Mechanism-based characterisation of toxicity for RepDose database substances: initial results on cytotoxicity and genomics.

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Abstract

Aim of this project is to predict repeated dose toxicity from in vitro toxicogenomics experiments. Many in vitro toxicogenomics initiatives focus on mechanistic studies and hazard identification, not risk assessment. Here, the RepDose database is taken as starting point, it comprises oral and inhalation studies in rodents (www.fraunhofer-repdose.de) and contains ~2200 studies for 650 mainly organic industrial chemicals. Analysis of RepDose revealed that six main targets (body weight, liver, kidney, clinical symptoms, clinical chemistry and haematology are most often affected at study Lowest Observed Effect Level (LOEL). Hence, for over 90% of e.g. subchronic rat studies, the study LOEL is predicted correctly if these six main organs/targets are taken into account. Based on these observations , this project focusses on liver, kidney, and lung models to predict systemic repeated dose toxicity from in vitro toxicogenomics data. Here, we present the project progress on chemicals selected from RepDose. Initial cytotoxicity experiments for selected chemicals in the *in vitro* models employed are performed: HepaRG cells to represent liver; RPTEC/TERT cells to represent kidney; and A549 cells for lung. Further, a start is made with the generation of lung RNA samples, for toxicogenomics analysis.

Goals

For selected chemicals the following questions are addressed:

- **Pathway extrapolation**. Is toxicogenomics-based pathway extrapolation from human in vitro models to in vivo possible? Do toxicogenomics derived mechanistic data corroborate these findings?
- **Inter-organ potency ranking.** Investigate the reproducibility of toxicity potency ranking of liver, kidney, lung from in vitro toxicogenomics data, (oral exposure: liver, kidney; inhalation exposure: lung, liver)
- **Intra-organ potency ranking** after oral/inhalation exposure. Investigate predictive value of in vitro toxicogenomics in ranking intra-organ potency of substances observed in inhalation and in vivo oral repeated dose toxicity studies, targeting the liver, kidney or lung.
- **Grouping**. Is grouping/read-across of chemicals possible based upon in vitro toxicogenomics read-out, in relation to their reported toxicity data and grouping available from the RepDose database.

Methods I
Selection of chemicals (WP2)

Criteria applied to select chemicals:

Inter-organ criteria

- Targets only liver or kidney or lung (respiratory tract such as nose) at LOEL
- Targets other target organ(s) of interest only at higher dose

Intra-organ criteria

- Select candidates of low, moderate and high toxicity (based on LOEL)
- Focus on critical adverse effects

Chemical space

- Include chemical structures differing with regard of major functional groups
- Avoid chemicals with structural alerts for genotoxicity
- Include selected pairs of chemicals, with similar/comparable structural properties

Results Lung cells: Cytotoxicity

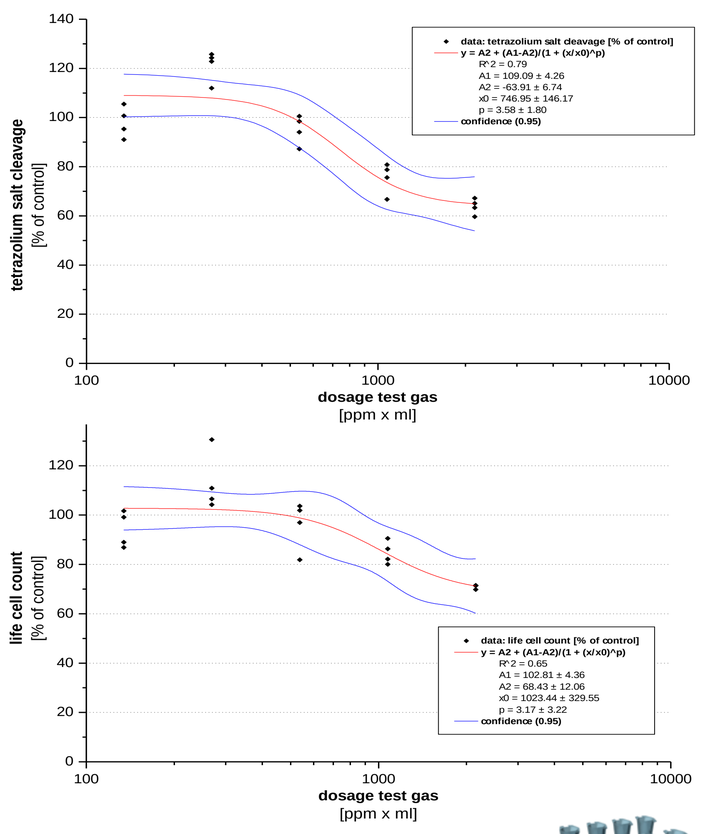


Figure 2, Typical dose effect graphs shown for exposure of A549 cells to formaldehyde.

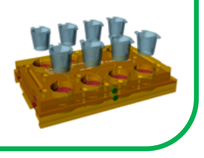


Figure 1, AIMT2 Project Overview

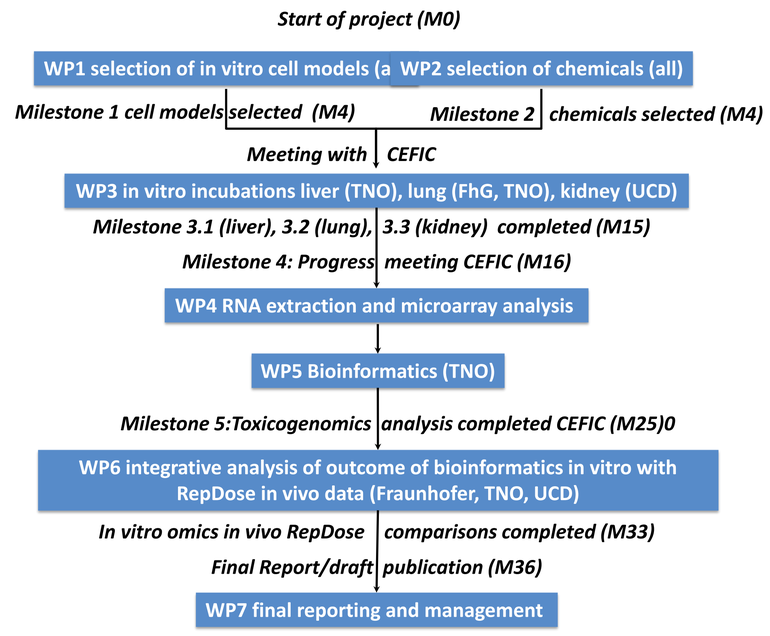


Table 1. Results chemical selection
IC10 estimation for RNA isolation

Liver				
Cas number	**Chemical**	**NOEL (mmol/kg bw/d)**	**IC10 (mM)**	
18181801	Acarol (Bromopropylate)	1.3 x 10-3 to 8 x 10-3	0.008	
131179	Diallyl phthalate	1.3 x 10-2 to 1.7 x 10-2	0.015	*
115322	Dicofol	2.3 x 10-5 to 2.3 x 10-3	0.029	
57-74-9	Chlordane		0.044	
2835394	Allyl isovalerate	0.05	0.2	*
80079	Bis(4-chlorophenyl) sulfone	3 x 10-4 to 9 x 10-4	0.35	
120-83-2	2,4-dichlorophenol	0.06-2.23	0.4	*
51-03-6	Piperonylbutoxide	1.3 x 10-2 to 2.2 x 10-2	0.74	
55934-93-5	Tripropylene glycol butyl ether	0.4	4.5	#
822366	4-Methylimidazole		6.4	
111-90-0	Diethylene glycol ethyl ether		10	#
34590-94-8	Dipropylene glycol methyl ether		10	#
107-21-1	Ethylene glycol		10	#
288-32-4	Imidazole	0.02-0.04	10	
112-50-5	Triethylene glycol monoethyl ether		10	#
112-35-6	Triethlylene monomethyl ether	n.d.	10	#
110-65-6	Butynediol	5 x 10-4 to 4.8 x 10-3	selected to be tested	
98-92-0	3-pyridine carboxamide	2.5 x 10-2	selected to be tested	
59080409	2,2',4,4',5,5'-Hexabromobiphenyl		selected to be tested	
67888997	2,3',4,4',5,5'-Hexabromobiphenyl		selected to be tested	
107186	Allyl alcohol	0.05	selected to be tested	
* to be tested in kidney cells as well				
# rejected for further study due to lack of toxicity or availability				
Kidney				
Cas number	**Chemical**	**NOEL (mmol/kg bw/d)**	**IC10 (mM)**	
136-77-6	4-Hexylresorcinol	1.5 x 10-2 to 7.6 x 10-3	0.006	
1897-45-6	Chorothalonil	7 x 10-4 to 3.9 x 10-2	0.020	
91-15-6	M-phthalodinitrile	2.3 x 10-2	0.035	
123-30-8	p-Aminophenol		0.035	
105-11-3	1, 4-Benzoquinone dioxime		0.048	
100-01-6	4-Nitrophenylamine		0.050	
93-76-5	2,4,5-Trichlorophenoxyacetic acid	3 x 10-3	0.063	*
87-68-3	Hexachloro-1,3-butadiene	1.3 x 10-3 to 5.5 x 10-5	0.200	*
2432-99-7	11-Aminoundecanoic acid	2.4 x 10-2 to 6.9 x 10-2	0.250	
96-31-1	1,3-Dimethylurea	2.3 x 10-2	1.500	*
271-89-6	Benzofuran	1.1 x 10-2 to 7.5 x 10-2	5.000	
108-31-6	Maleic Anhydride	4.2 x 10-2 to 1.1 x 10-1	5.000	
* to be tested in liver cells as well				
Lung				
Cas number	**chemical**	**NOEL (mmol/kg bw/d)**	**IC10 (ppm)**	
50-0-0	Formaldehyde	1.1×10^{-3} to 1.1×10^{-2}	5.97	
124-40-3	Dimethylamine	2.2×10^{-2} to 2.5×10^{-2}	150	
75-07-0	Acetaldehyde	0.18 to 1.8	210	
115-11-7	Isobutylene	0.45 to 1.2	>50000	

NOELs indicated are obtained from subacute to chronic studies. Either the lowest value or value range (obtained from multiple studies) is indicated. For some of the structural analogues, no NOEL was available.

Conclusions so far

Liver

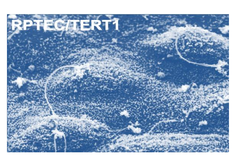
Preliminary conclusion for liver is that no clear association was observed between in vivo NOEL and in vitro IC10.

Kidney

Preliminary conclusion for kidney is that no clear association was observed between in vivo NOEL and in vitro IC10.

Lung

Interestingly, at least in terms of ranking, potency inferred from in vitro data reflected in vivo potency, with formaldehyde being most potent, followed by dimethylamine, acetaldehyde and isobutylene (compare with Table 1). Both tetrazolium salt conversion, as well as cell count gave very comparable results. Subsequently, RNA was successfully extracted for toxicogenomics experiments (microarray analysis ongoing)



Methods II
In vitro incubations liver, kidney and lung, cytotoxicity and extraction of RNA for microarray analysis (WP3, WP4)

- **Liver** HepaRG were cultured in special HepaRG-medium with serum and additives and plated in 96 or 24 well plates (three rounds). Plated cells were exposed to chemicals in triplicate for 72 hrs. After incubation, cytotoxicity and IC10 estimation was determined by the MTT and the LDH viability test.
- **Kidney** RPTEC/TERT1 cells were maintained under serum-free conditions in hormonally defined medium. After plating on 96- or 24-well plates, cells were grown to confluency and allowed to fully differentiate. Cells were then exposed to increasing concentrations of test chemicals in triplicate for 72hrs. After incubation, cytotoxicity and IC10 estimation was determined by Resazurin reduction assay and LDH release.
- **Lung** A549 cells were cultured in Dulbecco´s MEM medium supplemented with 10% FKS and antibiotics. Exposure to chemicals was performed employing the P.R.I.T.®-ALI Technology. Cells were exposed for 6 and 72 hrs. Cytotoxicity (IC10 value) was determined according to two methods: MTT and electronic cell counting (CASY®).
- **RNA isolation** For lung chemicals, pilot experiments were performed to determine if RNA isolation procedures (using Nucleospin RNA II kit from Machery-Nagel) were compatible upon exposure experiments using the P.R.I.T.®-ALI Technology. For RNA isolation (microarray) experiments, A549 was cultured/exposed at IC10 for 6h and 72 h. Triplicate independent experiments, containing four technical replicates each were performed.