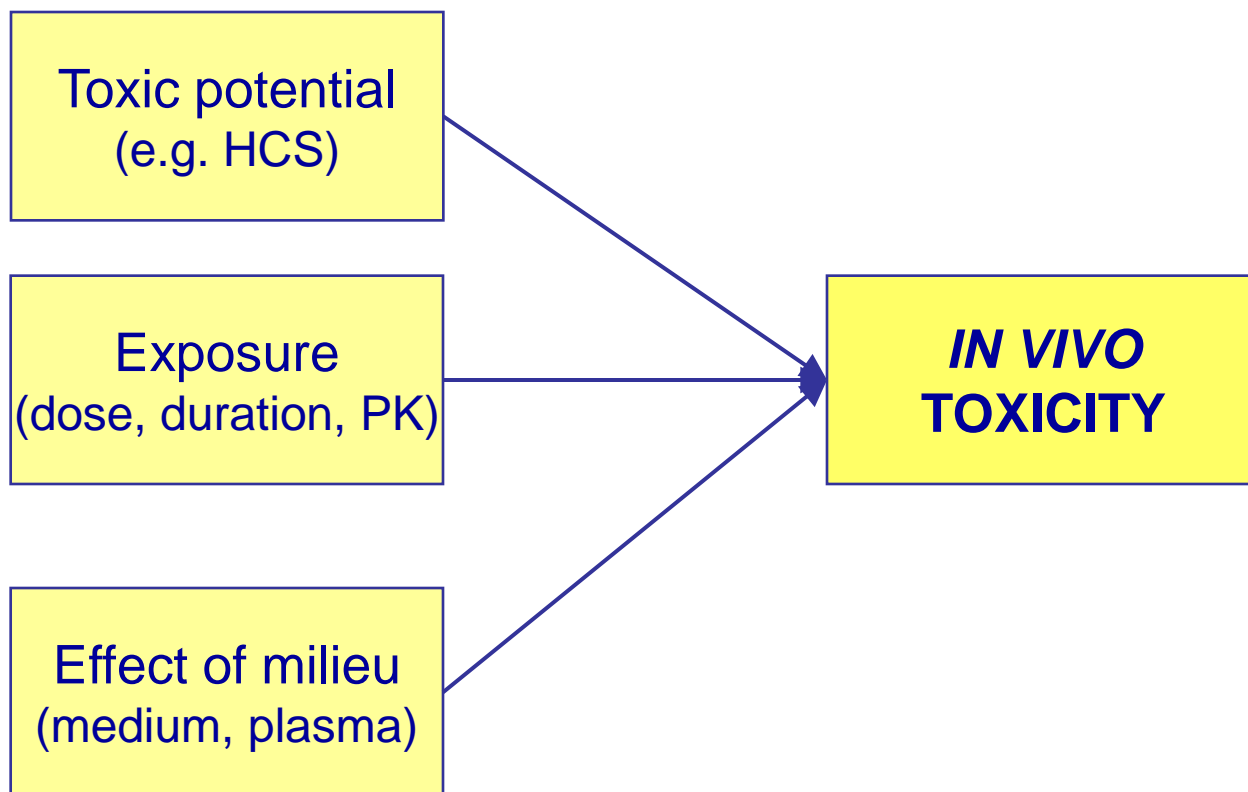# The Role of High Content Toxicology and *In Silico* Modelling in Identifying Toxic Liabilities

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In vivo toxicity is determined by xenobiotic toxicity, exposure and the modulating effects of environment

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CellCiphr™ High Content Toxicology

- High content screening (HCS) captures multiple mechanistic parameters covering a wide spectrum of cytopathological changes.

- CellCiphr™ comprises multiple cellular panels.

- HepG2 (Human hepatocellular carcinoma) – 10 endpoints; 1, 24 and 72h.
  - Insight into toxicity towards cycling cells.

- Rat primary hepatocytes – 8 endpoints; 1, 24 and 48h.
  - Primary cells with metabolic capability.
  - Investigate hepatocyte-specific toxicities.

- H9c2 cardiomyocytes – 8 endpoints; 1, 24 and 72h
  - Cardiomyocyte-specific toxicities.

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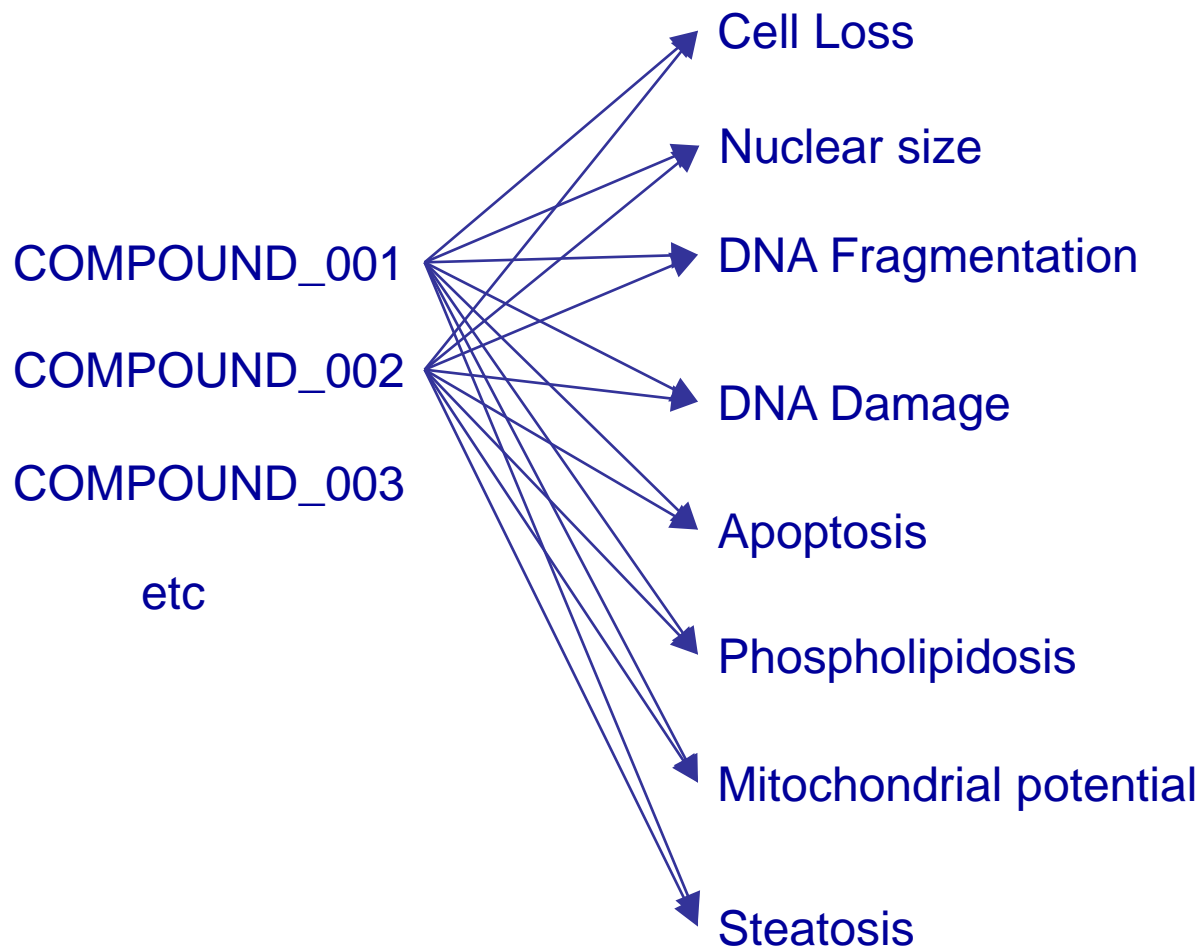
Example response image data
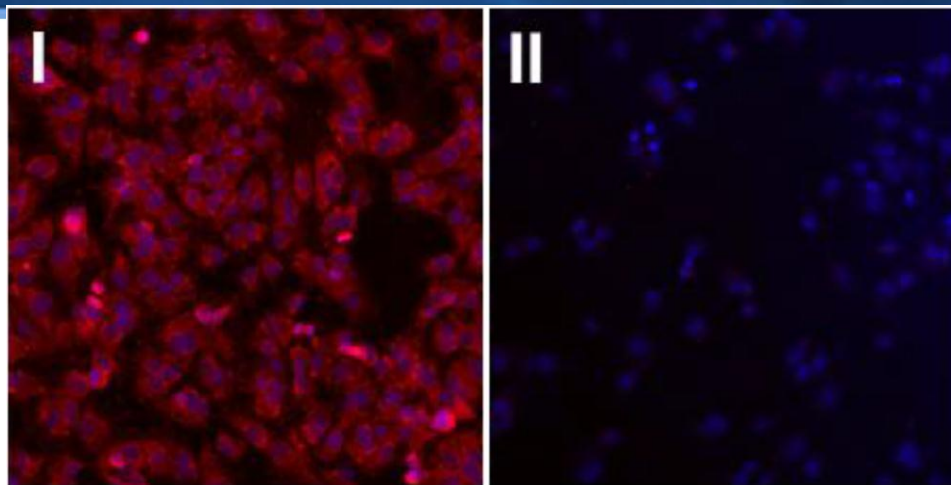
Mitochondrial Potential
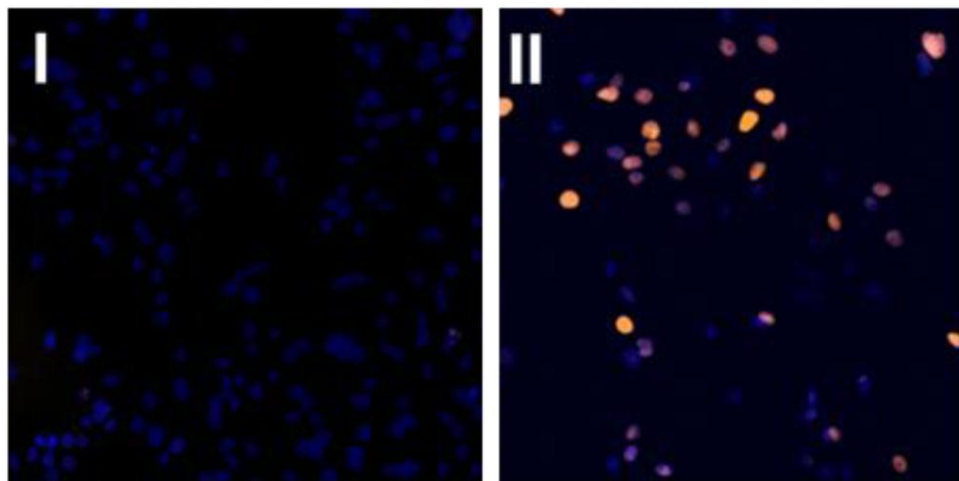
P53 activation

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Ranking toxicity based on a database of reference compounds

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Ranking toxicity based on a database of reference compounds

Cell Loss

Nuclear size

COMPOUND_001

DNA Fragmentation

COMPOUND_002

DNA Damage

COMPOUND_003

Apoptosis

etc

Phospholipidosis

Mitochondrial potential

Steatosis

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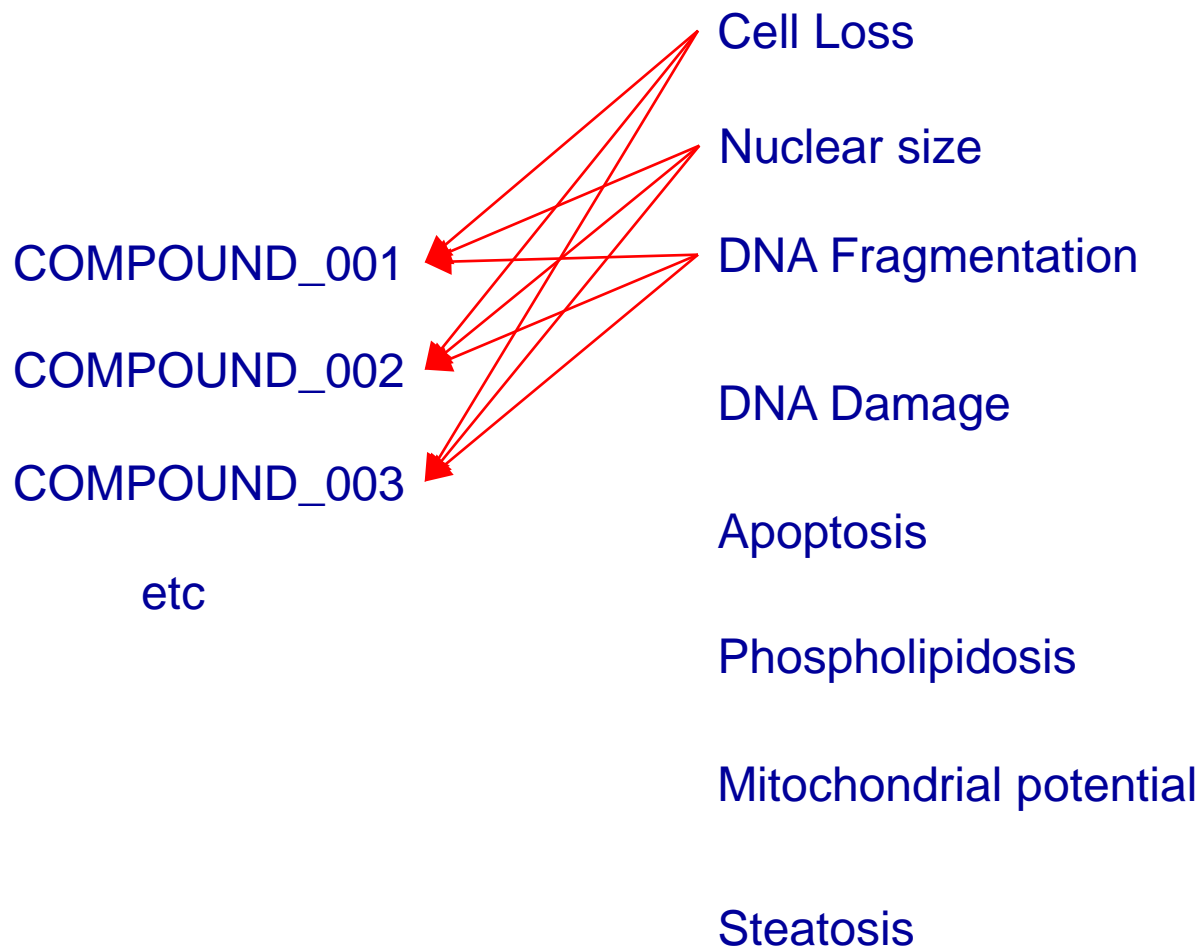
Ranking method – key features

- Key datum is the AC_{50}: the concentration at which response is 50% of that of a reference compound with high response against the endpoint.

- Method uses all AC_{50}s for <u>all</u> reference compounds for a cell type.

- Weightings are applied to the AC50 values:
    - Lower AC_{50}s have greater weight (more toxic).
    - Endpoints active for many compounds have lower weight (to reduce false positives).

- The basic model can be elaborated to include mechanistic effects, additional weighting etc.

- No reference to *in vivo* toxicity – based only on *in vitro* data

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Ranking method – example results

Toxicity Rank	HepG2 cells	Primary Rat Hepatocytes
1	paclitaxel	CCCP
2	amiodarone	terfenadine
3	nifedipine	chlorpromazine
4	etoposide	fluoxetine
	CCCP	chloroquine
	terfenadine	troglitazone
	chlorpromazine	amiodarone
	fluoxetine	ketoconazole
	propranolol	propranolol
	diethylstilbestrol	haloperidol
	haloperidol	etoposide
	ketoconazole	diclofenac
	chloroquine	trazodone
	troglitazone	diethylstilbestrol
	rosiglitazone	nifedipine
	quinidine	dexamethasone
	valproic acid	quinidine
	trazodone	paclitaxel
19	diclofenac	rosiglitazone
20	dexamethasone	valproic acid
21	carbamazepine	cyclophosphamide
22	acetaminophen	furosemide
23	cyclophosphamide	carbamazepine
24	furosemide	acetaminophen

18/30 endpoints activated, all …

19/30 endpoints activated with AC50s in the range 0.1 – 60uM. Cell loss is activated in the range 0.1 – …

9/30 endpoints activated with AC50s in the 40-250uM range.

13/24 endpoints acti…ted with AC50 i… the… Mi… affe… – … cel…

17/24 endpoints activated with AC50s in the 11-200uM range.

6/24 endpoints activated in the 1 – 1000uM range.

Only 2 endpoints activated with AC50s > 20 uM

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Ranking method – some example results

Paclitaxel:
- HepG2: 18/30 endpoints activated, all with sub-μM AC50s, many less than 10nM.
- Rat hepatocyte: only 2 endpoints activated, with AC50s > 20μM.

CCCP:
- HepG2: 21/30 endpoints activated with AC50s in the range 1 - 1400μM. Mitochondrial potential affected in the range 8 – 10μM. Cell loss activated in the range 2 - 10μM.
- Rat hepatocyte: 13/24 endpoints activated, with AC50s in the 0.1 - 10μM range. Mitochondrial potential affected in the range 1.6 - 11μM. Apoptosis and cell loss activated at sub-μM concentrations.

Troglitazone:
- HepG2: 9/30 endpoints activated with AC50s in the 40-250μM range.
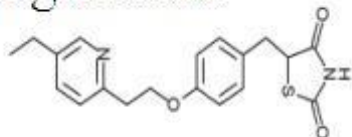- Rat hepatocyte: 17/24 endpoints activated with AC50s in the 11-200μM range.

Etoposide:
- HepG2: 19/30 endpoints activated with AC50s in the range 0.1 - 60μM. Cell loss is activated in the range 0.1 – 0.2μM.
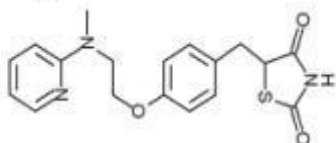- Rat hepatocyte: 6/24 endpoints activated in the 1 - 1000μM range.

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Rank order risk of development by CellCiphr™ Safety Risk

Compound	Trade Name	CellCiphr® Safety Risk	CellCiphr® Ranking	Commercial Status
pioglitazone	Actos®	0.414	Low	4	Occasional reversible cholestatic hepatitis
rosiglitazone	Avandia®	0.551	Moderate	3	Withdrawn Europe
ciglitazone	n/a	0.825	High	1=	Never used
troglitazone	Rezulin®	0.825	High	1=	Withdrawn

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CellCiphr™ screen quantitatively relates toxic endpoints to one another

AC50 for DNA damage at 24h (μM) vs AC50 for apoptosis at 24h (μM)

- These compounds cause apoptosis without DNA damage
- These compounds cause neither DNA damage nor apoptosis
- These compounds cause both apoptosis and DNA damage
- These compounds cause DNA damage but not apoptosis

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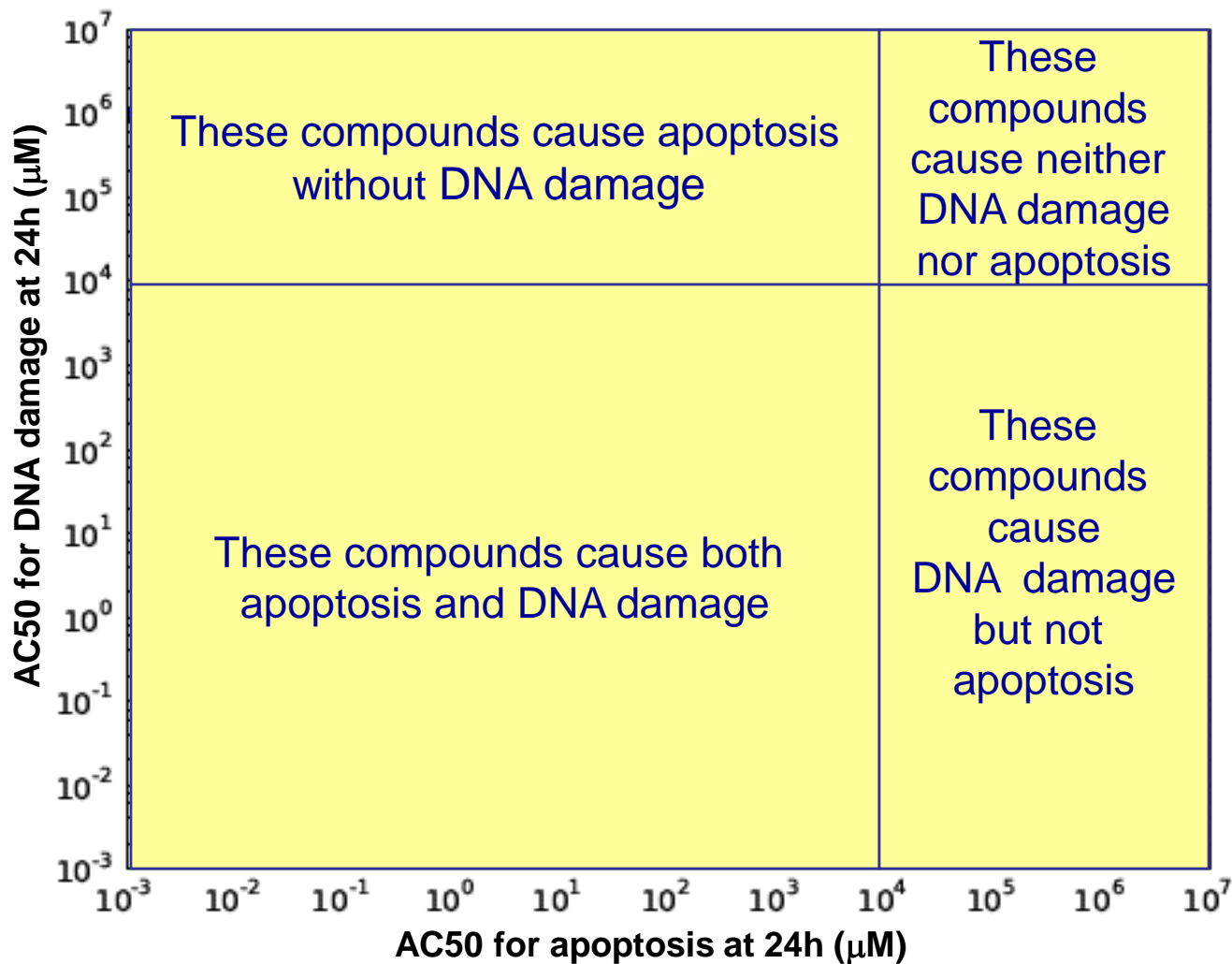
- CellCiphr™ results in <u>primary rat hepatocyte</u> were compared with endpoints for three preliminary *in vitro* screening assays.

- Between 31% and 41% of compounds that were negative in each of the preliminary screens showed a response in <u>at least</u> one CellCiphr™ endpoint.

- Less than 2% of compounds that were negative in the preliminary screens were also negative in all CellCiphr™ endpoints.

- The positive CellCiphr™ results were recorded as warnings that would require further investigation for any affected compound progressing down the pipeline.

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Summary of CellCiphr™ HCS

- CellCiphr™ HCS – generates quantitative data regarding:

  - The relationships between triggering of toxic responses in a particular cell type.

  - The time-courses of toxic response activation within a particular cell type.

  - Data on toxic responses across multiple cell types.

- The CellCiphr™ system uses its extensive database for reference compounds to rank and score test compounds, based on the HCS AC_{50}s.

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In vivo toxicity is determined by xenobiotic toxicity, exposure and the modulating effects of environment

Toxic potential (e.g. HCS)

Exposure (dose, duration, PK)

Effect of milieu (medium, plasma)

IN VIVO TOXICITY

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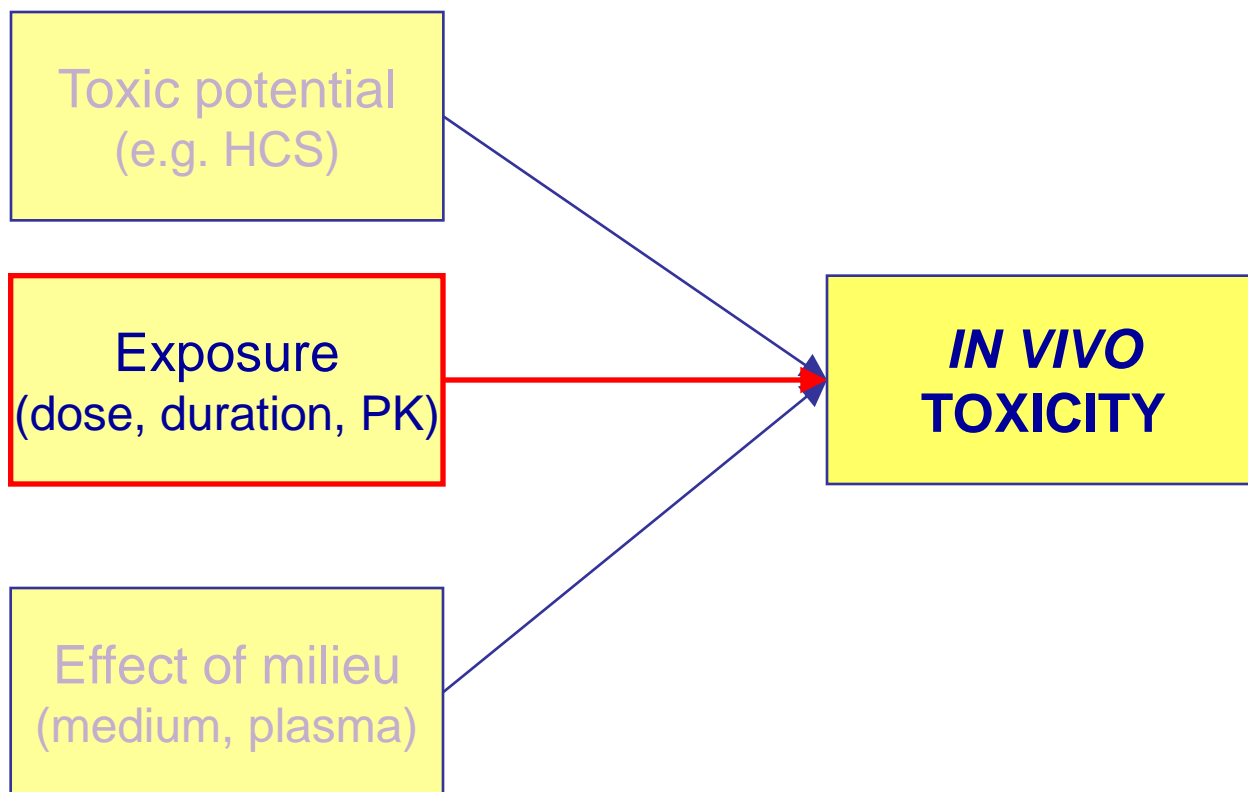
CellCiphr and exposure data are predictive of rat *in vivo* toxicity (big pharma case study)

- Relationships have been demonstrated between CellCiphr™ endpoints and specific *in vivo* toxicity markers in rat.

- These relationships are <u>considerably strengthened</u> when exposure (plasma C_{max}) is taken into account.

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CellCiphr data can be used to predict *in vivo* human drug-Induced liver injury (DILI)

- Data from Xu *et al* (2008)*:
  - 39 compounds labelled as safe (wrt DILI).
  - 98 compounds labelled as causing DILI.

- Use CellCiphr panels 1 and 2 data.

- Single dose C_{max} from the literature, or estimated where not available.

- AC_{50}s scaled by appropriate C_{max}.

- Build binary classification model to predict safe/DILI

*Toxicological sciences 105, 97–105.

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Interpretation of a binary classification model

		Observed *in vivo*	
		Safe	DILI
Predicted by model	DILI	False Positive	True Positive
	Safe	True Negative	False Negative

Sensitivity = fraction of toxic compounds detected = TP/(TP + FN).

Specificity = fraction of compounds predicted to be toxic that are toxic

= TP/(TP + FP)

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CellCiphr data can predict *in vivo* human DILI

		Observed *in vivo*	
		Safe	DILI
Predicted by model*	DILI	5	49
	Safe	34	49

Sensitivity = TP/(TP + FN) = 49/(49 + 49) = 50%

Specificity = TP/(TP + FP) = 49/(49 + 5) = 91%

*10-fold cross-validation on training set

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- 'False positives' are called safe by Xu et al, but predicted by the model to cause DILI:

  - **carbidopa** – labelled as 'most concern' for DILI by FDA.
  - **levodopa** – analogue of carbidopa.
  - **orphenadrine** – safety of long-term use has not been established: periodic monitoring of blood, urine and liver function values is recommended (FDA labelling).
  - **idarubicin** - chemotherapeutic, DNA intercalator, more potent in HepG2 then rat hepatocytes, expected to be toxic.
  - **pamidronate** – *in vivo* <u>decreases</u> in serum alkaline phosphatase; renal toxicity.

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Predictive models for *in vivo* toxicity require predictive modelling of exposure
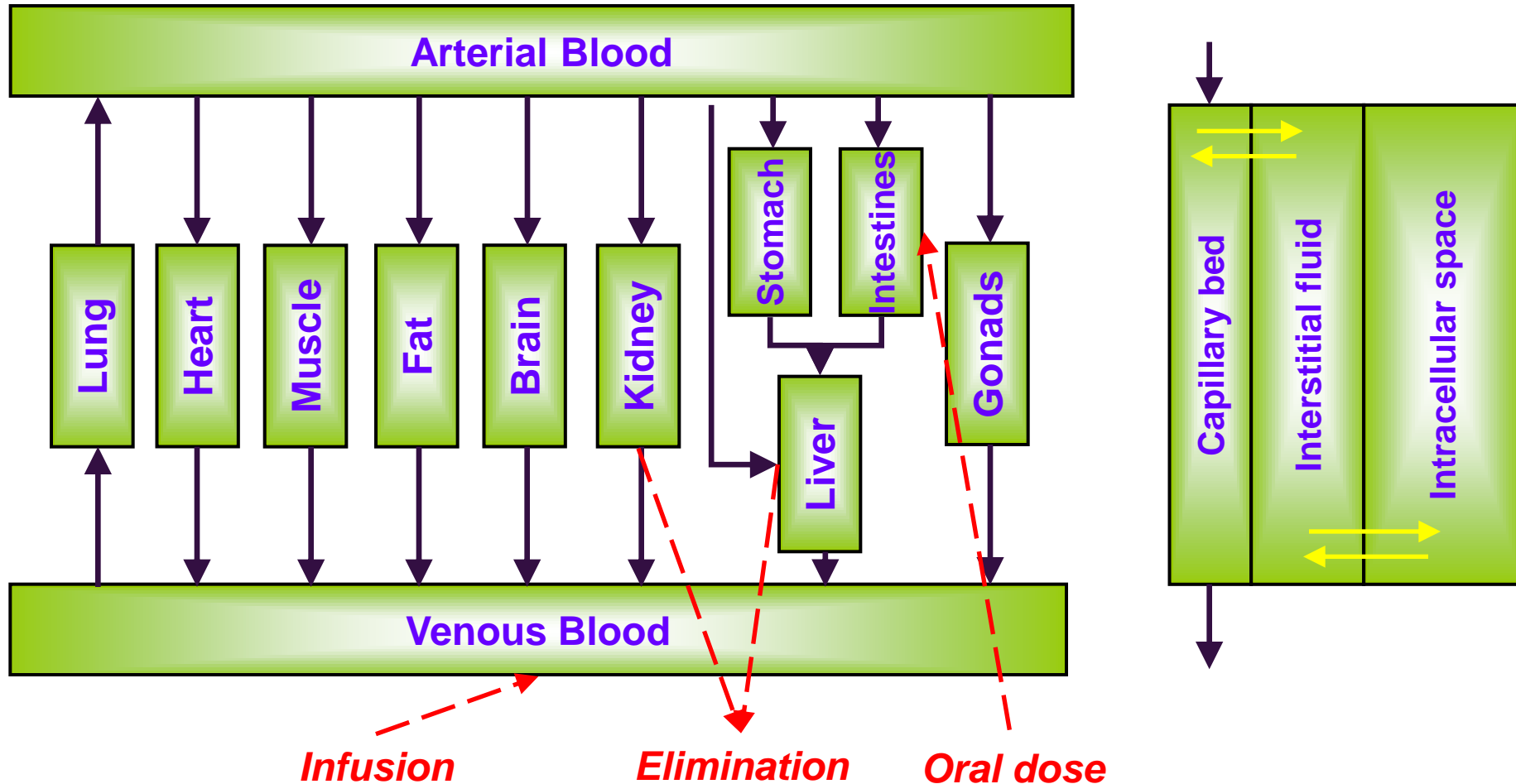
- A predictive screening approach should predict exposure (e.g. FA, C_{max}, AUC), and its link to dose, removing the need for *in vivo* PK data.

- Physiologically-based pharmacokinetic (PBPK) models satisfy these requirements.

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- PBPK models are mathematical simulation models.

- They are devised to predict the fate(s) of compound(s) in the bodies of humans, and other animals.

- Their primary output is the change over time following dosing of relevant quantities. e.g. the concentration of a compound in the plasma and other tissues.

- Simple physchem and *in vitro* ADME data can be used as inputs.

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A conceptual physiological model used to predict somatic distribution and elimination

Arterial Blood

Lung | Heart | Muscle | Fat | Brain | Kidney | Stomach | Intestines | Liver | Gonads

Venous Blood

Capillary bed | Interstitial fluid | Intracellular space

Infusion | *Elimination* | *Oral dose*

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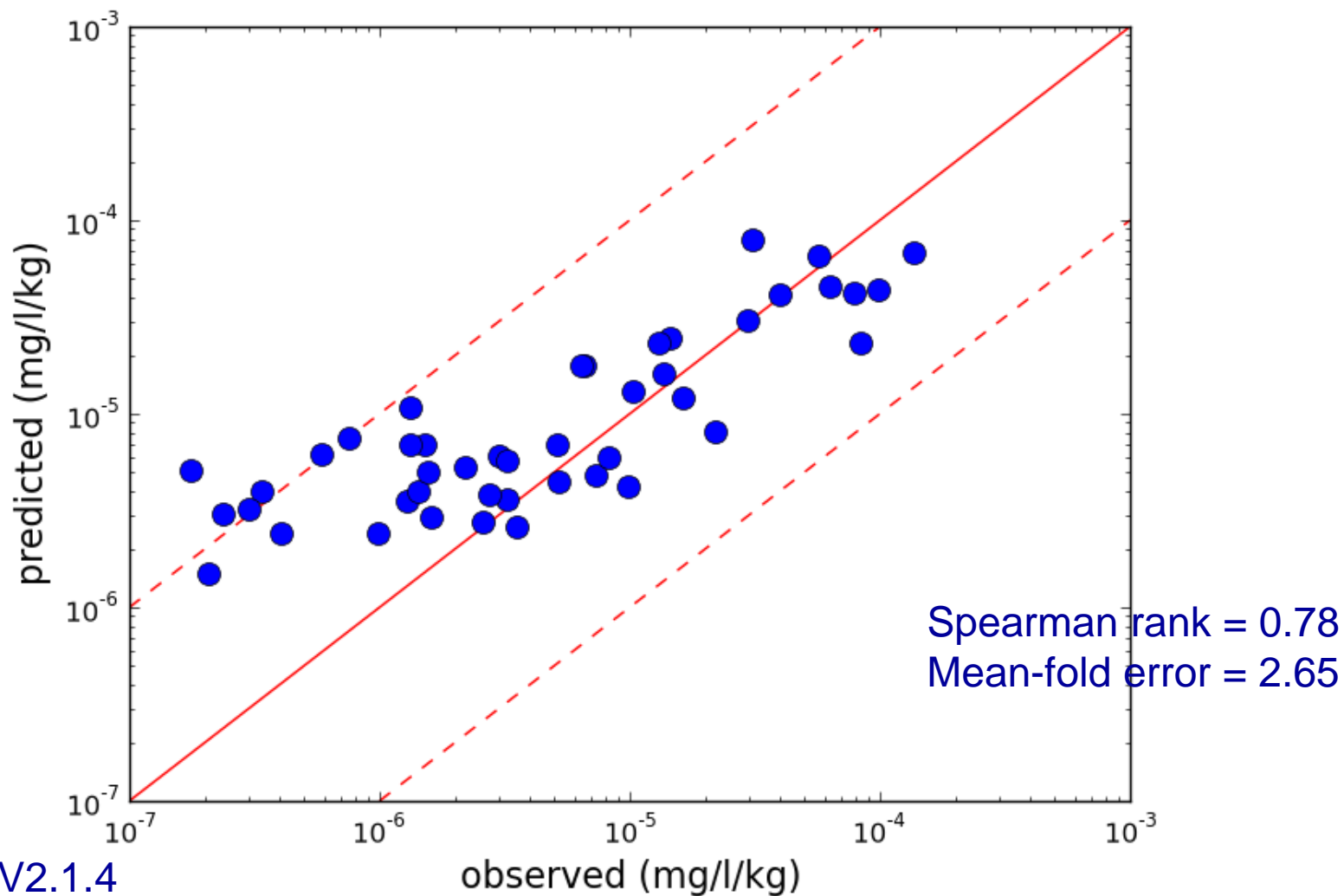# PBPK models inputs* for screening in drug discovery

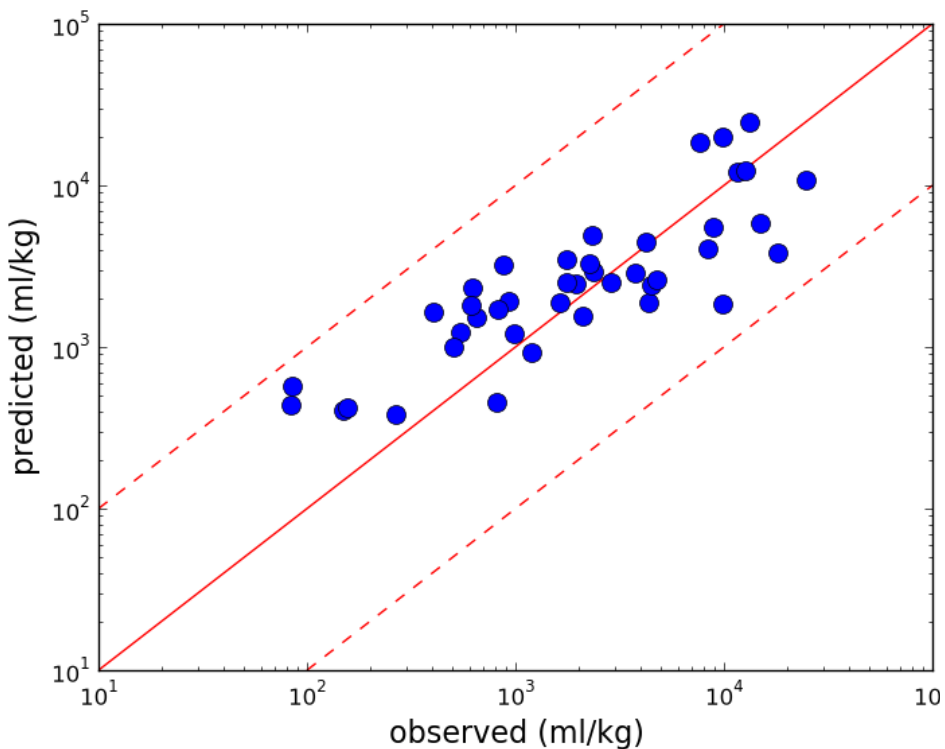| Input Property |
| --- |
| Hepatic microsomal intrinsic clearance (species-dependent) |
| Fraction unbound in plasma (species-dependent) |
| Blood:plasma ratio (species-dependent) |
| pKa(s) |
| logP octanol/water |
| Caco-2 permeability |
| Solubility (buffered) |

Prediction of i.v. dose, p.o. dose exposure

*Cloe® PK V2.1

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Prediction of Human Oral Dose Dose-Normalised C_{max} by PBPK Model*

Spearman rank = 0.78
Mean-fold error = 2.65

*Cloe® PK V2.1.4

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Prediction of Drug Distribution by PBPK Model*

Steady state volume of distribution

Elimination phase volume of distribution

*Cloe® PK V2.1.4

the ADME Tox specialists cyprotex
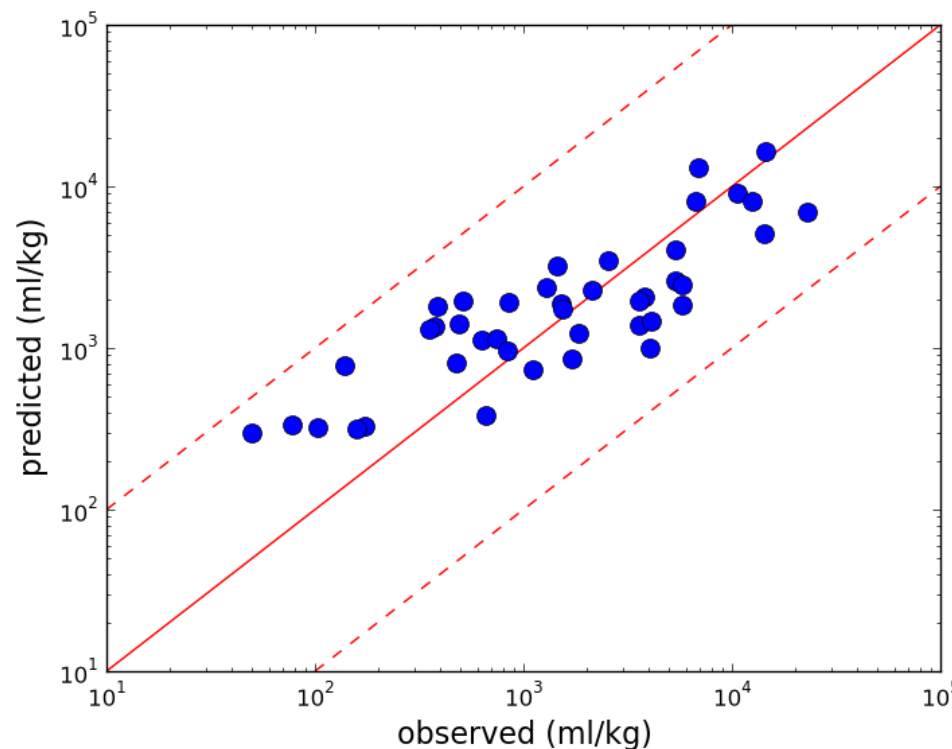
Summary of Exposure Prediction

- PBPK models can predict PK parameters, such as C_{max}, AUC, that are suitable for scaling *in vitro* HCS toxicity data.

- They can also provide more direct predictions of exposure relevant for hepatotoxicity prediction, e.g concentrations in the hepatic portal vein, in liver, etc.

- Distribution volume predictions provide confidence that intracellular exposure is predictable.

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In vivo toxicity is determined by xenobiotic toxicity, exposure and the modulating effects of environment

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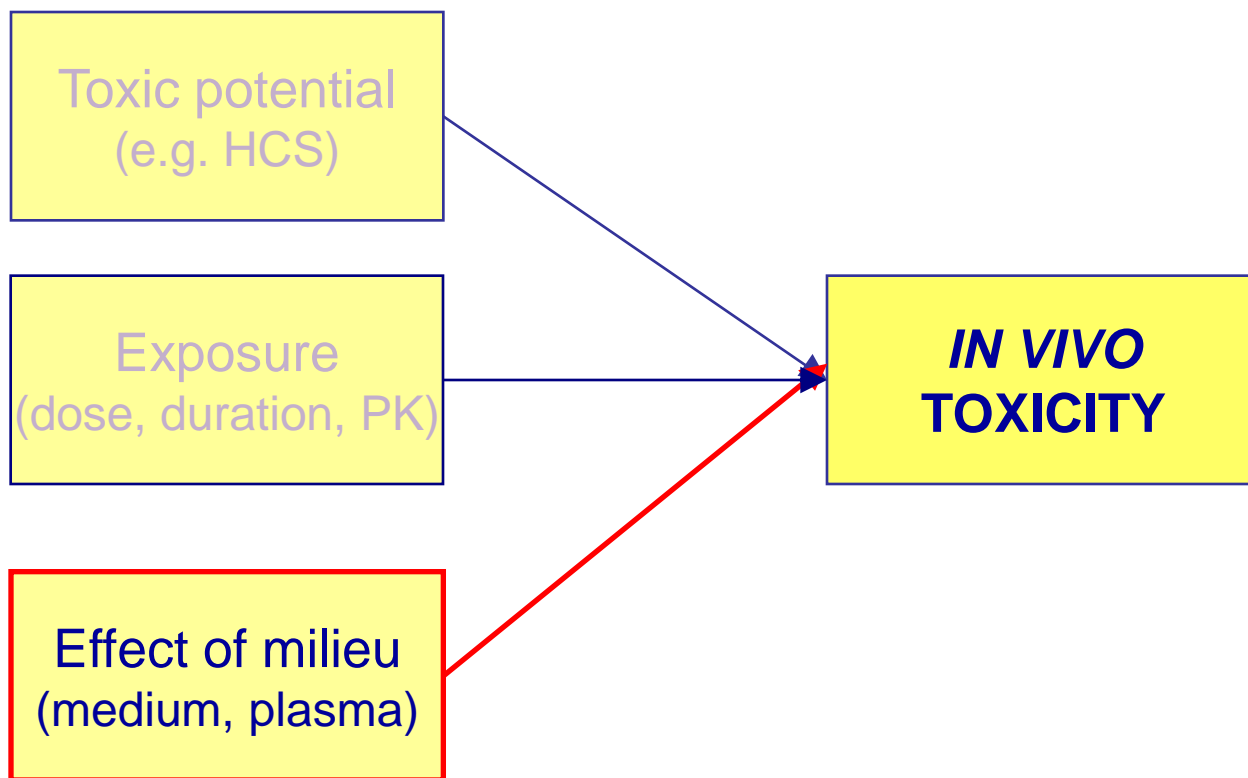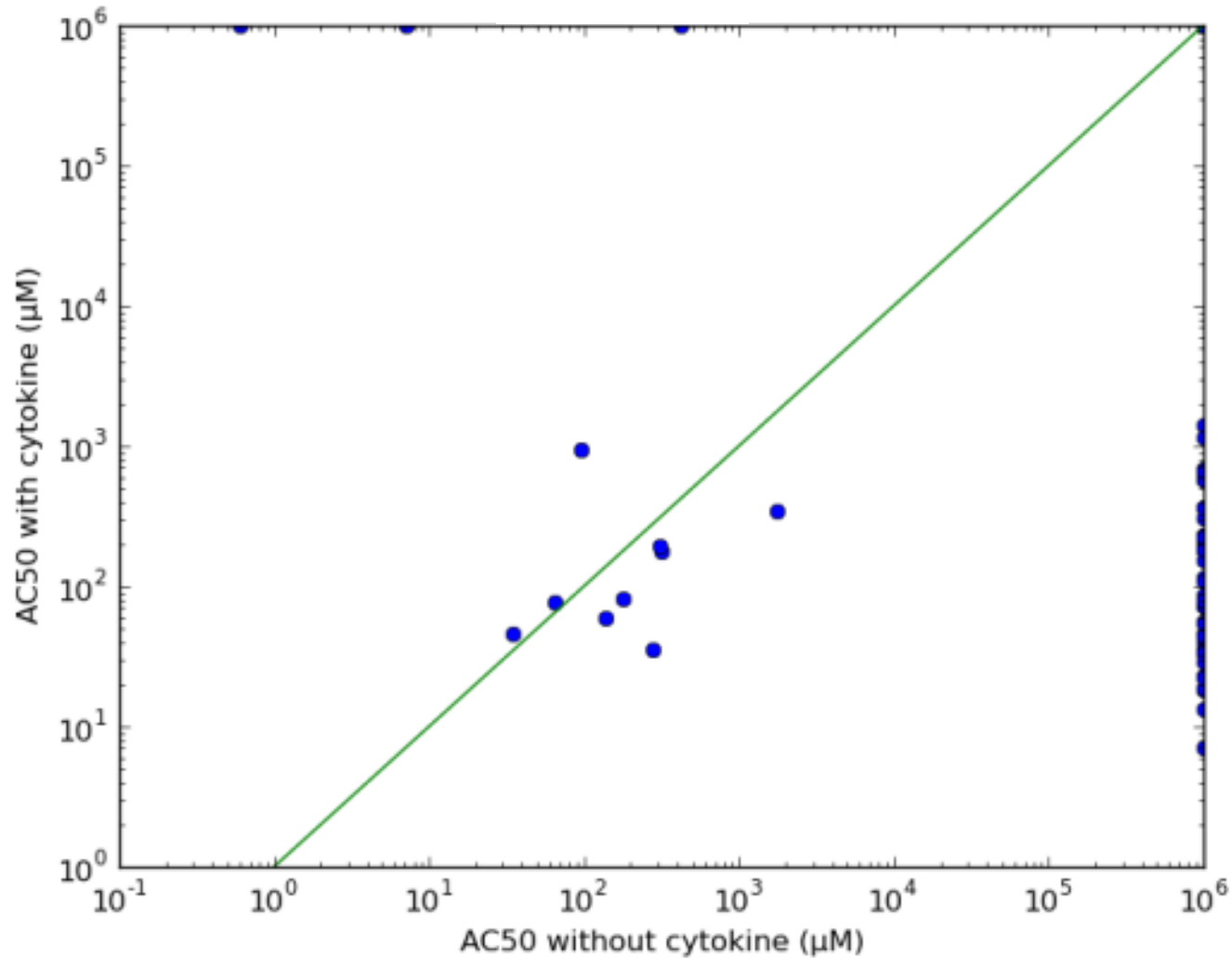
Summary of effect of milieu

- Xenobiotic effects, both *in vitro* and *in vivo* can be affected by the presence of bioactive molecules in the medium/plasma.

- This has been noticed in multiple CellCiphr™ HCS endpoints with cytokine exposure.

- The *in vitro – in vivo* interpretation of such data is in its infancy.

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Summary

- HCS captures multiple mechanistic parameters covering a wide spectrum of cytopathological changes.

- HCS data can be integrated, using machine-learning approaches to rank compounds on relative toxicity, compared to a reference database.

- Successful modelling of *in vivo* toxicity must account for exposure.

- Ongoing effort is to combine proven technologies – HCS, pattern recognition and PBPK modelling to predict *in vivo* toxicity from *in vitro* data.

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