WP7.2: Alerts and correctors in toxicity screening (IV): Nephrotoxicity (P18 and P25)

Selection of reference chemicals with nephrospecific potential

The following compounds were selected as potential nephrotoxic compounds namely cadmium chloride, ochratoxin A, cisplatin, diquat dibromide monohydrate, diethylene glycol.

The LLC-PK1 cells – TER assay

For the measurement of nephrotoxicity, transepithelial resistance (TER) was chosen as the functional assay and the LLC-PK1 proximal tubular cell line as the test system. The functional assay reflects the *in vivo* transporting capabilities of the renal proximal tubules. The functional assay was compared to a viability assay namely the resazurin (alamar blue) assay under exactly the same experimental conditions and testing was carried out in the 96-well plate format for both assays. The REMS automated device was selected for measurement of TER

Results WP7.2

The fifty seven reference chemicals (including some nephrotoxic) were tested and the overall results show that the TER is a sensitive predictor of nephrotoxicity. Using the data obtained, IC_{20}, IC_{50} and IC_{80} values were calculated for all chemicals tested (Table 8).

Table 8. Analysis of both non-nephrotoxic and nephrotoxic compounds using 96 well plate format.

	TER conc. in M			Resazurin conc. in M		
Reference Chemicals	**IC20 (M)**	**IC50 (M)**	**IC80 (M)**	**IC20 (M)**	**IC50 (M)**	**IC80 (M)**
Sodium Lauryl Sulfate (SLS)	2.25E-05	3.93E-05	1.27E-04	1.60E-04	3.00E-04	1.00E-03
Mercury II chloride	4.85E-05	6.54E-05	6.60E-05	4.90E-05	7.82E-05	1.05E-04
Cycloheximide	5.50E-05	8.74E-05	1.77E-04	4.78E-05	2.28E-04	NT
Phenobarbital	4.00E-05	1.20E-04	1.00E-03	4.00E-04	8.00E-04	NT
Pentachlorophenol	9.58E-05	1.22E-04	1.61E-04	1.38E-04	2.52E-04	4.00E-04
Digoxin	3.90E-04	1.60E-04	2.20E-04	3.50E-04	4.30E-04	NT
Diazepam	8.00E-05	1.67E-04	3.75E-04	4.00E-04	5.00E-04	8.00E-04
Atropine sulfate monohydrate	2.10E-04	3.00E-04	3.30E-04	2.40E-04	8.75E-04	NT
Carbamazepine	1.08E-04	3.25E-04	4.35E-04	9.10E-05	3.02E-04	6.03E-04
Acetominophen (Paracetamol)	3.00E-04	6.00E-04	9.50E-04	8.20E-05	6.00E-04	NT
Acetylsalicylic acid	7.00E-04	8.00E-04	9.00E-04	6.00E-04	7.00E-04	NT
Sodium valproate	4.50E-04	3.55E-03	ND	2.35E-04	5.90E-04	NT
Caffeine	NT	NT	NT	NT	NT	NT
Colchicine	NT	NT	NT	NT	NT	NT
Isopropyl alcohol	NT	NT	NT	NT	NT	NT
Malathion	NT	NT	NT	NT	NT	NT
Selected nephrotoxins						
Cadmium chloride	1.93E-06	2.42E-06	2.88E-06	2.17E-06	1.02E-05	1.37E-05
Diquat dibromide monohydrate	2.30E-05	2.98E-05	3.67E-05	1.75E-05	3.10E-05	8.13E-05
Cisplatin	1.66E-05	3.14E-05	5.48E-05	1.98E-05	3.44E-05	2.69E-04
Ochratoxin A	1.93E-05	3.67E-05	5.43E-05	NT	NT	NT
Diethylene glycol	NT	NT	NT	NT	NT	NT
Other Chemicals						
Cadmium chloride	1.93E-06	2.42E-06	2.88E-06	2.17E-06	1.02E-05	1.37E-05
thioridazine hydrochloride	9.50E-06	9.70E-06	2.00E-05	1.33E-05	3.40E-05	6.70E-05
thallium sulphate	1.50E-05	2.46E-05	3.00E-05	1.70E-06	7.50E-05	3.00E-04
paraquat dichloride	2.00E-05	2.80E-05	3.50E-05	3.60E-02	4.50E-05	1.50E-04
dichlorvos	4.25E-05	5.85E-05	8.00E-05	2.50E-05	1.25E-04	2.30E-04
Cyclosporine A	1.07E-04	7.30E-05	1.36E-03	ND	1.02E-06	ND
chloroquine diphosphate	2.90E-05	7.60E-05	1.50E-04	1.00E-04	1.35E-04	3.00E-04
amitriptyline hydrochloride	6.33E-05	8.20E-05	9.75E-05	6.00E-05	1.00E-04	2.00E-04
amiodarone hydrochloride	3.66E-05	9.30E-05	2.39E-04	ND	2.92E-05	ND

TER showed greater sensitivity for nephrotoxic chemicals compared to non-nephrotoxic chemicals. However, compounds requiring metabolism, such as diethylene glycol did not show toxicity at the highest concentration tested (Table 9).

Table 9. List of compounds where TER was found to be more sensitive than resazurin assay.

	TER	Resazurin
Chemical	**IC50 (M)**	
Cadmium chloride	2.42E-06 *	1.02E-05
thioridazine hydrochloride	9.70E-06	3.40E-05
thallium sulphate	2.46E-05 ***	7.50E-05
paraquat dichloride	2.80E-05 ***	4.50E-05
Diquat dibromide monohydrate	2.98E-05	3.10E-05
Cisplatin	3.14E-05	3.44E-05
Sodium Lauryl Sulfate (SLS)	3.93E-05 **	3.00E-04
dichlorvos	5.85E-05	1.25E-04
Mercury II chloride	6.54E-05	7.82E-05
chloroquine diphosphate	7.60E-05 *	1.35E-04
amitriptyline hydrochloride	8.20E-05	1.00E-04
Cycloheximide	8.74E-05 *	2.28E-04
Phenobarbital	1.20E-04 *	8.00E-04
Pentachlorophenol	1.22E-04 **	2.52E-04
Digoxin	1.60E-04 *	4.30E-04
Diazepam	1.67E-04 *	5.00E-04
orphenadrine hydrochloride	1.70E-04 ***	3.47E-04
(-)-epinephrine bitartrate	2.67E-04	5.30E-04
Atropine sulfate monohydrate	3.00E-04 *	8.75E-04
rifampicine	3.62E-04 ***	1.42E-02
sodium fluoride	4.00E-04 **	1.40E-03
tetracycline hydrochloride	9.50E-04	1.17E-03
nicotine	1.35E-03 ***	7.90E-03
theophylline	1.70E-03 *	8.00E-03
(±)-verapamil hydrochloride	2.33E-03 **	4.50E-03
lithium sulfate	1.20E-02 **	5.50E-02

Excellent inter-laboratory comparison of the TER was obtained between the two participating laboratories. (Figure 5)

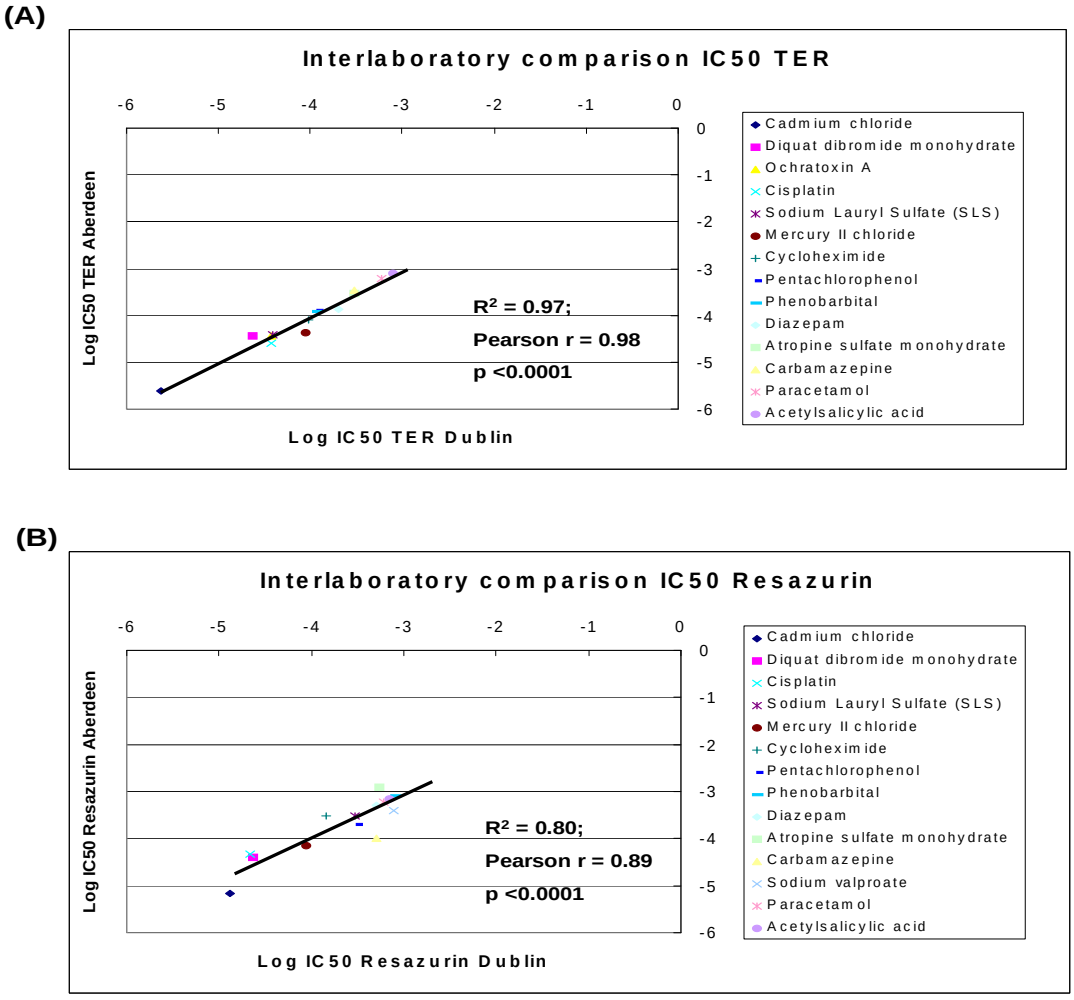

Figure 5. Inter-laboratory comparison of IC_{50} for TER and resazurin assays.

The results indicate that the TER functional assay is a very promising assay to detect nephrotoxicity *in vitro* and is more sensitive than the viability assay. The REMS automated device facilitates high throughput of the TER assay.

Two set-ups of the test system have been evaluated and compared. A 24-well Costar HTS polycarbonate filter plate system and a 96-well system. A comparison of the data obtained with the 96-well format and the 24-well format showed good correlation between the two systems.

Alerts for nephrotoxicity

The results indicate that the TER functional assay is a very promising assay to detect nephrotoxicity *in vitro* and is more sensitive than the viability assay.

The TER assay was set up specifically to detect nephrotoxicity rather than an alternative viability assay and it was not finally selected in the tiered testing strategy in comparison to the 3T3 fibroblast cell line viability assay (3T3/NRU) because the multivariate CART results did not change after removing TER from multivariate CART models.

In terms of alerts, all the known nephrotoxins had an IC_{50} value of less than 50μM in the TER assay. For the non-nephrotoxic reference compounds, this property was only shared by digoxin, where one of the mechanisms of action involves inhibition of the Na^+ /K^+ pump.