

## Survey of available *in vitro* and *in silico* methods developed by SEURAT-1 partners

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### Introduction and Aims

As an input to the *SEURAT-1 workshop describing mode of action in liver toxicity using adverse outcome pathways*, held in Ispra on the 24-25<sup>th</sup> October 2012, organised by the MoA working group and COACH, as well as the Safety Assessment Working Group meeting in Tübingen on 13-14 November 2012, the JRC asked all SEURAT-1 partners to provide a short description of methods (*in vitro* and *in silico*) being developed by them in the context of their projects, with a view to mapping available SEURAT-1 methods onto MoA-based integrated testing strategies.

### Methods

A questionnaire was sent to the SEURAT-1 partners, with questions focused on whether methods were developed to capture specific MoA, the target tissue/organ, chemicals used, which key event/s or biomarkers were detected, *in vitro* treatment protocols and which adverse effect/s could be predicted.

The data analysis was performed by grouping the methods on the basis of the answers given, in order to obtain a snapshot of the current methodology available within SEURAT-1.

### Results

We collected responses for a total of 29 methods, 4 of which were *in silico* assays and only 3 methods were developed in collaboration with other SEURAT-1 projects. The majority of the methods, 20, were developed by DETECTIVE (Fig.1) and only 9 were MoA-based (only 7 provided details about the MoA, Table 1).

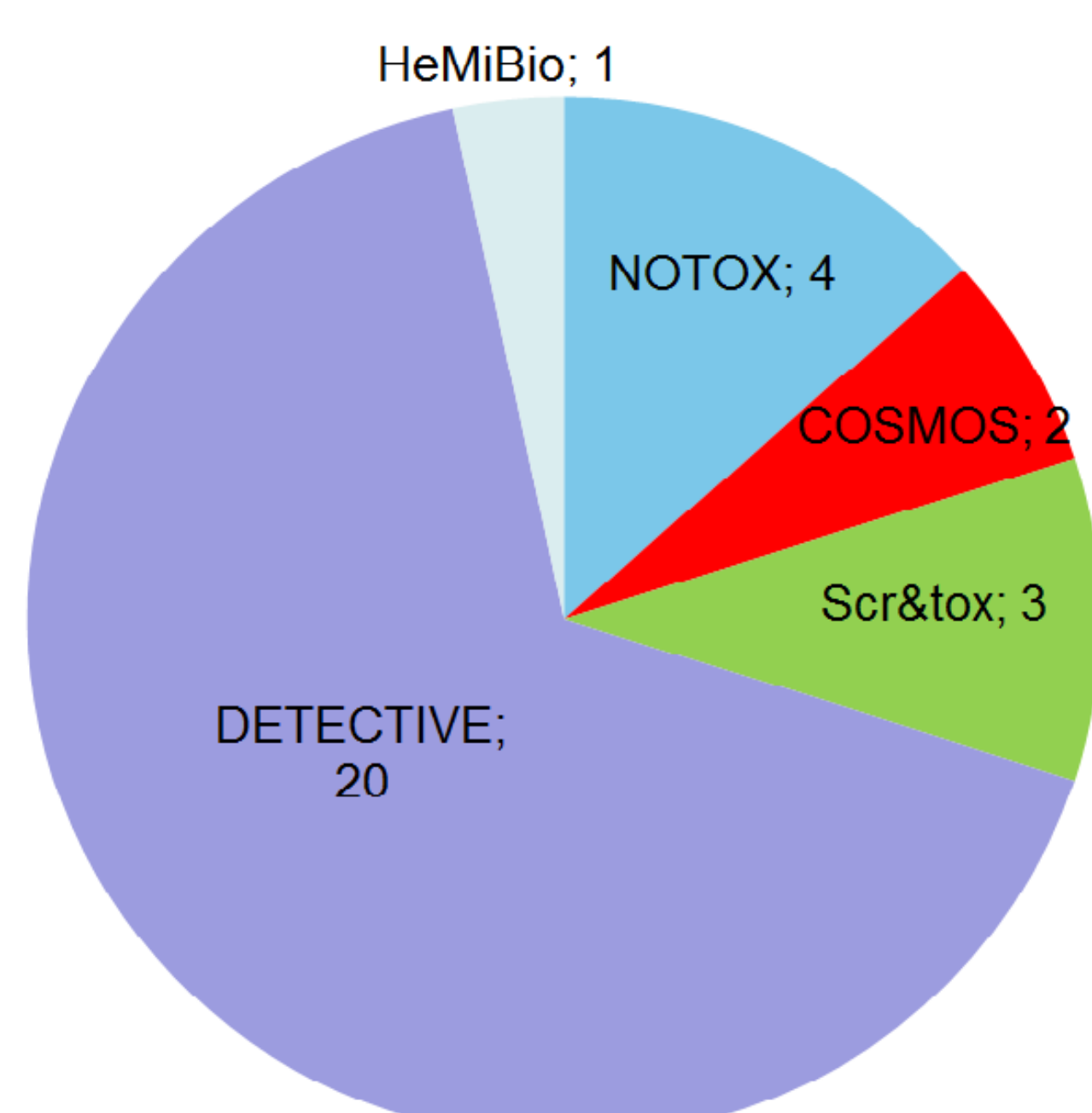


Figure 1. Methods developed by each SEURAT-1 project.

Twenty methods were tissue/organ specific and liver and heart were the main target organs considered (Fig. 2).

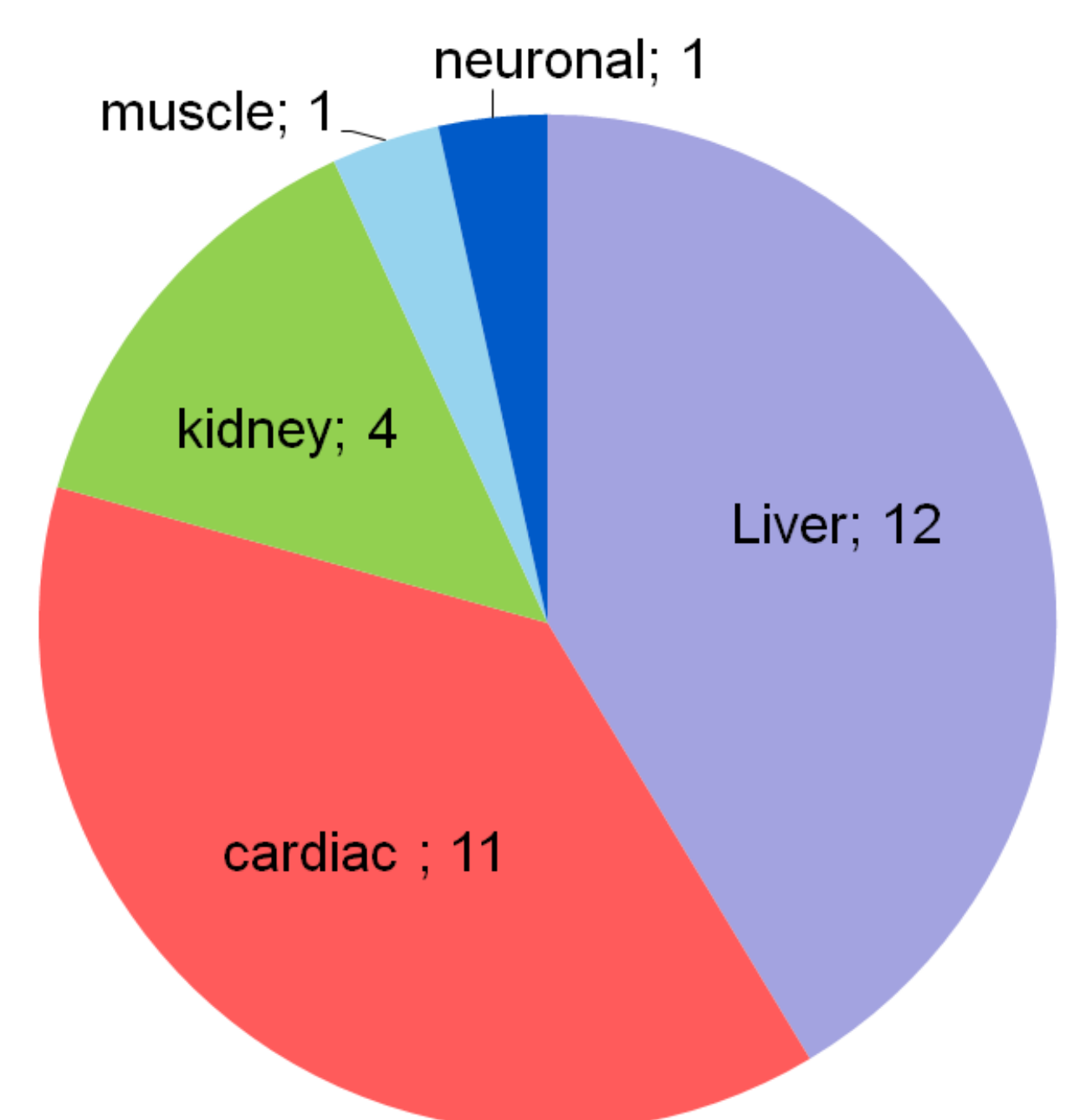


Figure 2. Methods focused on specific target organs related toxicity.

Table 1. MoAs considered during method development

- Oxidative stress
- ER stress
- Cytochrome P450 induction
- Myotoxicity of statins
- Inhibition of neuronal relevant pathway (e.g. CREB) and of synaptogenesis
- Arrest of protein synthesis through ribosome inhibition
- Mitochondrial dysfunction

For each target organ, the following *in vitro* cell model were used:

- Liver: HepG2 (2); HepaRG (2); primary human hepatocytes (8) and hepatocyte cell like derived from skin stem cells (1)
- Heart: IPSC-cardiomyocytes
- Kidney: RPTEC/TERT1
- Muscle: IPCS-MSC
- Neurons: IPCS-neurons

### Results

Most partners had selected chemicals from the gold compound list and in Table 2 are reported the most used chemicals for each target organ.

Table 2. Most used chemicals for *in vitro* methods

Target organ	Chemical	Number of methods that used the chemical
Liver	Paracetamol	7
	Acetaminophen	3
	Tunicamycin and Thapsigargin	2
Heart	Doxorubicin	8
Kidney	Potassium Bromate	6
Muscle	Simvastatin	1
Nervous system	Naphthol and Propofol	1

The treatment protocols varied considerably between projects and only 10 methods provided sufficient information (Table 3).

Table 3. *In vitro* treatment protocols used

Treatment protocol	number of methods
Different <i>in vitro</i> model for liver, heart and kidney exposed with several chemicals at 3 h and 1, 2, 4, 6 and 14 days. IC 10 and IC 10*0.25.	4
HepG2 singly exposed with chemicals at different concentrations (analysis of Nrf2 activity)	2
HepaRG treated for 20 h with 15 mM paracetamol in duplicates (analysis DNA methylation)	1
Pluripotent stem cells derived MSC exposed with simvastatin for 17 days, treated every 2 days (myotoxicity of statins)	1
Pluripotent stem cells derived neurons (H9-syn-GFP) exposed for 14 days, treated every 3 days (analysis of decrease in % of synapsin/GFP+ cells)	1
Primary human hepatocytes exposed with 0,2 and 1 mM paracetamol at each medium change (daily change) for 7 days (analysis of mRNA, proteins, DNA and histone modifications)	1

The main key events/biomarkers measured were: Nrf2 activity, ER stress, changes in DNA methylation; changes in gene-, miRNA- and protein-expression; ROS formation; high temporal resolution of epithelial cell impedance, contractile properties of cardiomyocytes; mitochondrial damage and decrease of synapsin. Not all the methods provided a detailed description of the methodology used to measure the biomarkers/endpoints. Most of the assay did not indicate which adverse outcome they could predict.

The available *in silico* methods were focused on:

1. Systematic knowledge extraction to build database
2. Cell based model to determine the cell bioavailable concentration of a chemical
3. PBPK model to calculate concentration at tissue level
4. R-package (RnBeads) for comprehensive analysis of Illumina Infinium Human Methylation450 BeadChip data.

A more detailed description of the available methods is available on request.

### Conclusions

This survey represents the first effort to map the methods available within SEURAT-1 in order to start designing integrated testing strategies and therefore to move from the first to the second level of the proof of concept objective.

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