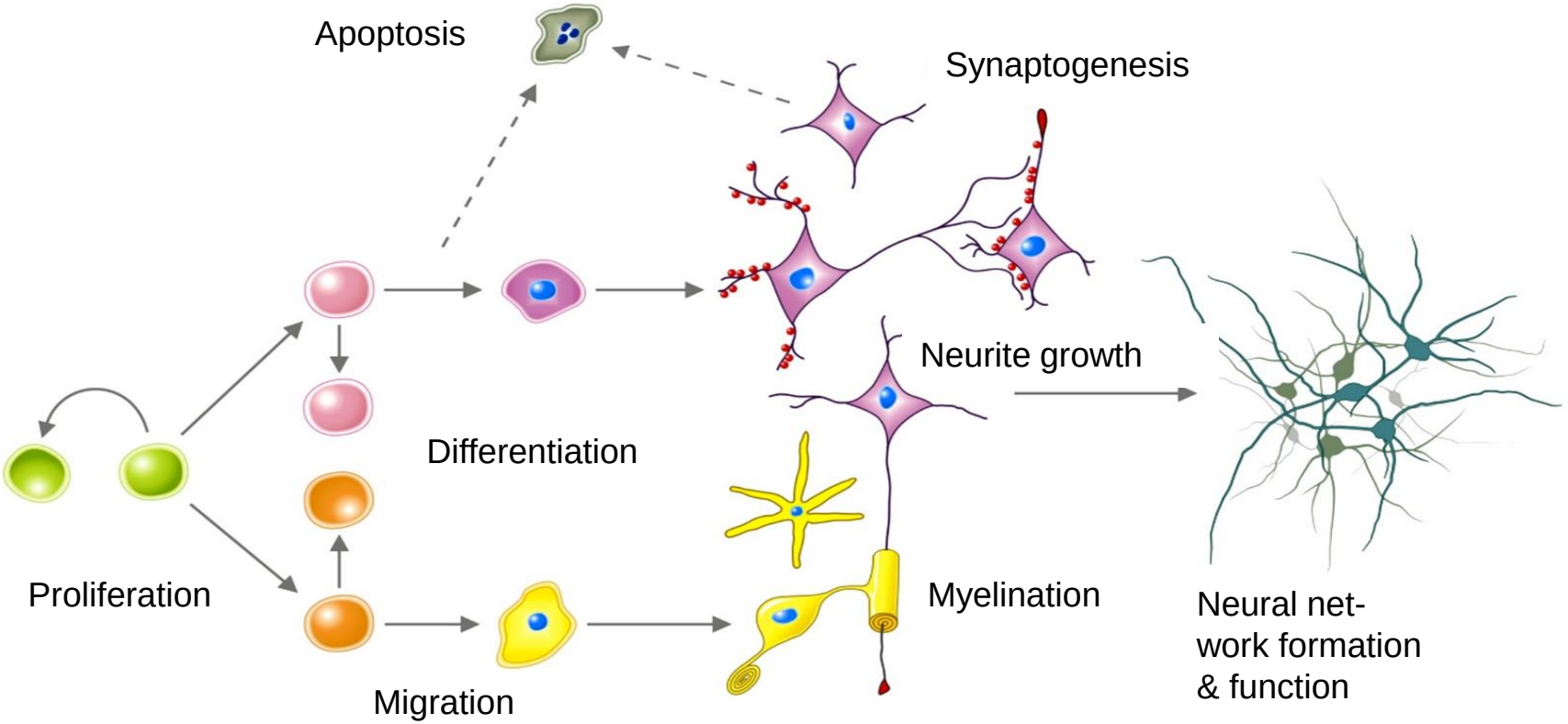
*The exophenotypes (reduced verbal memory, diminished executive functions, social anxiety etc.) are hard to model.*

# What determines normal or non-normal brain structure (functional or structural connectivity)?

Right or wrong:
defined by the **integrity of the processes** leading
to the final state



# Any toxicity endophenotype is the result of disturbances of one or more fundamental neurodevelopmental processes




*Aschner et al. (2016) ALTEX, in press*

# Eventually, any DNT finding (man or animal) must be due to a combination of disturbed neurodevelopmental processes

**If a compound does not disturb at least one process, it cannot be associated with a DNT hazard**

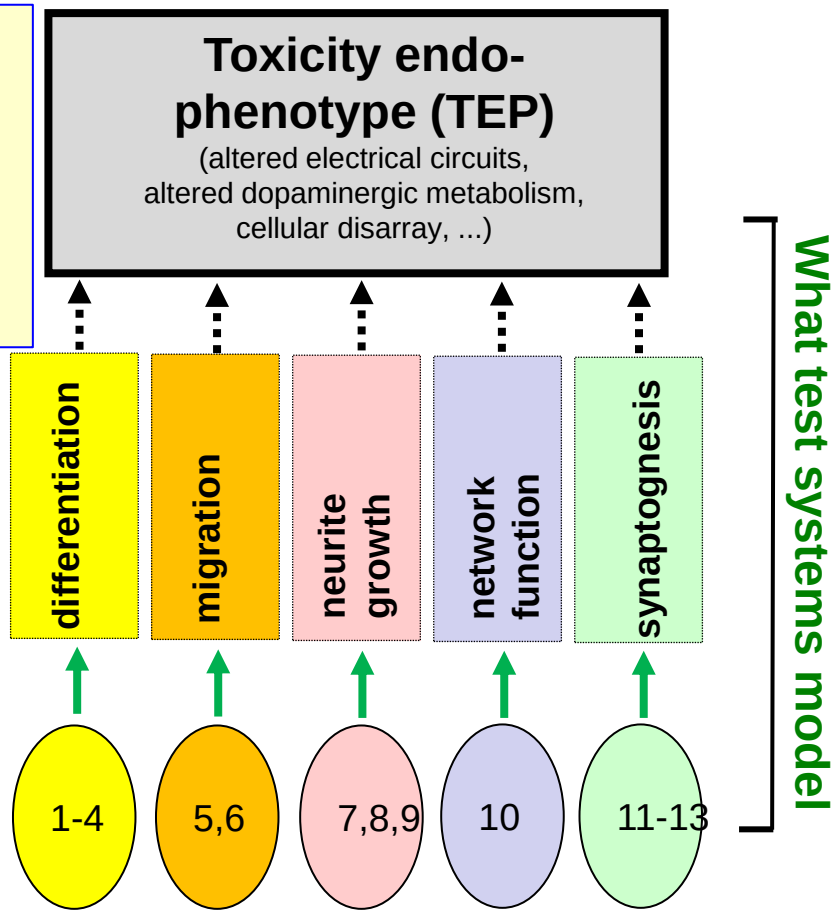
| In vivo Finding | Disturbed neurodevelopmental processes |
|---|---|
| Brain weight up/down | **Proliferation**, **Apoptosis** |
| Holoprosencephaly | **Apoptosis**, **Neurodifferentiation** |
| Lissencephaly | **Apoptosis**, **Neurodifferentiation**, **Migration** |
| Neuroinflammation | **Astrocyte activation, Gliosis**, **Neurodegneration** |
| Cortical layer thickness | **Proliferation**, **Migration**, **Myelination** |
| Disturbed reflexes | **Neurodifferentiation**, **Myelination**, **Synaptic transmission** |
| Anxiety behaviour | **Neurodifferentiation**, **Synaptic transmission, Synapse formation** |

# Toxicity endophenotypes (TEP) are linked to human and animal outcomes. How are test systems linked to TEP?

**Each fundamental neurodevelopmental process can be reflected by a test system**

**Fundamental neurodevelopmental processes**

**Test systems battery**
(Test No. #)



# Lessons from the development of biologics

*(Erytropoietin, vaccines, blood factors,...)*

**End control:**

The final product cannot be sufficiently controlled / described

**Process control:**

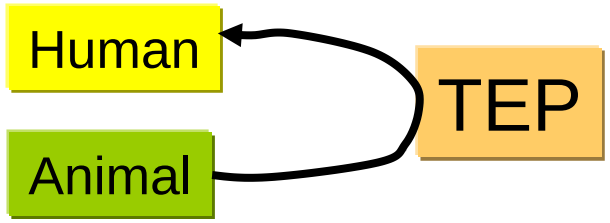
If every production step is OK, then end product is OK.

**Process control for DNT hazard evaluation: measuring whether a compound disturbs any of the key neurodevelopmental**

John Meynard Keynes:

**‚The difficulty lies, not in the new *ideas*, but escaping the *old* ones‘**

**Old idea:**
**Prediction of safety from undisturbed phenotype**

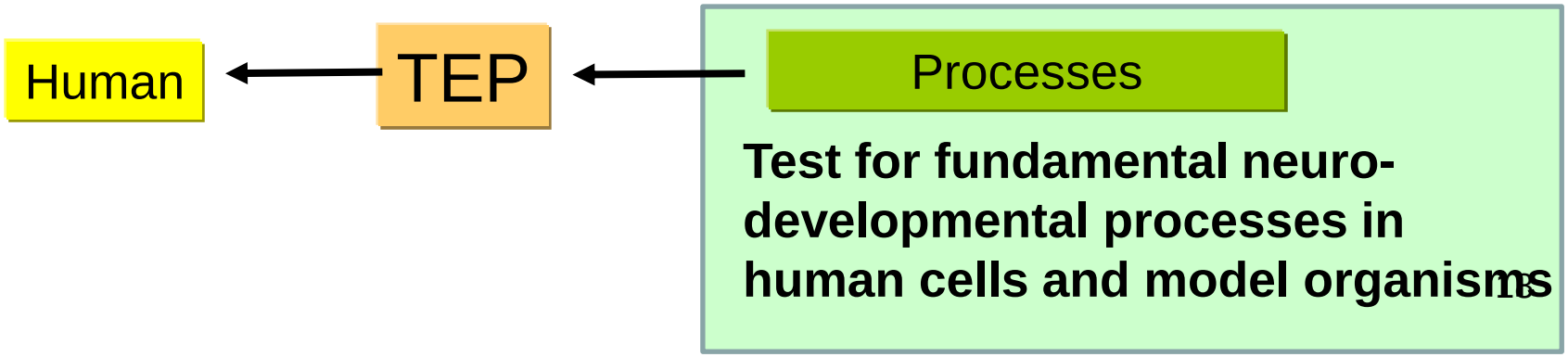



*TEP: toxicity endophenotype*

John Meynard Keynes:

**‚The difficulty lies, not in the new *ideas*, but escaping the *old* ones‘**

**New idea:**
**Prediction of safety from undisturbed key processes**

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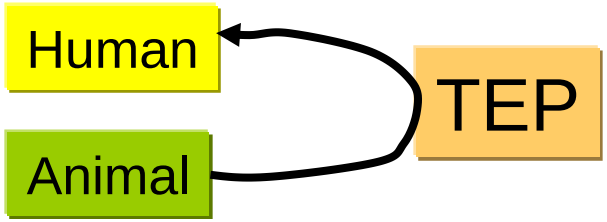


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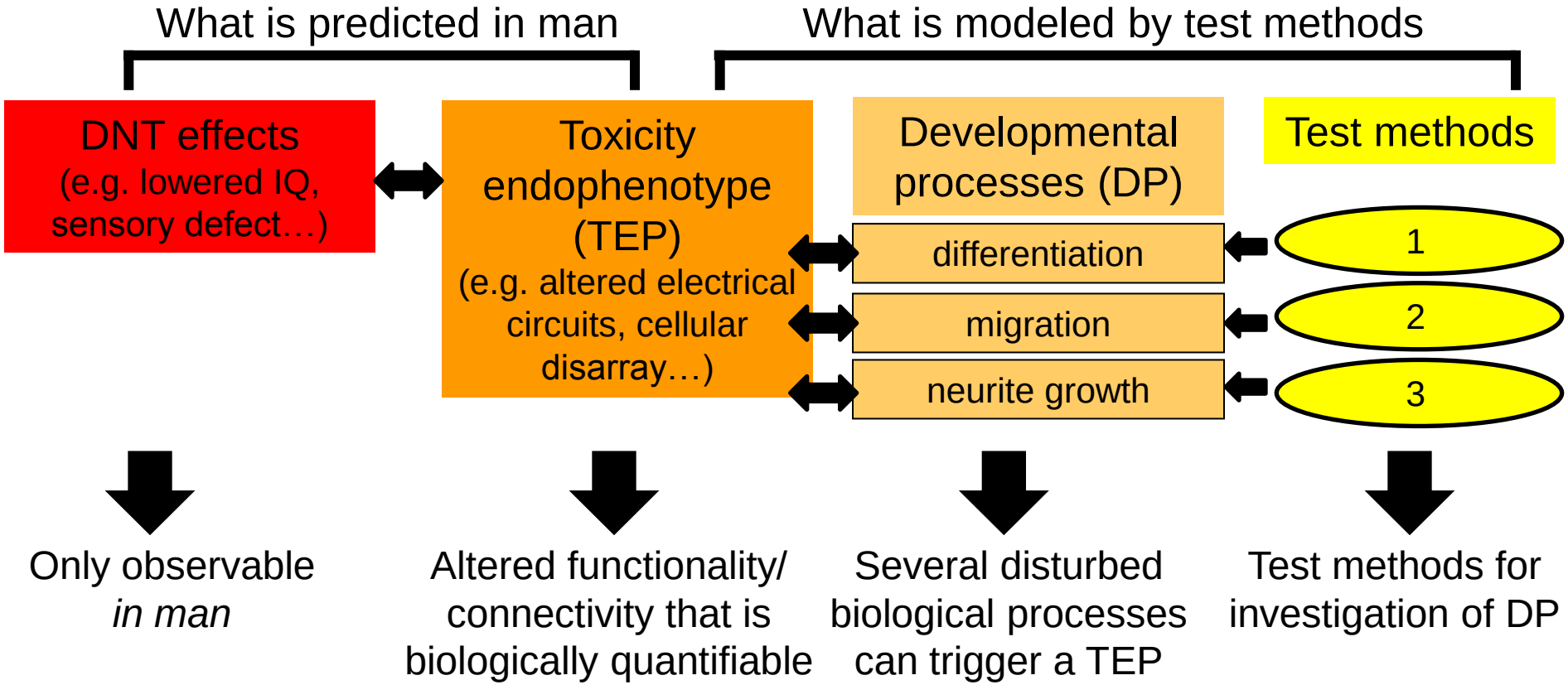


**Final conclusion: a process control-based test strategy for DNT**