



# Public-private platform for the pre-validation of endocrine disruptors characterization methods.

Philippe HUBERT, PEPPER

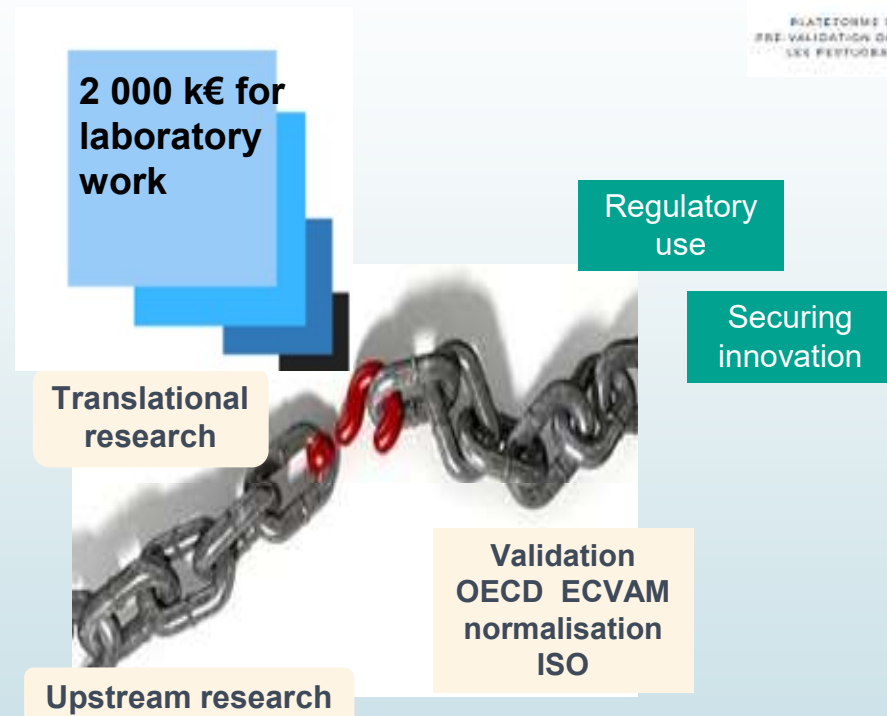
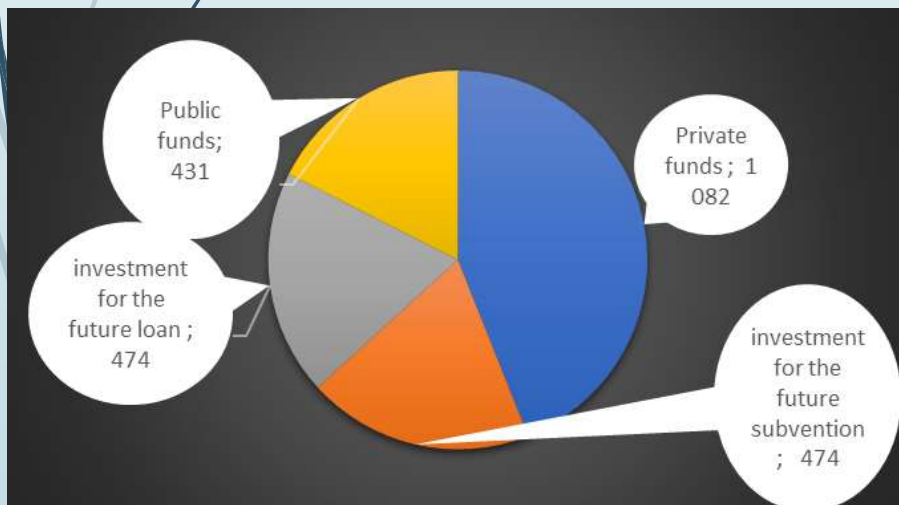
<https://ed-pepper.eu/>



# A unique Public Private Partnership



- Non profit association dedicated to prevalidation
- Helping, organizing and funding labs in prevalidation field operations
- Speeding up international validation
- Private/public resources



Average 3 first years ; k€

**Part of French National Strategy on Endocrine Disruptors**

## PEPPER within Europe



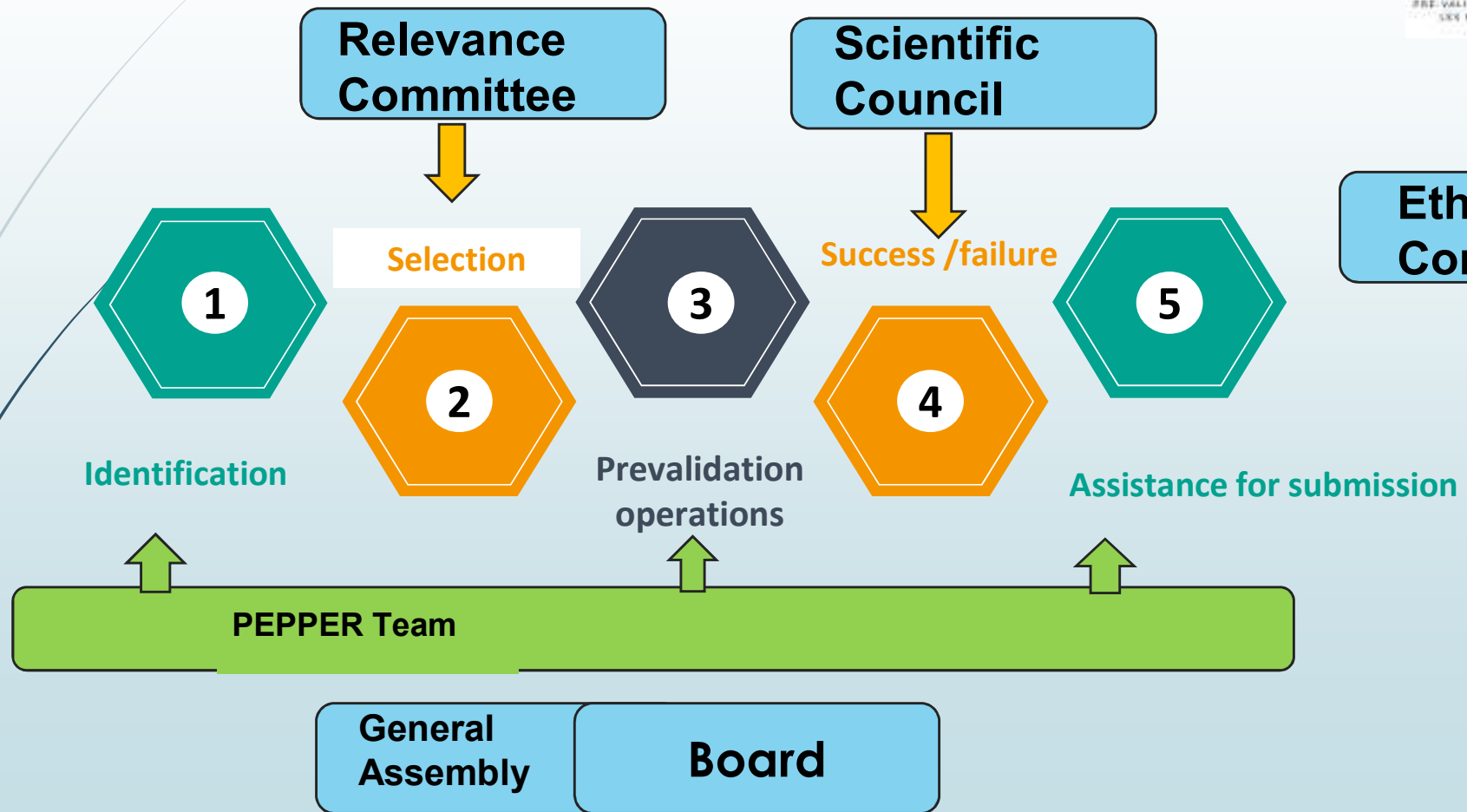
- A shared concern : more validated methods are needed on **EDs**
- A **request**: 2018 EC communication en Eds : *“The Commission will **step up its support to the work of relevant international organisations** and **encourages Member States to do the same**. Of particular importance is the need to provide the Organisation for Economic Co-operation and Development with the necessary support to progress in the development of internationally agreed test guidelines.”*
- **PEPPER** targets weaknesses recognized by institutions
- A French initiative, supported at Member States and EU levels



Participants to PEPPER governance

- Industry, retailers, NGOs, Government, members of parliament (EU & French) launched together the process in 2014
- Build up thanks to a task force and Ineris studies
- Funded in 2018 and launched **December 2<sup>nd</sup> 2019**

## Governance and Work Flow



# Identification and preselection of methods : spring and summer 2020



ALERTOX  
academy

Interviews of stakeholders

data base and literature  
survey  
IA aided on 12000 papers

Analysis of identified gaps

259

Broad list of methods  
Assessment of Readiness  
level  
75

Sort list for Relevance  
Committee  
With descriptions  
17



- answering expressed needs by institutions & filling recognized gaps
- not validated or undergoing a validation process (e.g. ECVAM Work on Thyroid)
- mature enough for prevalidation measured by Test Readiness Criteria (Bal-Price et al)



†4 Workshop Report\*

**Recommendation on Test Readiness Criteria for New Approach Methods in Toxicology: Exemplified for Developmental Neurotoxicity**

Anna Bal-Price<sup>1</sup>, Helena T. Hogberg<sup>2</sup>, Kevin M. Crofton<sup>3</sup>, Mardas Daneshian<sup>4</sup>, Rex E. FitzGerald<sup>5</sup>,



# Sources of information on needs


6



Screening of available evidence on chemical substances for the identification of endocrine disruptors according to different options in the context of an Impact Assessment

Specific Contract SANTE/2015/E3/SI2.706218

Final report



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Cooperation with authorities and Endocrine Disruptor Expert Group



European Food Safety Authority

Adverse Outcome Pathway WIKI



BETTER POLICIES FOR BETTER LIVES

Plant Protection Products



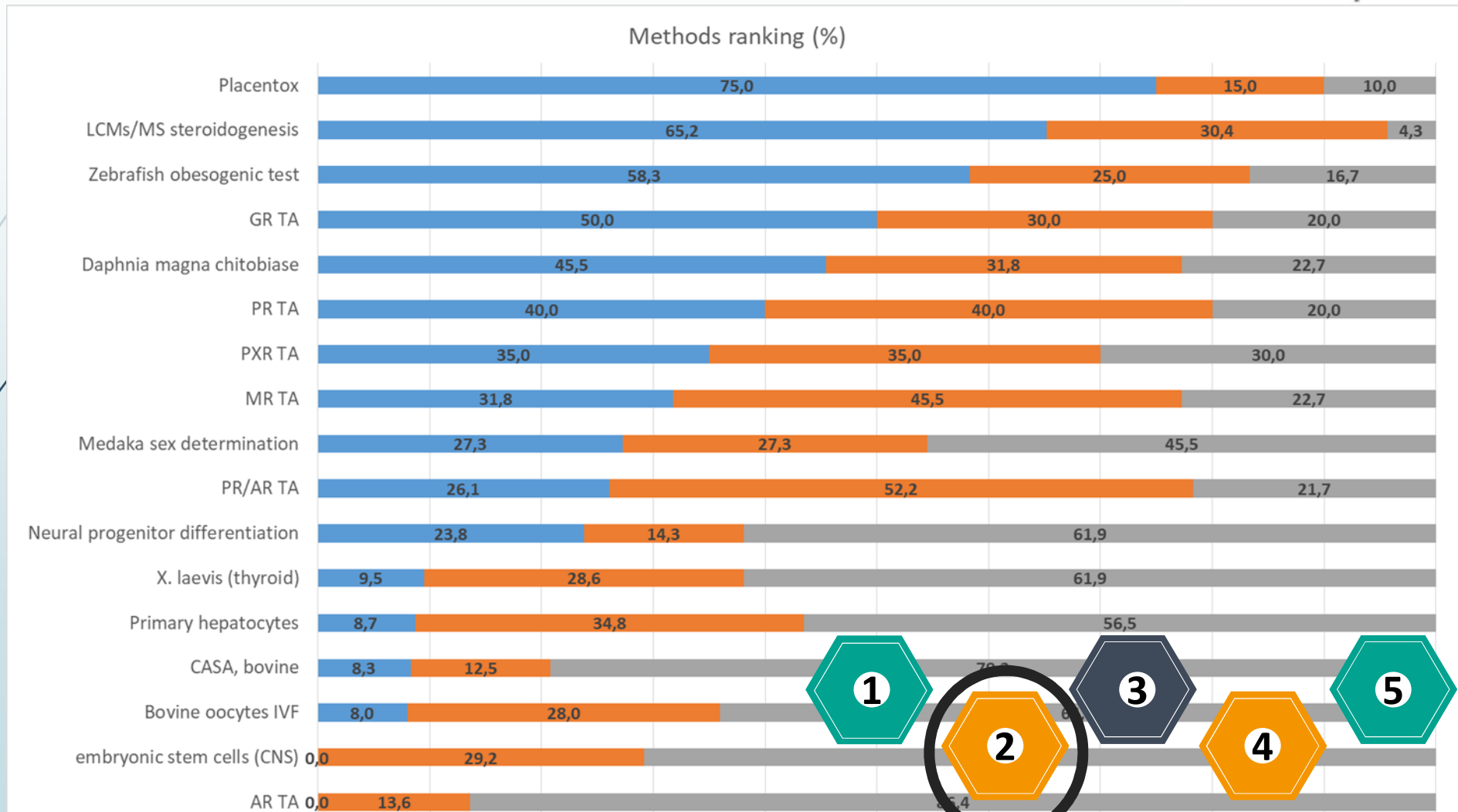
United States Environmental Protection Agency

Endocrine Disruptors Testing and Assessment Advisory Group

Toxcast



# September : Presentation and selection by the relevance committee



TRC= 71,43%

# hPLACENTOX-PE

*In vitro complex*

➤ **Principle / Endpoint**

- Human physiological **placental function** and **women reproduction/fertility**
- Assessment of secretion of relevant hormones (progesterone, Beta hCG, hPL, Estradiol) by placental cells (supernatant) and activation of P2X7 receptors, implicated in placental pathologies (pre-eclampsia, miscarriages, premature births)

➤ **Strength**

- Human placental cell line, commercially available (JEG-3)
- Endocrine pathway(s)
- High throughput (P2X7 activation)
- 12 substances tested (at least)

➤ **Limitations**

➤ **Protocol**

- Publication, patent

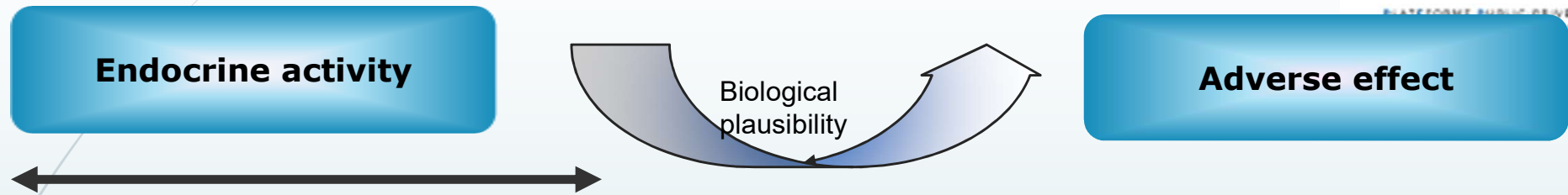




# hPLACENTOX-PE



PLATFORME PUBLIQUE SUR LA  
D'ESSAIS SUB  
CRINIENS



Mammalian and non-mammalian Toxicology	
<b>Level 1</b> Existing Data and Non-Test Information	
<b>Level 2</b> <i>In vitro</i> assays providing data about selected endocrine mechanism(s) / pathway(s)	
Mammalian Toxicology	Non-mammalian Toxicology
<b>Level 3</b> <i>In vivo</i> assays providing data about selected endocrine mechanism(s) / pathway(s)	
<b>Level 4</b> <i>In vivo</i> assays providing data on adverse effects on endocrine relevant endpoints	
<b>Level 5</b> <i>In vivo</i> assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism	



placental function, women reproduction/fertility



# LC-MS/MS Based Profiling and Dynamic Modelling of the Steroidogenesis Pathway in Adrenocarcinoma H295R Cells

TRC= 68,25%

10

*In vitro complex*



## ➤ Principle / Endpoint

- Measuring the levels of steroids produced by human adrenal cells
- 19 measurements across steroidogenesis pathway (precursors, intermediates and end-products)

## ➤ Strength

- High throughput, high accuracy
- Commercially available cell line
- Specific endocrine pathway
- Enhancement of OECD TG 456

## ➤ Limitations

- No xenobiotic tested

## ➤ Protocol

- Publication (test based on OECD TG)



# Zebrafish obesogenic test: a tool for screening molecules that target adiposity

11

TRC= 66,07%

## ➤ Principle / Endpoint

- Zebrafish larvae (>5 day post-fertilisation)
- Characterisation of potential obesogenic or anti-obesogenic substances (**obesity and metabolic dysfunction**)
- Observation of adipocyte lipid droplet size and measurement of adiposity by fluorescence microscopy

## ➤ Strength

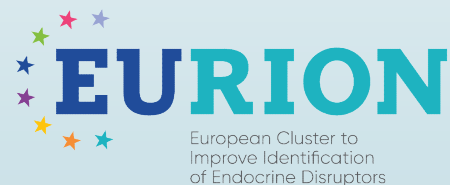
- Whole organism mechanism based

## ➤ Limitations

- Low throughput

## ➤ Protocol

- Publication (and video)



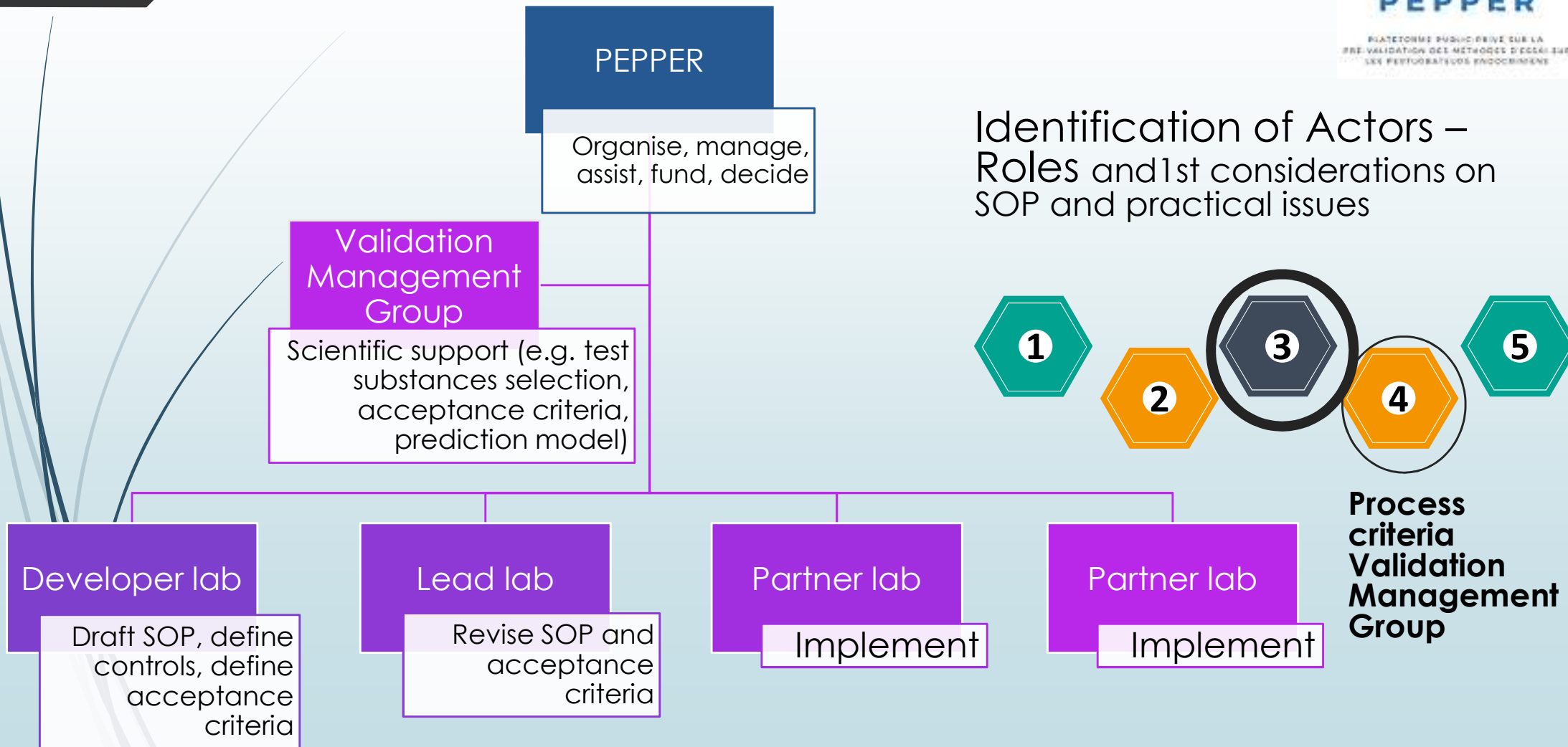
In vivo



PEPPER

PLATEFORME PUBLIC-PRIVE SUR LA  
PRE-VALIDATION DES METHODES D'ESSAI SUR  
LES PERTURBATEURS ENDOCRINIENS

# November : Starting prevalidation



# Objectives and Timelines (as in December)



- Define contact person
- Cost evaluation
- Provide historical data
- Draft Standard Operating Procedure
- Define positive and negative controls

Draft and send  
collaboration  
agreement

Plan training  
of partner  
labs

March 2021  
transfer  
finalised

November 2021  
Submission



## Next steps and ambitions



- Feeding OECD with mature submissions (possibly CEN/ISO, ECVAM) for the **first 3 methods**
- Starting a new action for the next 3 methods
- Contributing to fill gaps in validated methods
- Help and encourage research teams in very practical prevalidation operations
  - Both with funding and advices
- Act as an accelerator for regulatory toxicology
- Reduce the « opposition » between regulatory science and academic science

# Conclusions

## ➤ Lessons learned

- Finding methods both relevant and mature is a difficult task, with a low efficiency
- The level of requirement on practical issues seems underestimated by many teams...and sometimes overestimated and seen as an obstacle.
- But once confidence is established, cooperation with PEPPER team is efficient
- But cooperative laboratory networks exist at the European Level.

## ➤ Expectations

- Availability of resources such as PEPPER will motivate research teams to developing assays and practical tools
- Other partners will join and the approach can be applied to other fields
- Such an improvement in regulatory science will help facing the challenges in the Green Deal, and Chemical Strategy for Sustainability



The work in PEPPER is possible thanks to the founding members and sponsoring members and other financial support



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**RI  
SE**