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Alternative methods for acute systemic toxicity testing

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European Union Reference Laboratory for Alternatives to Animal Testing

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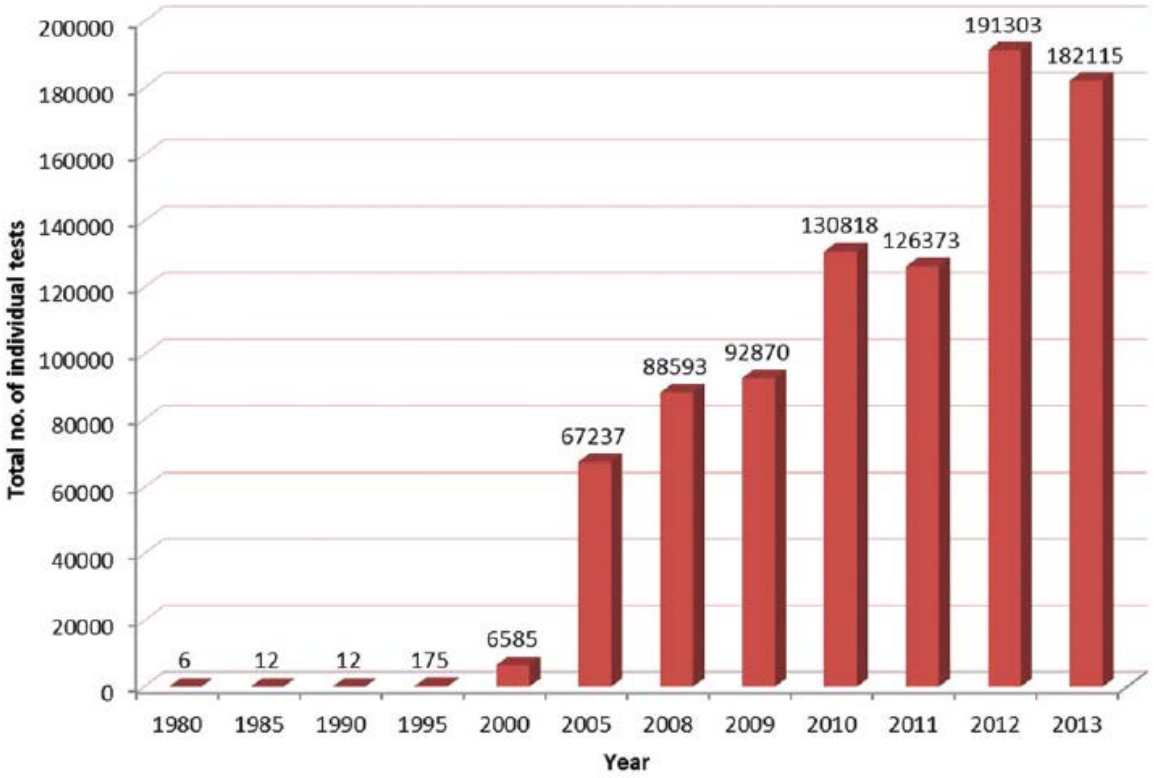
Use of *in vitro* methods – pharmaceutical industry



Goh *et al*., *Toxicol. Res*. 2015

- **7 UK companies**
- **Number of *in vitro* tests carried out**
- **Genotoxicity, ADME & safety pharmacology**

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Introduction – *In vitro* methods for acute systemic toxicity testing

- **DB-ALM** (http://ecvam-dbalm.jrc.ec.europa.eu/) – 25 protocols

Toxicokinetics
(3 protocols)

Specific target organs
(18 protocols)

Basal cytotoxicity
(4 protocols)

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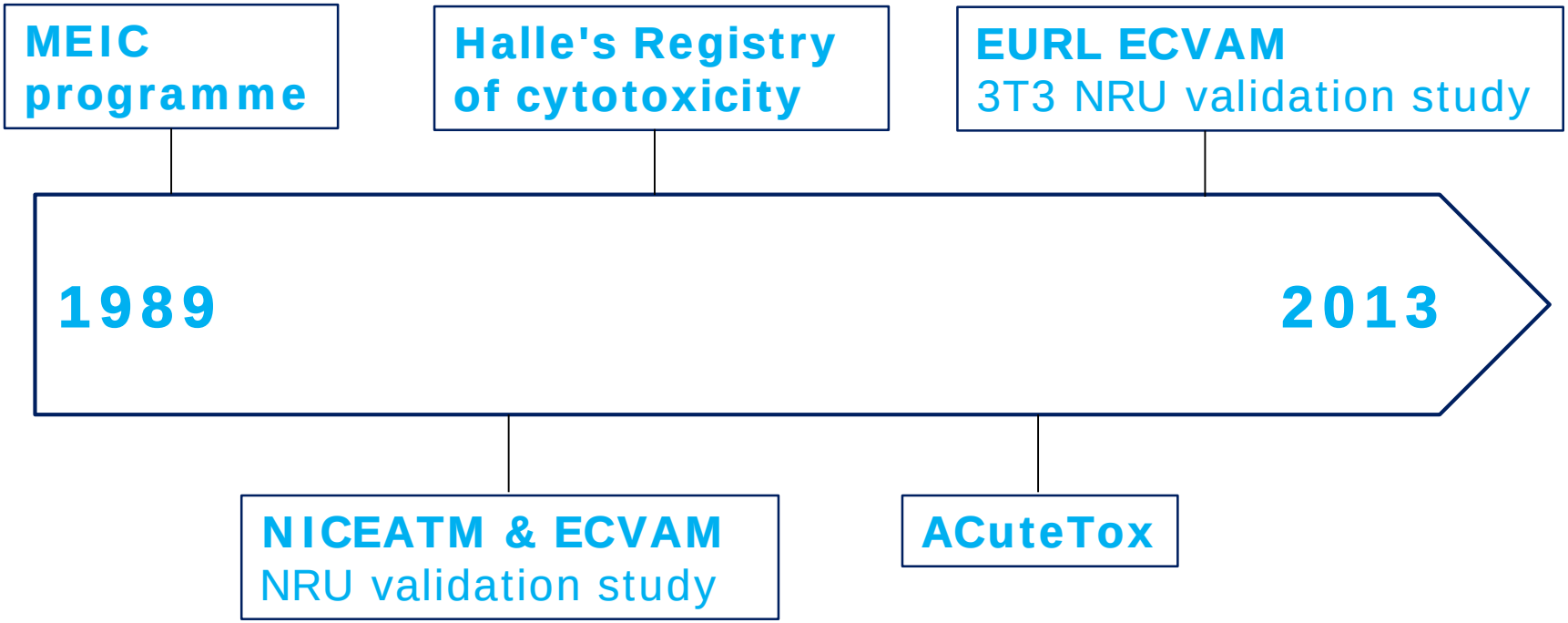
Research efforts into alternative methods for acute systemic toxicity testing



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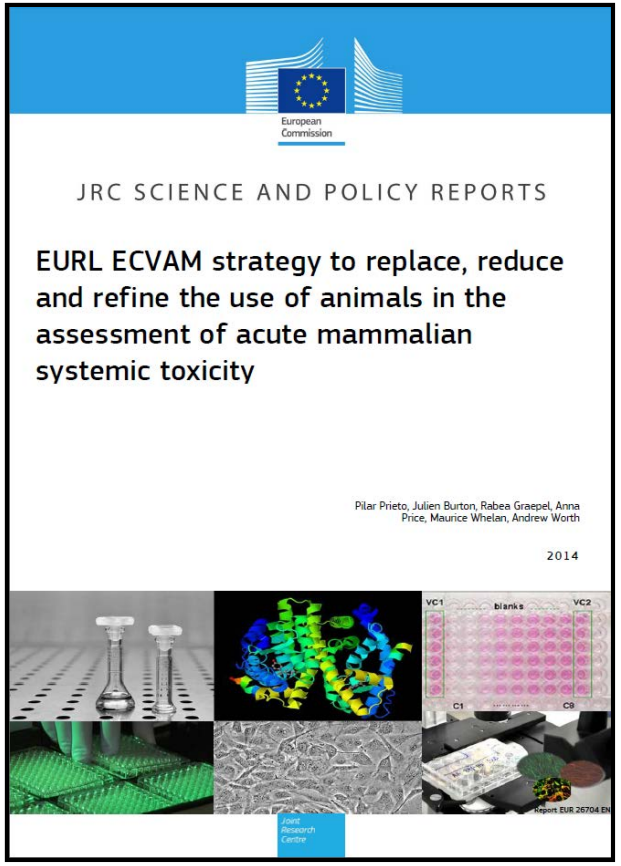
EURL ECVAM strategy paper - 2014

- **3Rs** in acute systemic toxicity testing

- **Aim 1**: **reduction** & **replacement** of animal testing

- **Aim 2**: **refinement** of animal studies



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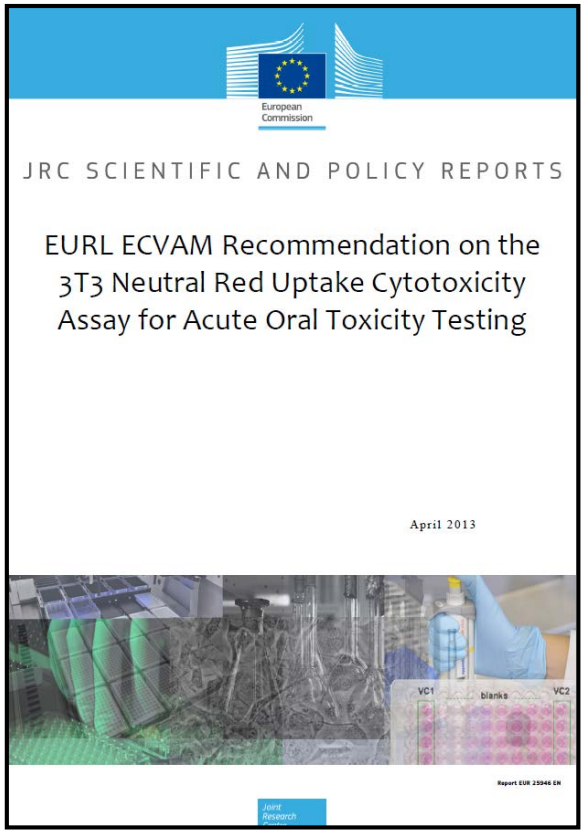
3T3 Neutral Red Uptake (NRU) test method

- BALB/c 3T3 cells + Neutral Red Uptake (fixation of red dye)
- Validated on **56** industrial chemicals
- High sensitivity (92-96%) for identification of non-classified (**oral** LD_{50}>2000mg/kg)
- **Take home –** 3T3 NRU as part of WoE/ ITS to identify non-classified chemicals



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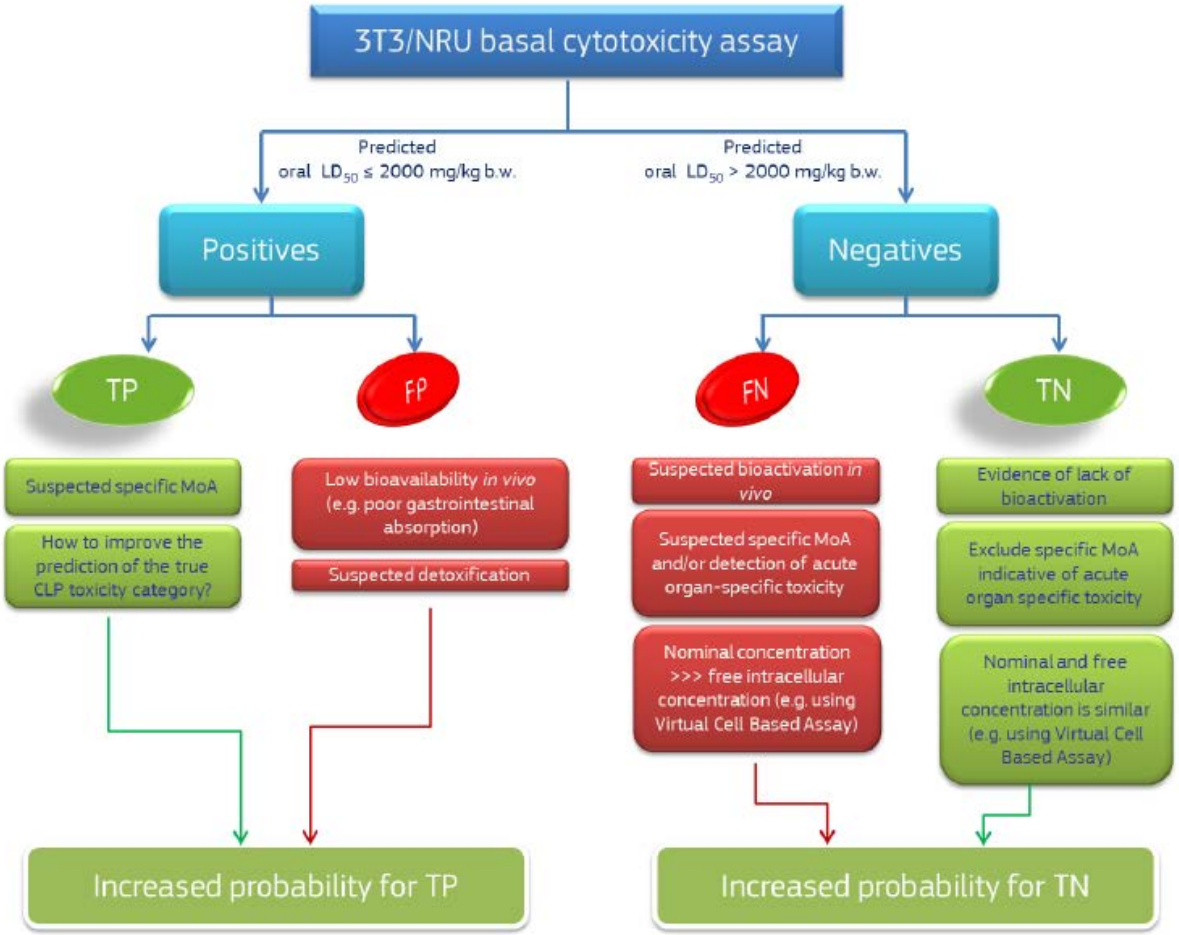
EURL ECVAM strategy – Aim 1 - Reduction



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Increasing confidence - 3T3 NRU + QSAR methods

- 3T3 NRU & LD_{50} data for **181** chemicals
- Threshold POS/NEG: LD_{50}=2000mg/kg
- 5 false negatives results
- "correction" for metabolism

Thanks to Julien Burton

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Increasing confidence - 3T3 NRU + QSAR methods

Compound name	Nb unique metabolites	Min predicted LD50 (mg/kg)	Max predicted LD50 (mg/kg)	Avg predicted LD50 (mg/kg)	Oral cat1 < 5	Oral cat2 <50	Oral cat3 <300	Oral cat4 <2000	Oral cat5 <5000	In vivo LD_{50} (mg/kg)
Digoxin	777	**30.11**	1429.32	294.47	0	**114**	362	301	0	28
Aconitine	677	**23.55**	9218.3	325.24	0	**119**	297	243	15	6
Benzylbenzoate	69	**815.43**	6511.15	2257.27	0	0	0	**35**	31	1990
5,5-Diphenylhydantoin	25	**1088.63**	3620.21	1783.76	0	0	0	**18**	7	1360
Disopyramide	188	**266.83**	6960.22	1393.25	0	0	**1**	159	25	333

- Extended to all negatives (automated process)

- Limitations - QSAR on oral for rats
  - Metabolites generated “in situ” (oral model accounts for ingestion of the chemical)

Thanks to Julien Burton

Reduction – use of existing repeated-dose toxicity data

- Analysis of New Chemical Database – relation 28 day oral NOAEL & oral LD_{50} (Bulgheroni *et al.*, 2009)
  - **NOAEL ≥ 200mg/kg bw - LD_{50}>2000mg/kg bw** (63% correct, n=1436)
- European Chemicals Agency (ECHA) – REACH registration dossiers
  - 28 day oral LOAEL & oral LD_{50}
  - Klimisch scores 1 & 2
  - Rat & oral gavage
  - **96 chemicals**

Reduction – use of existing repeated-dose toxicity data

LOAEL (mg/kg b.w./day)	EU CLP categories (LD50 mg/kg b.w.)					Total
	1 (<5)	2 (5-50)	3 (50 – 300)	4 (300-2000)	NC (>2000)	
< 5	**0**	**0**	0	1	0	**1**
5 – 50	0	**0**	**1**	5	0	**6**
50 – 300	0	0	**2**	**10**	3	**15**
300 – 2000	0	0	0	**4**	**17**	**21**
>2000	0	0	0	0	**0**	**0**
Total	**0**	**0**	**3**	**20**	**20**	**43**

LOAEL (mg/kg b.w.)	LD50 (mg/kg b.w.)		Total
	≤ 2000	>2000	
<200	**20**	7	27
≥200	18	**40**	58
Total	38	47	85

- poor **direct** correlation between the two data sets
- correctly predict **85% non-classified** substances

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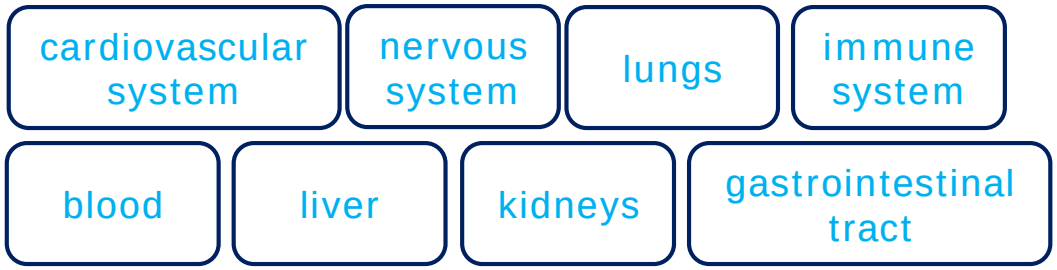
3T3 NRU dataset - mechanism mapping

- Lack of mechanistic knowledge for acute systemic toxicity
- **Data rich** set of **181** chemicals
  - IC_{50} values; oral LD_{50} values & functional information
  - 99 industrial chemicals & 82 "others" ie biocides, pharmaceutical
  - 66 non-classified & 115 "toxic"

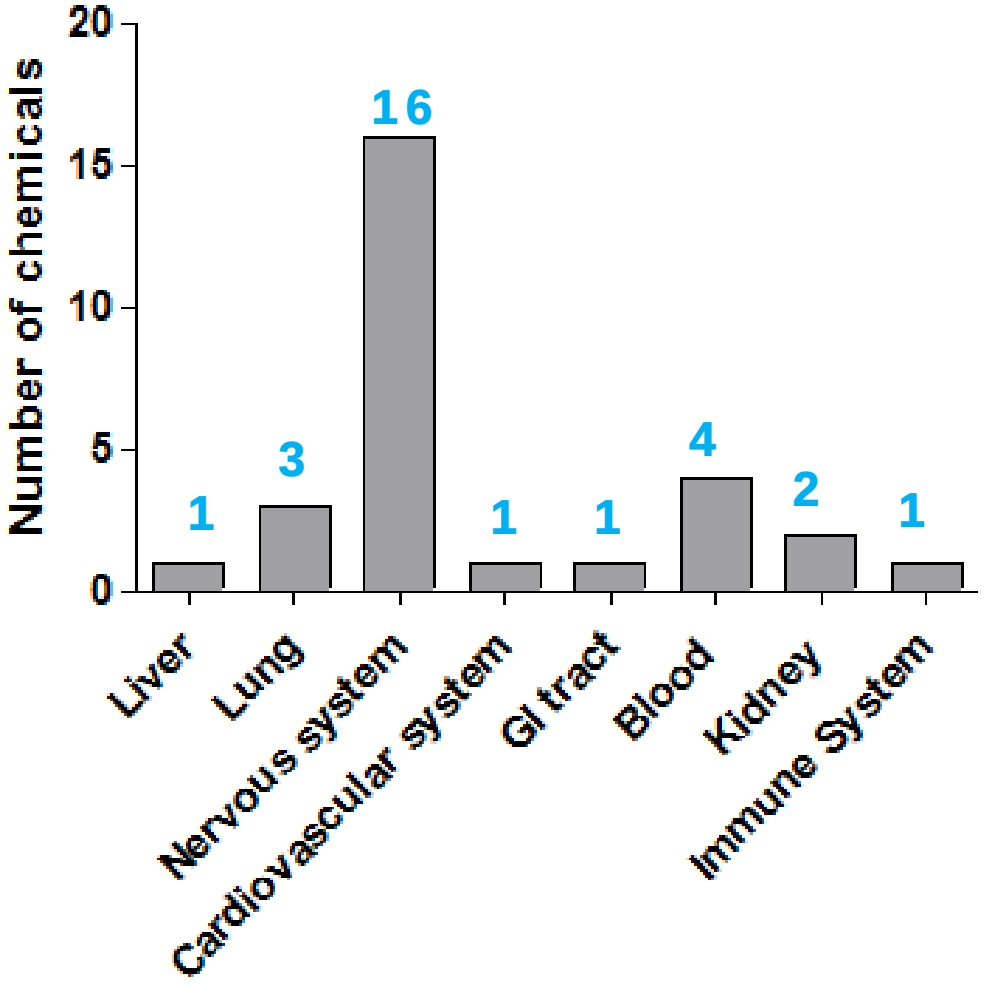
- 8 target organs:



- Aim: Complement 3T3 NRU results with mechanistically relevant information

3T3 NRU dataset - mechanism mapping

How often are the 8 organs the SINGLE targets of toxicity?



- ***in vitro*** **methods for target organ toxicity**
- **brain aggregates for neurotoxicity**

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Conclusions & summary

- *In vitro* methods are relevant and useful in safety assessment

- **3T3 NRU** method could form valuable part of an **integrated testing strategy** to identify non-classified compounds
  - QSAR modelling of metabolism
  - Existing *in vivo* **LOAEL** data from repeated-dose studies
  - Mechanistic data on specific target organs

Thanks

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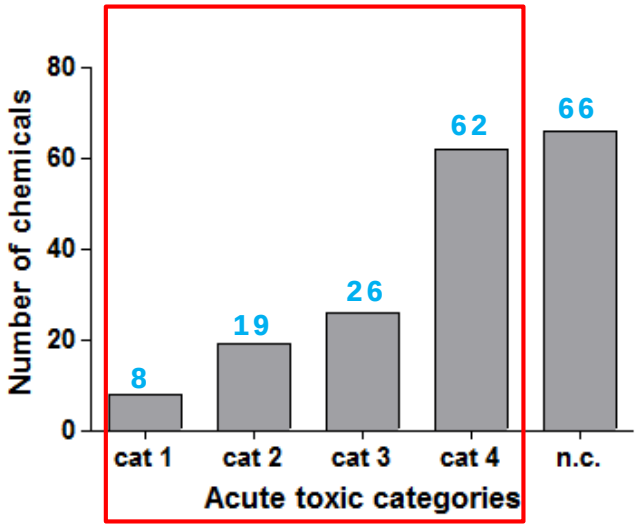
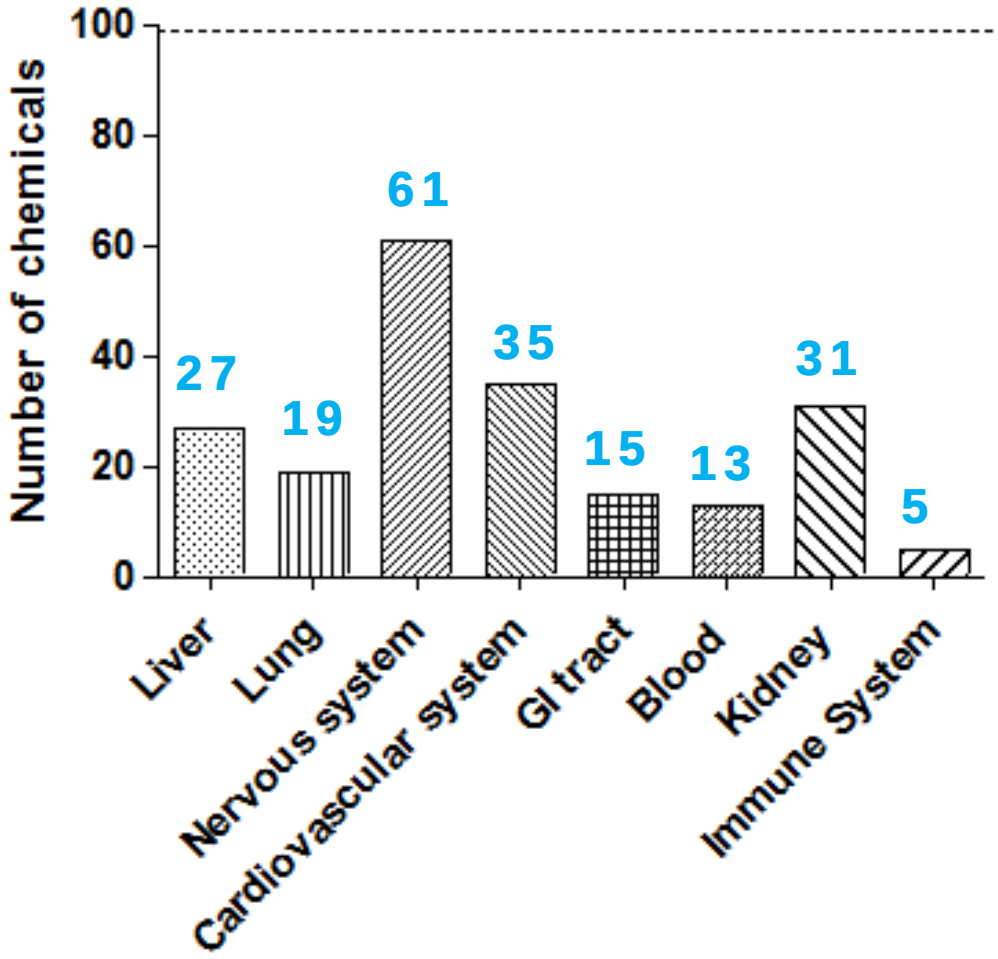
3T3 NRU dataset - mechanism mapping



3T3 NRU dataset - mechanism mapping



26 of these chemicals were identified to have a mechanism of general cytotoxicity.

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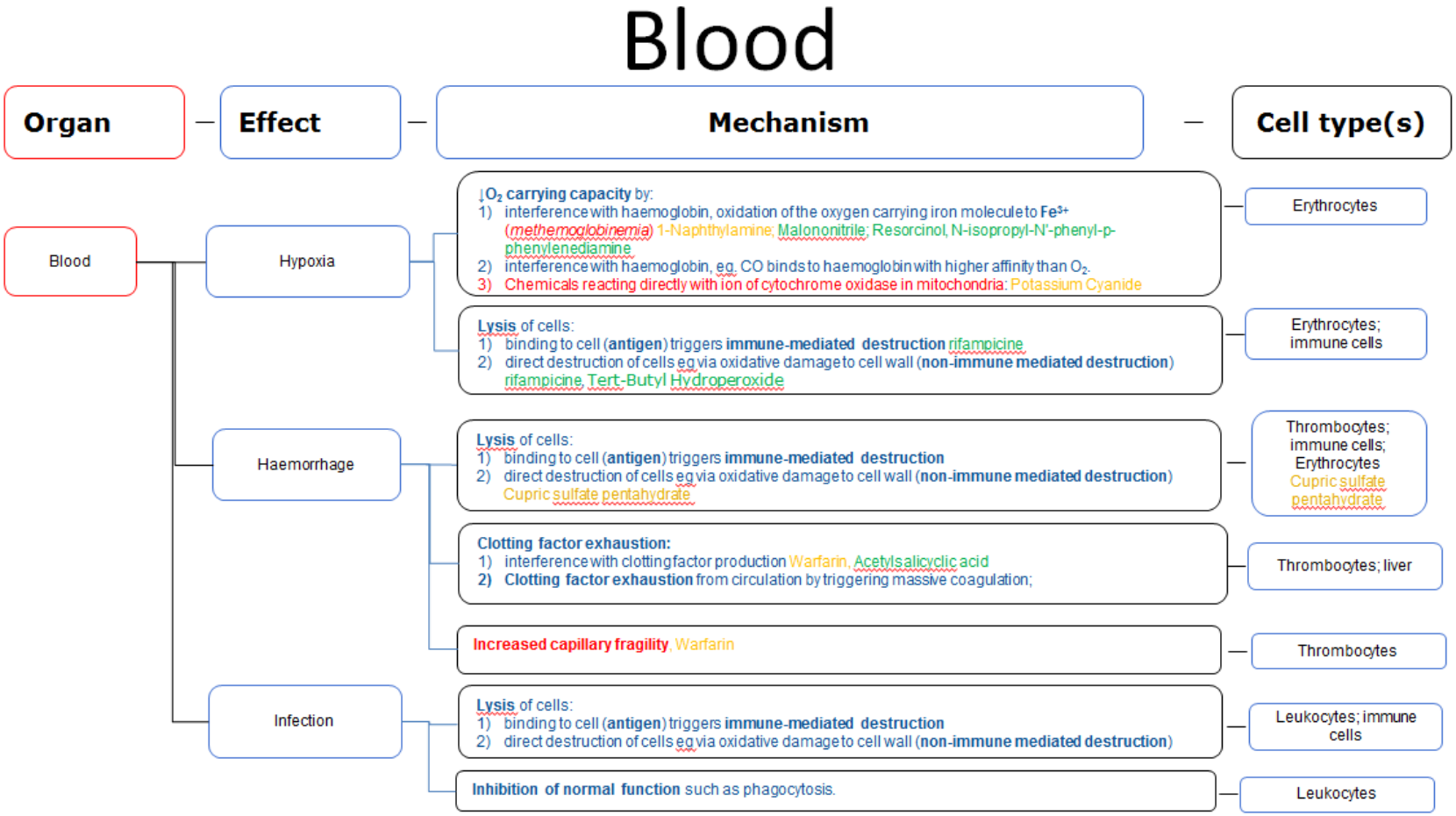
3T3 NRU dataset - mechanism mapping



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Increasing confidence - 3T3 NRU + QSAR methods

Simulations for all 19 true negatives

Name	CAS N°	N° of unique metabolites	Min predicted LD_{50} (mg/kg)	Max predicted LD_{50} (mg/kg)	Avg predicted LD_{50} (mg/kg)
Dichloromethane	75-09-2	4	106	567	289
***1,2-Dichlorobenzene**	95-50-1	4	248	578	413
Gibberellic acid	77-06-5	5	264	553	418
1,1,1-Trichloroethane	71-55-6	5	263	906	469
Benzene	71-43-2	5	536	1219	994
***Ethylene glycol**	107-21-1	3	585	2065	1185
***2,6-Diethylaniline**	579-66-8	8	1311	2624	1804
2-Ethylhexyl acrylate	103-11-7	4	685	4200	2586
Tris(nonylphenyl)phosphite	26523-78-4	13	374	3699	2950
Glycerol	56-81-5	3	2705	4634	3436
Glycerol triacetate	102-76-1	8	752	5720	3744
Tripotassium Citrate	866-84-2	1	3837	3837	3837
1,2-Benzenedicarboxylic Acid	68515-48-0	6	2715	5620	3901
Di-"isodecyl" phthalate	26761-40-0	6	2976	6145	4226
Tween 20	9005-64-5	16	752	8970	4759
2-(2-Butoxyethoxy)ethanol	112-34-5	15	752	7389	4854
Triethanolamine	102-71-6	1	9307	9307	9307
Sodium bicarbonate	144-55-8	0	NA	NA	NA
Urea	57-13-6	0	NA	NA	NA

*Officially classified as Acute Tox. 4/H302 – harmful if swallowed in Annex VI of the Regulation (EC) No. 1336/2008 (EU, 2008a). OECD's QSAR Toolbox is the metabolism simulator (rat liver S9 metabolism profiler).