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 ONTOX



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Bob van de Water



# EU-ToxRisk Achievement: Guidance on method description.

**150** test method descriptions uploaded on the EU-ToxRisk Knowledge Platform

Archives of Toxicology (2020) 94:2435–2461  
<https://doi.org/10.1007/s00204-020-02802-6>

IN VITRO SYSTEMS



## The EU-ToxRisk method documentation, data processing and chemical testing pipeline for the regulatory use of new approach methods

Alice Krebs<sup>1,2</sup> · Barbara M. A. van Vugt-Lussenburg<sup>3</sup> · Tanja Waldmann<sup>1,20</sup> · Wiebke Albrecht<sup>4</sup> · Jan Boei<sup>5</sup> · Bas ter Braak<sup>6</sup> · Maja Brajnik<sup>7</sup> · Thomas Braunbeck<sup>8</sup> · Tim Brecklinghaus<sup>4</sup> · Francois Busquet<sup>9</sup> · Andras Dinnyes<sup>10</sup> · Joh Dokler<sup>7</sup> · Xenia Dolde<sup>1</sup> · Thomas E. Exner<sup>7</sup> · Ciarán Fisher<sup>11</sup> · David Fluri<sup>12</sup> · Anna Forsby<sup>13,21</sup> · Jan G. Hengstler<sup>4</sup> · Anna-Katharina Holzer<sup>1</sup> · Zofia Janstova<sup>10</sup> · Paul Jennings<sup>14</sup> · Jaffar Kisitu<sup>1,2</sup> · Julianna Kobolak<sup>10</sup> · Manoj Kumar<sup>15</sup> · Alice Limonciel<sup>14</sup> · Jessica Lundqvist<sup>13,21</sup> · Balázs Mihalik<sup>10</sup> · Wolfgang Moritz<sup>12</sup> · Giorgia Pallocca<sup>9</sup> · Andrea Paola Cediel Ulloa<sup>13</sup> · Manuel Pastor<sup>16</sup> · Costanza Rovida<sup>9</sup> · Ugis Sarkans<sup>17</sup> · Johannes P. Schimming<sup>18</sup> · Bela Z. Schmidt<sup>19</sup> · Regina Stöber<sup>4</sup> · Tobias Strassfeld<sup>12</sup> · Bob van de Water<sup>18</sup> · Anja Wilmes<sup>14</sup> · Bart van der Burg<sup>3</sup> · Catherine M. Verfaillie<sup>15</sup> · Rebecca von Hellfeld<sup>8</sup> · Harry Vrieling<sup>5</sup> · Nanette G. Vrijenhoek<sup>18</sup> · Marcel Leist<sup>1,9</sup>

Krebs et al. Arch Toxicol. 2020  
Jul;94(7):2435-2461.



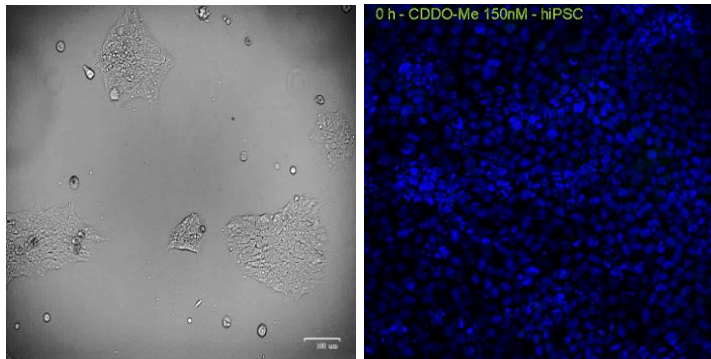
## Template for the Description of Cell-Based Toxicological Test Methods to Allow Evaluation and Regulatory Use of the Data

Alice Krebs<sup>1,2</sup>, Tanja Waldmann<sup>1</sup>, Martin F. Wilks<sup>3</sup>, Barbara M. A. van Vugt-Lussenburg<sup>4</sup>, Bart van der Burg<sup>4</sup>, Andrea Terron<sup>5</sup>, Thomas Steger-Hartmann<sup>6</sup>, Joelle Ruegg<sup>7</sup>, Costanza Rovida<sup>8</sup>, Emma Pedersen<sup>9</sup>, Giorgia Pallocca<sup>1,8</sup>, Mirjam Luijten<sup>10</sup>, Sofia B. Leite<sup>11</sup>, Stefan Kustermann<sup>12</sup>, Henicke Kamp<sup>14</sup>, Julia Hoeng<sup>14</sup>, Philip Hewitt<sup>15</sup>, Matthias Herzler<sup>16</sup>, Jan G. Hengstler<sup>17</sup>, Tuula Heinonen<sup>18</sup>, Thomas Hartung<sup>8,19</sup>, Barry Hardy<sup>20</sup>, Florian Gantner<sup>21</sup>, Ellen Fritsche<sup>22</sup>, Kristina Fant<sup>9</sup>, Janine Ezendam<sup>10</sup>, Thomas Exner<sup>20</sup>, Torsten Dunkern<sup>23</sup>, Daniel R. Dietrich<sup>24</sup>, Sandra Coecke<sup>11</sup>, Francois Busquet<sup>8,25</sup>, Albert Braeuning<sup>26</sup>, Olesja Bondarenko<sup>27</sup>, Susanne H. Bennekou<sup>28</sup>, Mario Beilmann<sup>29</sup> and Marcel Leist<sup>1,2,8</sup>

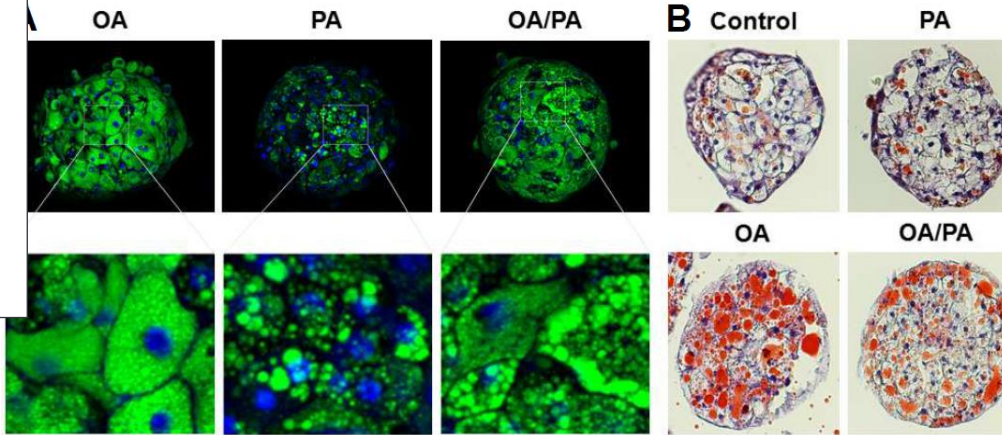
Krebs et al. ALTEX. 2019;36(4):682-699.

# EU-ToxRisk Achievement: Advanced novel test methods

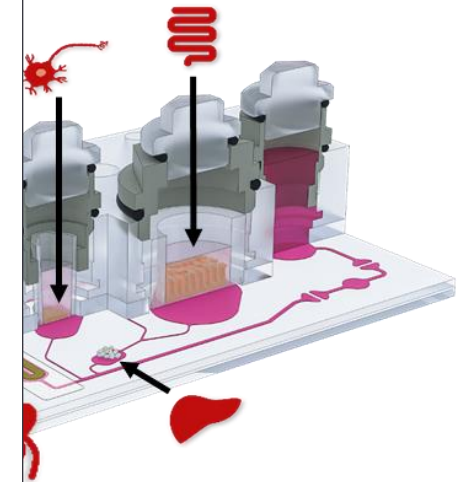
## CRISPR-based fluorescent reporters in stem cells



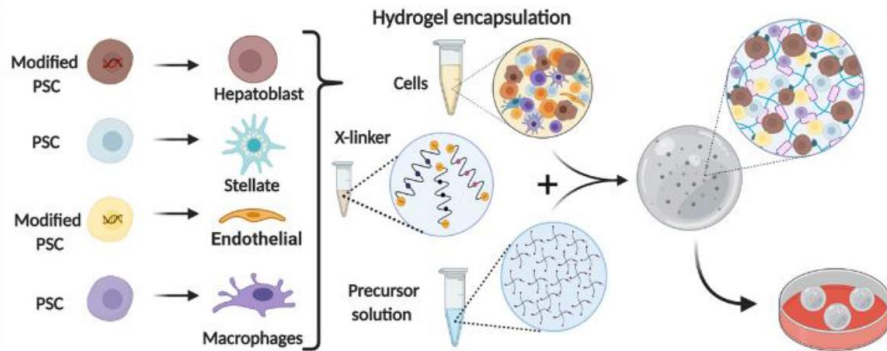
## Diseased liver microtissues



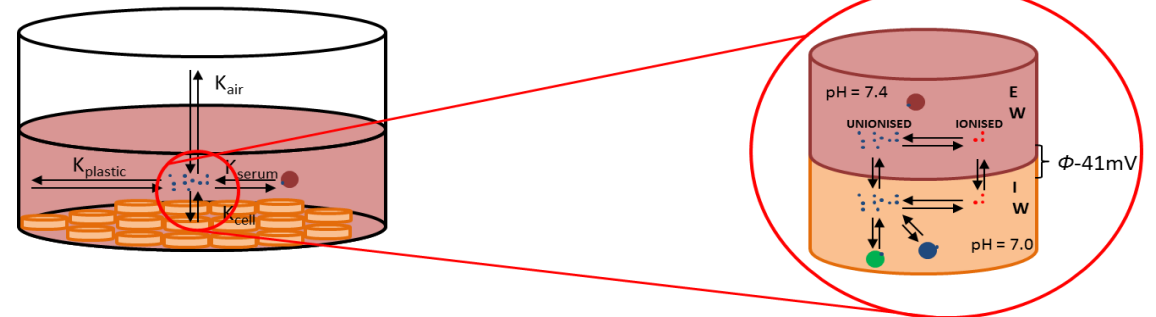
## Four-organ-chip



## Stem cell-derived multi-liver-cell model



## Virtual in vitro cell disposition model



# EU-ToxRisk Achievement: Advisory document on regulatory requirements for acceptance of NAM-assisted RAX

## Recommendations of the EU-ToxRisk Regulatory Advisory Board (RAB) on how to document case studies for regulatory evaluation

27 April 2018

Archives of Toxicology (2019) 93:3643–3667  
https://doi.org/10.1007/s00204-019-02591-7

GUEST EDITORIAL



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### Towards grouping concepts based on new approach methodologies in chemical hazard assessment: the read-across approach of the EU-ToxRisk project

Sylvia E. Escher<sup>1</sup>,  
Jan G. Hennekamp<sup>2</sup>,  
Manuel Pastor<sup>1</sup>

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### Meeting Report

## NAM-Supported Read-Across: From Case Studies to Regulatory Guidance in Safety Assessment

doi:10.14573/altex.2010062

The use of new data is a main goal of the collaboration within the European Committee on Toxicology (ECT) in the USA, as well as actively participating in the discussion. There was general agreement as well as characterization of similarity or difference data correct the uncertainty. An interesting point was avoiding the data in every case, but the case is still an



### t4 Workshop Report\*

## Internationalization of Read-Across as a Validated New Approach Method (NAM) for Regulatory Toxicology

Costanza Rovida<sup>1</sup>, Tara Barton-Maclaren<sup>2</sup>, Emilio Benfenati<sup>3</sup>, Francesca Caloni<sup>4</sup>, P. Charukeshi Chandrasekera<sup>5</sup>, Christophe Chesné<sup>6</sup>, Mark T. D. Cronin<sup>7</sup>, Joop De Knecht<sup>8</sup>, Daniel R. Dietrich<sup>9</sup>, Sylvia E. Escher<sup>10</sup>, Suzanne Fitzpatrick<sup>11</sup>, Brenna Flannery<sup>11</sup>, Matthias Herzler<sup>12</sup>, Susanne Hougaard Bennekou<sup>13</sup>, Bruno Hubsch<sup>14</sup>, Henricke Kamp<sup>15</sup>, Jaffar Kisitu<sup>16</sup>, Nicole Kleinstreuer<sup>17</sup>, Simona Kovarich<sup>18</sup>, Marcel Leist<sup>1,16</sup>, Alexandra Maertens<sup>19</sup>, Kerry Nugent<sup>20</sup>, Giorgia Pallocca<sup>1</sup>, Manuel Pastor<sup>21</sup>, Grace Patlewicz<sup>22</sup>, Manuela Pavan<sup>23</sup>, Octavio Presgrave<sup>24</sup>, Lena Smirnova<sup>19</sup>, Michael Schwarz<sup>25</sup>, Takashi Yamada<sup>26</sup> and Thomas Hartung<sup>1,19</sup>

Prediction of a 90 day repeated dose toxicity study (OECD 408) for 2-Ethylbutyric acid using a read-across approach to other branched carboxylic acids.

Read-across based filling of developmental and reproductive toxicity data gap for methyl hexanoic acid.

Identification and characterization of parkinsonian hazard liability of deguelin by an AOP-based testing and read across approach

### Waiving of repeat-dose neurotoxicity study (TG 424) for azoxystrobin based on Read-Across to other strobilurins

#### 1 Abstract / Synopsis / Executive summary

This section should provide a brief overview of the case study, including the objectives, concepts, methodologies, outcomes, and conclusion in about 300 words.

The synthetic strobilurin fungicides are derived from the naturally occurring strobilurin A and B. The strobilurins bind to the quinol oxidation site of cytochrome b of complex III of the mitochondria which is also their fungicidal mode of action. There are some signals of potential neurotoxicity from in vitro studies by a CILI-mediated mechanism.

The objective of this read-across case is to justify the waiving of an OECD TG 424 study for azoxystrobin by means of NAM data. The source compounds are other strobilurin fungicides. The formation of the category is based on the hypothesis that the compounds share similar chemical structure, similar pesticidal mode of action, similar toxophore, similar neurotoxic potential and similar toxicokinetics to azoxystrobin. The source compounds chosen were pyraclostrobin, picoxystrobin, trifloxystrobin, and kresoxim-methyl. Furthermore, testing was conducted on Antimycin A, a well-established CILI inhibitor with neurotoxic effects, which serves as a reference compound for this mode of action. The degree of in vivo inhibition of the mitochondrial respiratory system depends on the respiratory activity and thus the tissues like brain can be more susceptible.

Existing regulatory in vivo data was collected for the source and target compounds with a focus on ADME, neurotoxicity as well as target organ toxicity data. The source compounds do neither show signs of neurotoxicity in neurotoxicity studies nor in other repeat dose toxicity studies.

The scientific hypothesis is: Can the absence of a neurotoxic potential (as detected with a TG424 study) mediated by inhibition of Complex III of the mitochondria be predicted by toxicodynamic and toxicokinetic NAM data?

The hypothesis is supported by mechanistic data, anchored to a putative AOP (based on the recently OECD adopted AOP on CILI inhibition leading to parkinsonian disorder), and kinetic PBTK data. Thus, the following data was obtained: physicochem, structural similarity (animoto-index), effects on oxygen consumption (mitochondrial complexes and whole cells), effects on mitochondrial membrane potential, cellular damage measured by effects on glycolysis and cell viability in three different cell types including neuronal cells, neuronal degeneration and neurite outgrowth.

The overall structural similarity of the compounds, although having the same pesticidal mode of action and toxophore is less.

Inhibition of CILI complexes measured by oxygen consumption, by the target compound azoxystrobin seemed to be slightly less strong than by the source compounds pyraclostrobin and picoxystrobin, while antimycin A resulted in a much stronger inhibition. This was confirmed with whole cells as well. Effects on membrane potential were marked by Antimycin A and orders of magnitude less with the target and source compounds. Effects on glycolysis and cell viability were similar between the compounds. The target compound was negative in the neurite outgrowth assay in SH-SY5Y cells, while some of the source compounds did show weak effects, and neither the target nor the source compounds were regarded as neurotoxic in the neurotoxic assay in LUNHES cells.

ak, Enrico Mombelli, Frederico Boia, Paul Jennings, Rabaea Graepel, Ulf Henrich Gladstetter, Thomas Eimer, ...

butyric acid (2-EBA) has to be more than 100 µg/kg bw/day with oral exposure. We use a read-across approach to other branched carboxylic acids. We use a scenario based on a consistent trend identified in the in vivo studies in silico models are used in the characterization.

a short chain, branched carboxylic acid with different branched carboxylic acid (2-EBA) has to be more than 100 µg/kg bw/day with oral exposure. We use a scenario based on a consistent trend identified in the in vivo studies in silico models are used in the characterization.

chondrial respiratory system and Parkinson disease. It has been accepted and validated in the OECD AOP template. The identification of an AOP and the subsequent testing and read across approach are currently on the AOP list. The and routinely applied that reflect complex human (deguelin). Moreover, used approaches to of cellular exposure the relevance of the specific that rotenone re for the binding to mitochondrial behavior in cell dysfunction, with so more potent than the mode-of-action as application of an AOP of the AOP MIE and structurally related

is correctly predicted as in vivo toxicants. The NAM results are more light on the structural carboxylic acids. On the basis of this in vivo reproductive toxicant.

is describing the development of a model was established, based on the PBTK models were all properties and in vitro clearance (CL<sub>int, liver</sub>) were good predictive and. Based on this proof of concept for all analogues

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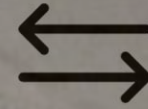
endpoints covered, as well as the class of insecticides and pesticide. The two since 2008



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Animal-free  
Safety assessment of Chemicals:  
Project cluster for  
Implementation of novel  
Strategies

# ASPIS

*Revolutionary defensive tool, designed to push forward into the opposing army, joining forces for better protection*



## **ASPIS:** “Animal-Free Safety Assessment of Chemicals: Project Cluster for Implementation of Novel Strategies”

- 2021-2026 under H2020
- €60M funded budget
- 70 institutions united in 3 projects across 16 EU countries + US

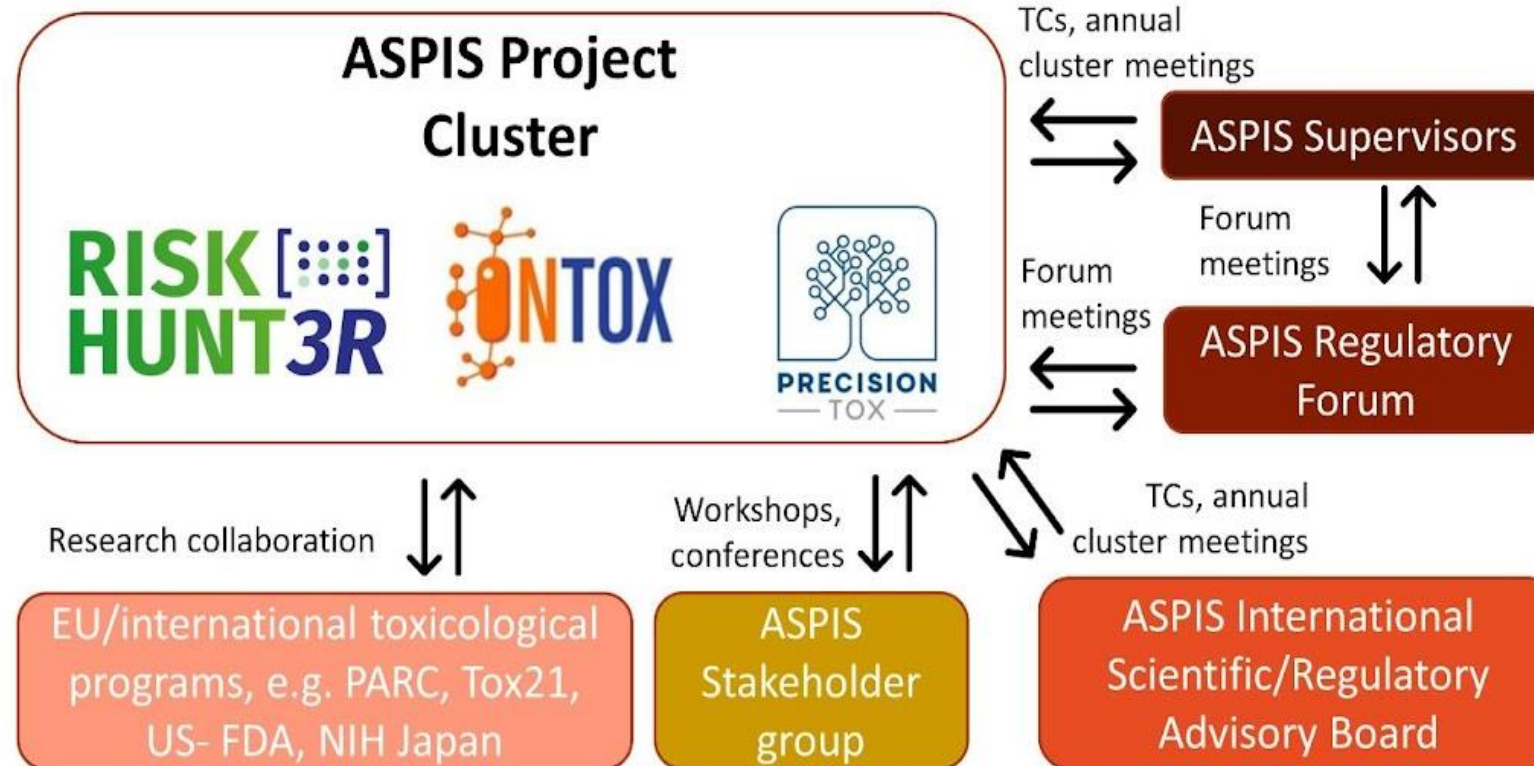
# Objectives



- **advance NAMs** for the protection of human health and the environment
- **improve certainty** in the safety assessment of chemicals
- facilitate practicably implementable **non-animal solutions** in various public (e.g. regulatory agencies) and private (e.g. industry) sectors
- **translate results, methods and solutions** from the scientific research community into **safety assessment practice**
- **promote regulatory uptake** and **commercial exploitation of NAMs**
- contribute to the **3R principles**



# ASPIS interaction with satellite entities



# Background of NGRA approaches

Food for Thought ...

## Ready for Regulatory Use: NAMs and NGRA for Chemical Safety Assurance

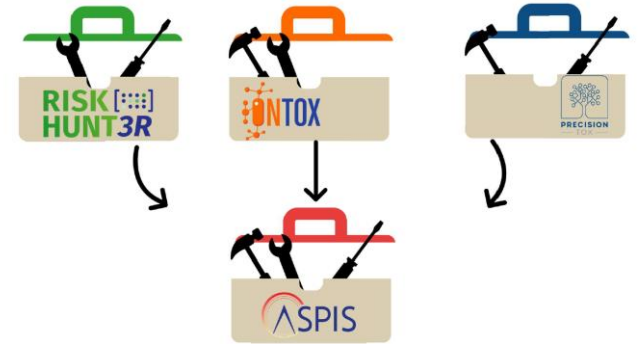
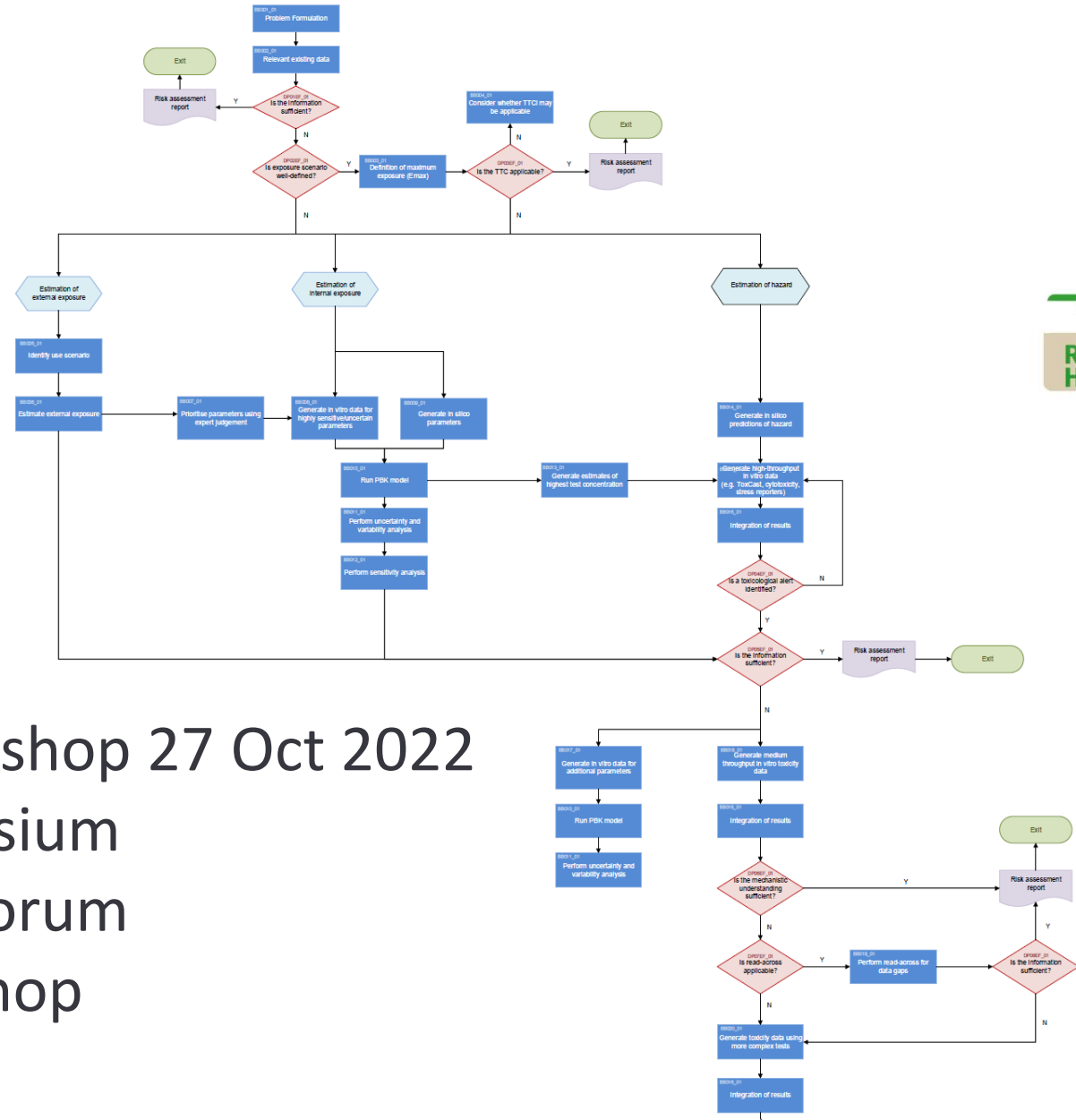
Paul L. Carmichael<sup>1,2</sup>, Maria T. Baltazar<sup>1</sup>, Sophie Cable<sup>1</sup>, Stella Cochrane<sup>1</sup>, Matthew Dent<sup>1</sup>, Hequn Li<sup>1</sup>, Alistair Middleton<sup>1</sup>, Iris Muller<sup>1</sup>, Georgia Reynolds<sup>1</sup>, Carl Westmoreland<sup>1</sup> and Andrew White<sup>1</sup>

<sup>1</sup>Safety & Environmental Assurance Centre (SEAC), Unilever, Sharnbrook, Bedfordshire, UK; <sup>2</sup>Toxicology, Wageningen University & Research, Wageningen, The Netherlands

ALTEX (2022), 399, 419	The assessment is...
OBJECTIVES	1. focused on safety
	2. exposure-led
	3. hypothesis-driven
	4. based on adversities (rather than “perturbations”)
PROCEDURE	The assessment uses...
	5. consideration of all existing info
	6. tiered and iterative approaches
DOCUMENTATION	7. robust and relevant methods and strategies
	The assessment includes...
	8. documentation and quantification of uncertainty
	9. documentation of all steps and the rationale for conclusions

# ASPIS NGRA workflow

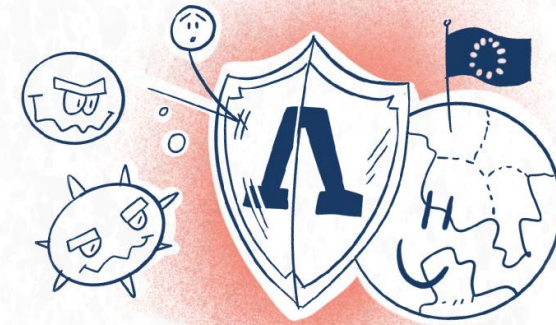
ASPIS NGRA workflow draft v1



- ASPIS partner workshop 27 Oct 2022
- ASPIS Open Symposium
- ASPIS Regulatory Forum
- Stakeholder workshop
- Case studies



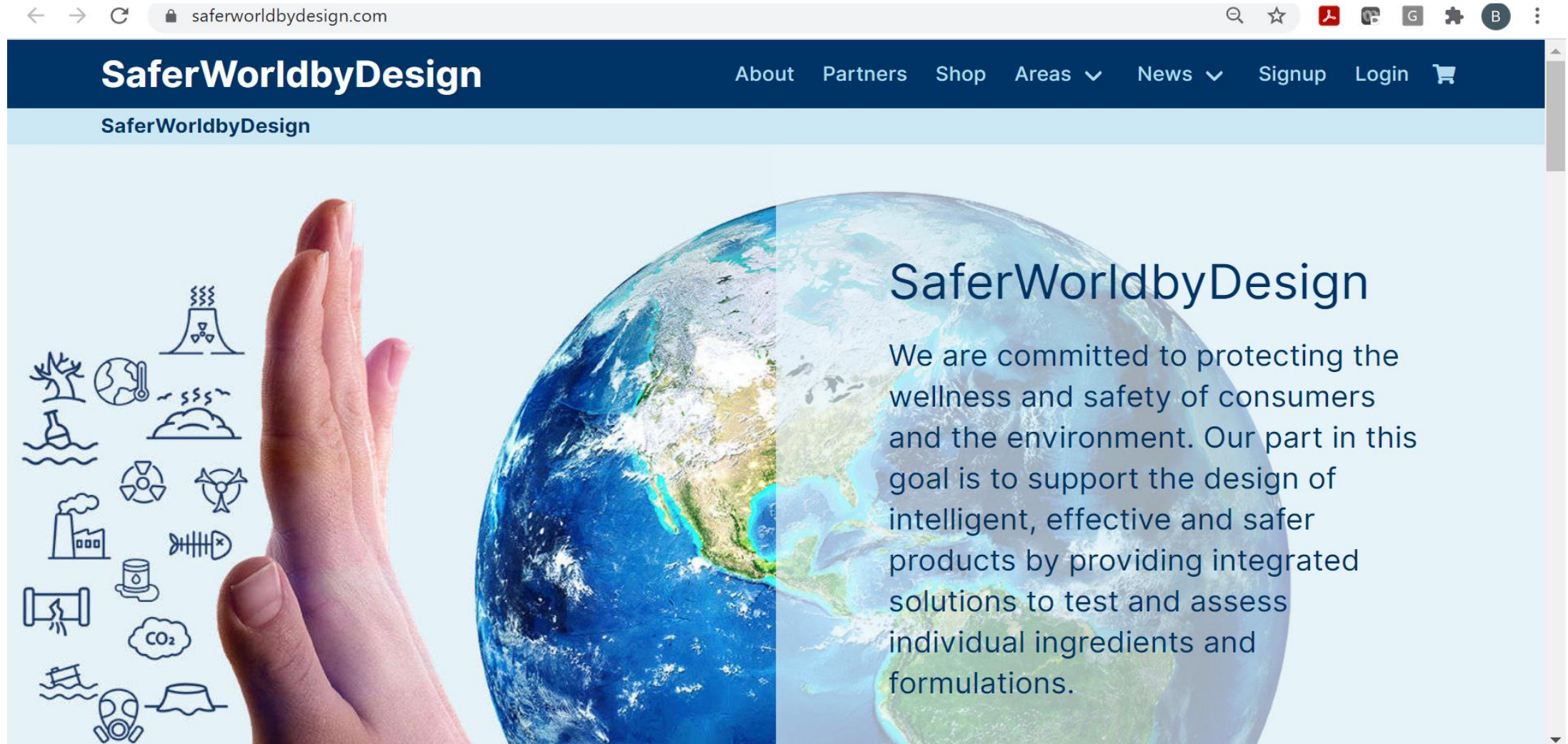
# ASPIS open symposium



[www.aspis-cluster.eu](http://www.aspis-cluster.eu)

24-25 nov 2022  
Sitges - Spain

# EU-ToxRisk Achievement: SaferWorldbyDesign




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SaferWorldbyDesign



**SaferWorldbyDesign**

We are committed to protecting the wellness and safety of consumers and the environment. Our part in this goal is to support the design of intelligent, effective and safer products by providing integrated solutions to test and assess individual ingredients and formulations.

# Critical issues for discussion.

- Need to facilitate ‘validation’ of science driven test methods.
- Requirement to enhance access of test methods for stakeholders.
- Need to break the (stakeholder) barrier for NAM application in NGRA.



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# Thank you!



*This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 964537.*