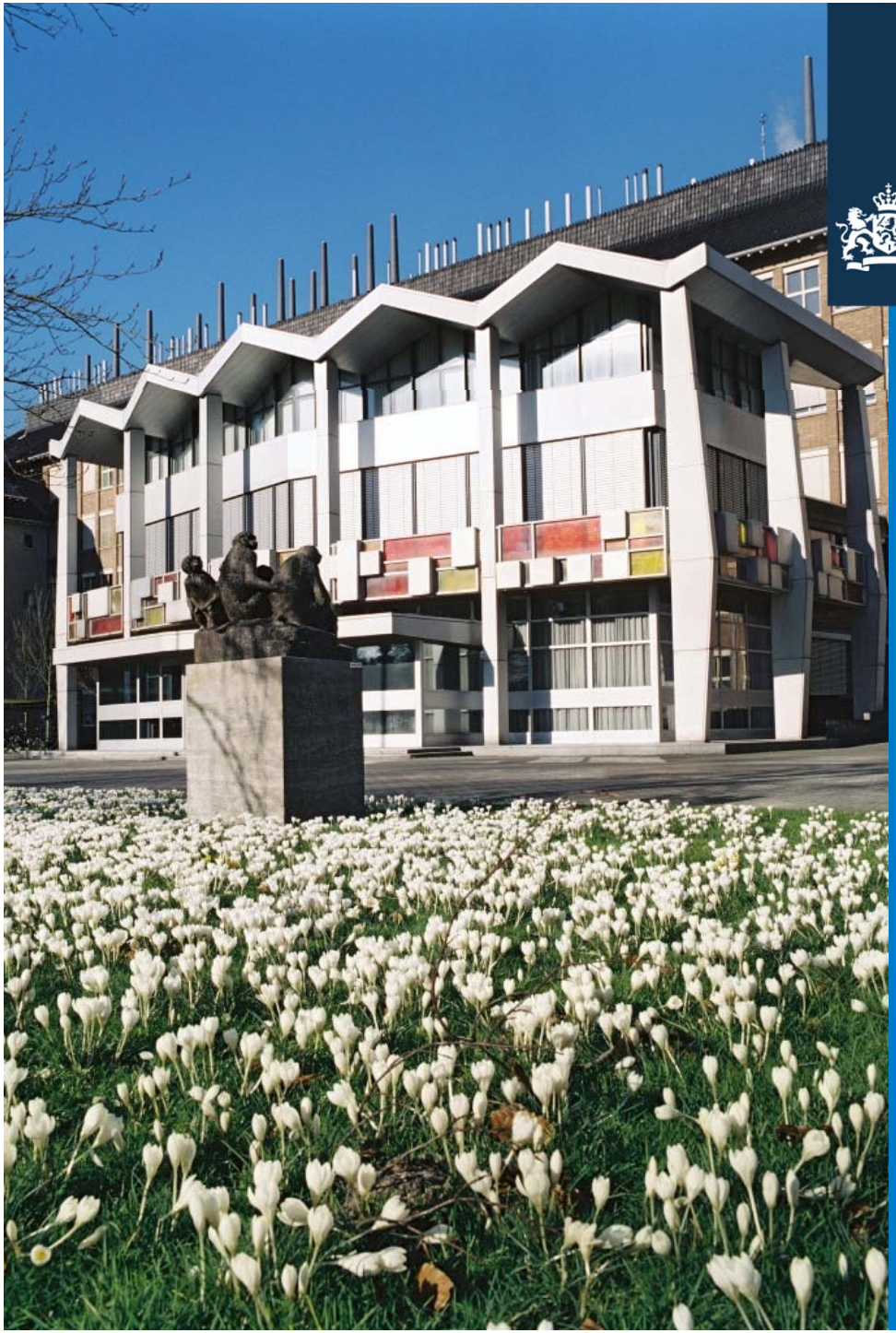


National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Organ-on-a-Chip: What is needed from a risk assessment perspective?

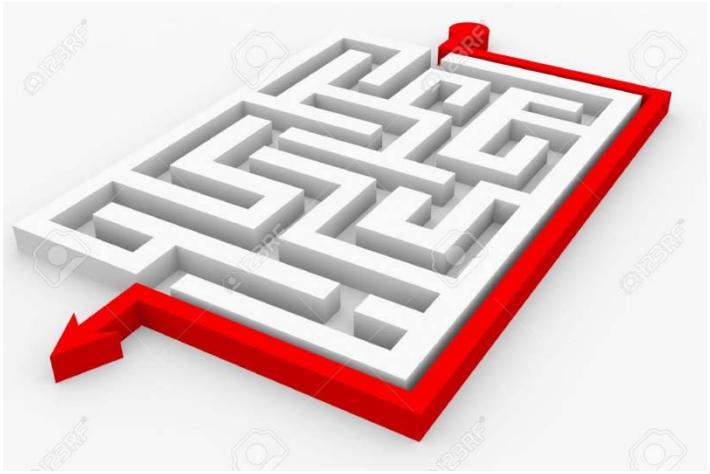
Minne Heringa, Rob Vandebriel, Adrienne Sips, Flemming Cassee, Wim de Jong, Margriet Park, Anne Kienhuis

Organ-on-a-Chip: symposium NVT| 10 april 2018

Challenges for RIVM

- Government aim to have animal toxicity tests replaced
- Desire for better prediction effects in humans
- Nanomaterials: so many forms for which toxicity data are needed → animal testing for all unrealistic

→ Can organs-on-a-chip (OC) be part of the solution?



Available alternatives for regulatory toxicity data

Endpoint	Accepted non-animal method available?
Acute toxicity	
Irritation/Corrosion	√
Sensitisation	√
Repeated dose toxicity	Alternatives necessary!
Mutagenicity	√
(Carcinogenicity)	
Reproduction toxicity	
(Neurotoxicity + Immunotoxicity)	

In addition: kinetics/ biodistribution

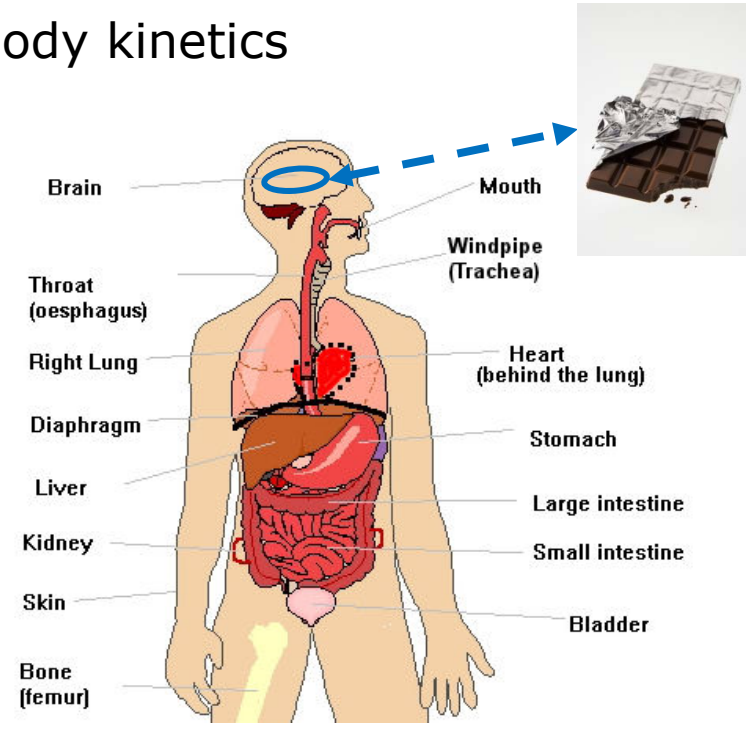
Testing tissues separately → miss whole body kinetics

→ Test for absorption/translocation, (distribution), metabolism and excretion and use kinetic models to combine

lung, skin, intestine, liver, kidney

or

→ Combine tissues and substance flows in human-on-a-chip



Promising aspects organs-on-a-chip

- Longer viability cell culture (already 28 days)
  → repeated dose toxicity testing possible

- Can use human tissue → better prediction human effects/kinetics than in animal tests?

- Different toxicity and kinetics seen for substances in OC than in 2D or static 3D or OC without lung strain → better prediction of human effects/kinetics than in 2D, static 3D and OC without strain?
  - Easier acceptance?

- Can be performed high-throughput → efficient testing of many substances or nanomaterial forms?

1. Which tissues?

- Current *in vivo r*epeated dose toxicity tests (e.g. OECD TG 407, 28d oral test): 21 tissues for histopathology

  Brain (including cerebrum, cerebellum and medulla/pons), spinal cord, stomach, (small and large intestines (including Peyer's patches)), liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea, lungs, ovaries, testes, seminal vesicles, uterus, prostate, urinary bladder, lymph nodes, peripheral nerve, bone marrow , (larynx), (nasopharyngeal tissue), (oesophagus)

- Analysis RepDose database Fraunhofer ITEM (Batke et al., 2013):

  Liver, kidney, clinical chemistry, body weight, clinical symptoms and hematology are the first affected endpoints oral studies, at the Lowest Observed Effect Level (LOEL).

  → probability of 86% to detect the LOEL

  → probability of 98% for detecting LOAEL + 1 dose level

  - Body weight and clinical symptoms not analyzable with organs-on-a-chip → need to find replacement
  - From which tissues do the affected clinical chemistry and hematology parameters originate?

Table 3
Fractions of targets affected in old ($n = 56$) and comprehensive subchronic oral rat studies ($n = 88$). Fractions are given at the LOEL (old and comprehensive studies) as well as at the LOEL + 1 and for LOELs determined by a single target (comprehensive studies).

Target	Fraction (% of studies)			
	Old	Comprehensive		
	LOEL	LOEL	LOEL + 1	LOEL single target
Clinical chemistry	17.9	38.6	56.8	9.1
→ Liver	44.6	31.8	55.7	5.7
Haematology	16.1	29.5	46.6	11.4
→ Kidney	37.5	23.9	40.9	4.5
Body weight	37.5	21.6	44.3	2.3
Clinical symptoms	17.9	18.2	42.0	1.1
→ Testes	10.7	8.0	17.0	
Urine analysis	17.9	6.8	13.6	1.1
→ Forestomach		6.8[a]	9.1[a]	3.4[a]
→ Heart	3.6	5.7	13.6	1.1
→ Spleen	7.1	4.5	15.9	2.3
→ Brain	5.4	2.3	8.0	
→ Stomach	1.8	2.3	5.7	
→ Intestine		2.3	3.4	1.1
→ Adrenal gland	5.4	1.1	8.0	1.1
→ Thyroid gland	5.4	1.1	4.5	
→ Thymus		1.1	5.7	

In inhalation or dermal studies:

- Lung ←
- Skin ←
- Other order

Caution: probably 28d tests included <2008, less organs included then → Other order now?

Batke et al., 2013

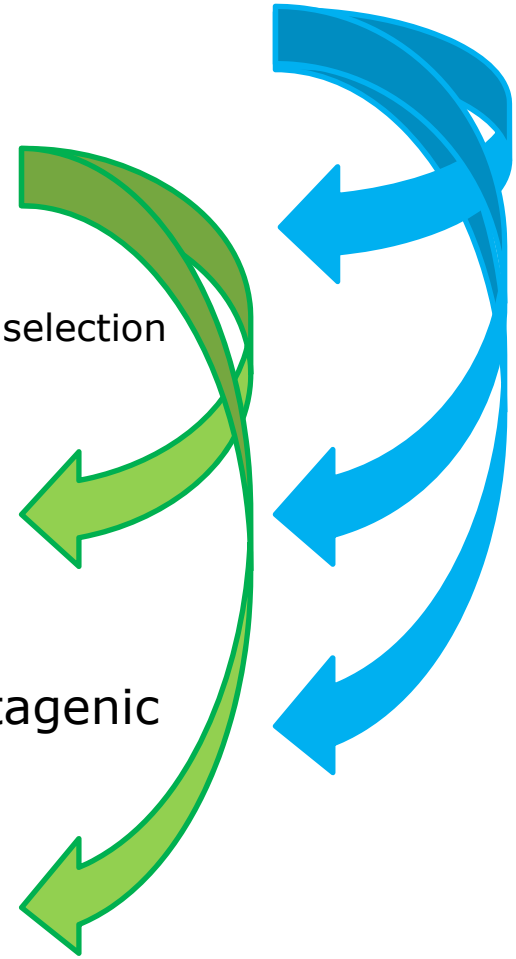
Envisaged roadmap OCs for tox. risk assessment

1. OC models for toxicokinetics
   - Necessary to validate the rest

2. OC models for repeated dose toxicity
   - Now highest demand
   - RepDose database with effects per tissue available → tissue selection possible

3. OC models for acute toxicity
   - See if tissues from repeated dose are sufficiently predictive

4. OC models for reproduction toxicity and non-mutagenic carcinogenicity??
   - Usually required only for high production volume chemicals
   - Reprotox very complicated, ethical barriers
   - Carcinogenicity may be included in repeated dose testing



Tissues of interest and priority

Need	Tissue	OC developed?
Kinetics and repeated dose toxicity	1. Liver 2. Kidney 3. Intestine 4. Lung 5. Skin	Yes Yes Yes Yes Yes
Repeated dose toxicity and (developmental) immunotoxicity	6. Spleen 7. Thymus (+ rest immune system)	No (except physical filter) No
Repeated dose toxicity and (developmental) neurotoxicity	8. Brain	No (except BBB)
Repeated dose toxicity and reproductive toxicity	9. Testes (+ rest reproductive system)	No
Repeated dose toxicity Workshop June 2017	10. Heart 11. Stomach 12. Adrenal gland 13. Thyroid gland	Yes No No No

Preliminary outcome workshop

- For some tissues: time to optimize and standardize
  → Risk assessors (users) need to indicate precise needs
- For other tissues/endpoints: development needed
  → Mostly academic research needed
- For immune system: rather function-on-a-chip
- Research agenda: to be published

- Questionable whether we need viability for 90 days

- Not only focus on OoaC, but all advanced in vitro methods: only as complex as necessary

2. What level of complexity is needed?

Types of risk assessment questions

(from Peter Bos and Jacqueline van Engelen)

1. Classification and labelling
   - Qualitative, only hazard assessment (no exposure), high doses
2. Limit value
   - Quantitative, only hazard and kinetics assessment, per route of exposure, precision needed for key effect
3. Actual risk assessment
   - Quantitative, when there is exposure already; compare exposure to limit value, all possible effects matter

→ Need different types of data for different cases → different demands on test system

Necessary complexity depends on more factors

Example for absorption assessment

In silico: Lipinski rule of 5 → absorption likely or unlikely

a. Result can be used in design of new substances

In vitro 2D Caco2 test: absorption high, medium or low (<20%)

a. If high or medium: exact % usually not of significance → use 2D result
b. But if around MOS of 100 or RCR of 1, then exact % absorption may be of importance → proceed with OC
c. If low: exact % is of significance → proceed with OC

In vitro OC with strain: exact % absorption and flux

a. If flux necessary for e.g. acute risk: start directly with OC

→ Research need: show where OC models have an added value over 2D, static 3D, OC without strain, etc.

Conclusion: much to do towards OECD TGs, but we have a plan!

Thanks!

- Organ-on-a-chip team RIVM: Rob Vandebriel, Flemming Cassee, Adrienne Sips, Wim de Jong, Anne Kienhuis, Margriet Park

- You for your attention

Further contact:

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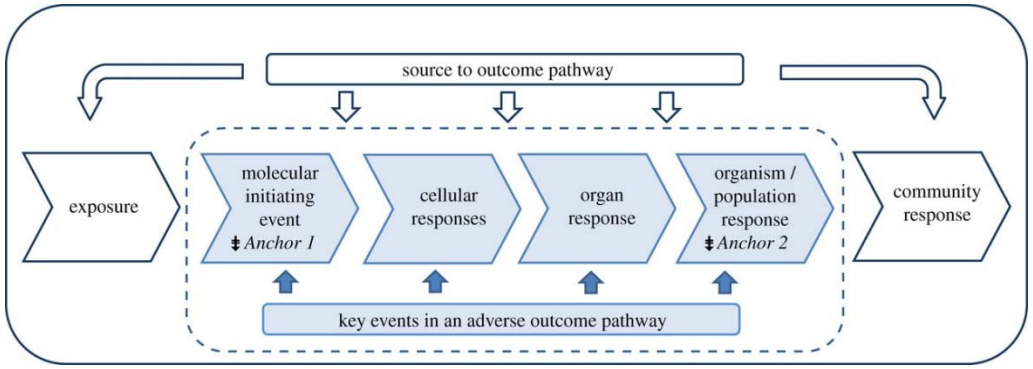
Other needs

- Costs? (compared to animal study)
- Reproducibility and robustness
- Validation: how?
  - Leave this for later, in future workshop perhaps
- Need Adverse Outcome Pathways to determine which initiating/key events to measure in OC, as predictor for clinical effect (e.g. change in behaviour)



- Finally need to place OC among other methods in an integrated testing strategy (ITS or IATA)

Tissue selection: other considerations

- Currently growing interest for developmental neurotoxicity and (developmental) immunotoxicity
  - Not covered well in current tests or regulations
  - Societal costs of IQ-loss, ADHD, mental retardation, obesity and diabetes are much higher than for cancers or other curable or fatal diseases (report Rijk et al., 2016)

→ Future toxicity testing should include the detection of (the initiating events of) these types of effects

- Other effects in humans may not have been picked up by animal tests

→ stay open to include those