Prieto et al.:

Investigating cell type specific mechanisms contributing to acute oral toxicity

Supplementary Data

Tab. S1: Overview of collected information on specific target organ/system and general cytotoxicity for chemicals correctly assigned to the CLP acute oral toxicity category by the 3T3 NRU cytotoxicity assay

Chemical	General cytotoxicity	Liver	Lung	Nervous system	Cardiovascular system	GI system	Blood	Kidney
(±)-Propranolol hydrochloride				x	[a]x			
(4-Ammonio-m-tolyl)ethyl(2-hydroxyethyl)ammonium sulfate	x							x
1,2,4-Trichlorobenzene	x	x						
1,2-Dichlorobenzene	x	x						
2,4,6-Tris(dimethylaminomethyl)phenol			x	x				
2,4-Dichlorophenoxyacetic acid	x	x		x				
5,5-Diphenylhydantoin				x	x			
5-Fluorouracil	x		x		x	x		
Acetophenone				x				
Acetylsalicylic acid	x	x	x	x			x	x
Acrolein	x		x					
Acrylamide	x	x		x				
Ammonium chloride			x		[a]x			[a]x
Atropine sulfate monohydrate				x	x			
Benzaldehyde	x			x				
Cadmium (III) chloride	x	x	x		x			x
Caffeine	x			x	x			
Chloroform	x	x	[a]x	x	x	x		x
Chloroquine bis(phosphate)	x				x			
Chlorpromazine	x	x		x				
Codeine		x		x	x			x
Colchicine	x	x		x		x		
Copper sulfate	x	x				x		
Cupric sulfate pentahydrate	x						x	
Cyclosporin A	x	x			x			x
Diazepam				x				
Diphenhydramine hydrochloride				x	x			
Disopyramide				x	x			
Ethyl chloroacetate			x			x		
Ferrous sulfate	x	x				x		
Glufosinate-ammonium				x				
Glutethimide	x		[a]x	x				
Hexachlorophene	x			x				
Lithium carbonate				x	x			x
Lithium sulfate				x	x			x
Malathion				x	x			
Maleic acid	x							x
Meprobamate				x	x			
Orphenadrine hydrochloride	x	x		x	x			
p-Benzoquinone	x		x					x
Phenol	x	x		x		x		x
Procainamide hydrochloride				x	x			x
Quinidine sulfate dehydrate				x	x		x	

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ALTEX 36(1), SUPPLEMENTARY DATA

Chemical	General cytotoxicity	Liver	Lung	Nervous system	Cardiovascular system	GI system	Blood	Kidney
Resorcinol				x			x	
Rifampicin		x					x	
Sodium Cyanate	x			x				
Sodium oxalate	x				x			x
Sodium valproate	x	[b]x		x	x			
Thioridazine hydrochloride				x	x			
Valproic acid	x	x		x	x	x		

GI: Gastrointestinal; CLP: Classification, labelling and packaging; NRU: Neutral Red Uptake; [a] Indirect effect: [b] chronic effect

Tab. S2: Overview of collected information on specific target organ/system and general cytotoxicity for chemicals with CLP acute oral toxicity category under-predicted by the 3T3 NRU cytotoxicity assay

Chemical	General cytotoxicity	Liver	Lung	Nervous system	Cardiovascular system	GI system	Blood	Kidney	Immune system
Potassium cyanide	x			x			x		
(±)-Verapamil hydrochloride					x				
1-Phenyl-2-thiourea	x		x						
1-Phenyl-3-pyrazolidone			x	x					
Barium chloride	x	x			[a]x	x			
Brucine	x			x				x	
Chloral hydrate	x	x		x	x	x			
cis-Diammineplatinum (II) dichloride	x							x	
D-Amphetamine				x					
Dichlorvos	x		x	x					
Disulfoton				x					
Endosulfan	x			x					
Epinephrine hydrogen tartrate				x	x				
Fenpropathrin				x					
Lindane	x	x		x				x	
Malononitrile	x	x	[a]x	x	x	x	x	x	
Mercury II chloride	x					[b]x		x	
Methadone hydrochloride			x	x					
Nicotine	x			x					
Ochratoxin A	x							x	x
Paraquat dichloride	x	x	x					x	
Parathion			[a]x	x					
Pentachlorophenol	x	x		x				x	
Phenobarbital			[a]x	x	[a]x				
Physostigmine				x					
Sodium pentobarbital			[a]x	x					
Sodium salt of chloroacetic acid	x			x				x	
Strychnine	x			x					
Thallium sulfate	x			x	x				
Theophylline	x			x	x	x			
Triethylenemelamine	x					x			x
Warfarin					x		x		

GI: Gastrointestinal; CLP: Classification, labelling and packaging; NRU: Neutral Red Uptake; [a] indirect effect; [b] corrosive

Tab. S3: Overview of collected information on specific target organ/system and general cytotoxicity for chemicals with CLP acute oral toxicity category over-predicted in the 3T3 NRU cytotoxicity assay

Chemical	Cytotoxicity	Liver	Lung	Nervous system	Cardiovascular system	GI system	Blood	Kidney	Immune system
17α-Ethynyloestradiol	x	x				x			
1-Naphthylamine							x		
Amitriptilyne hydrochloride	x			x	x				
Diquat dibromide	x	x				x		x	
Haloperidol	x			x					
Maprotiline	x			x					
N-isopropyl-N'-phenyl-p-phenylenediamine							x		
Tert-butyl hydroperoxide	x		x				x		
Triphenyltin hydroxide									x

GI: Gastrointestinal; CLP: Classification, labelling and packaging; NRU: Neutral Red Uptake

Tab. S4: Specific target organ/systems and general cytotoxicity reported for the chemicals falsely predicted as negatives (i.e. LD_{50} > 2000 mg/kg) by the 3T3 NRU cytotoxicity assay

Chemical	General cytotoxicity	Liver	Lung	Nervous system	Cardiovascular system	GI system	Kidney
Aconitine	x			x	x		
Isoniazid	x	x		x			
Diethylene glycol			x	x			x
Digoxin					x		
Ethylene glycol			x	x	x		x
Paraldehyde				x		x	

GI: Gastrointestinal; NRU: Neutral Red Uptake

Tab. S5: Adverse outcome pathways outlined or fully developed with potential relevance to acute oral toxicity

Adverse outcome	Molecular initiating event (MIE) / Key events (KEs)	Stressor	References
Cell death (Apoptosis/necrosis) [1](AOP 205) In support of cytotoxicity as general mechanism (non-cell specific)	• Decompartmentalization • Direct mitochondrial inhibition • Narcosis • Mitochondrial impairment (key event)		Vinken and Blaauboer, 2017
Hematotoxicity [1](AOP 31)	• Parent compound is converted to the reactive metabolite and forms free radicals leading to oxidation of heme iron(II) in hemoglobin to iron(III) • Altered regulation of alpha-hemoglobin • Oxidative stress propagation • Damaging of red blood cells; hemolysis • Formation of hemoglobin adducts • Down regulation of glucose-6-phosphate dehydrogenase • Increase RBC congestion in liver • Increase liver and splenic hemosiderosis • Decrease in hemoglobin, hematocrit, and red blood cell number (methemoglobinemia)		
Acute liver response [1](AOP 11)	• PXR/SXR activation • Key events unknown		
Cholestatic liver injury [1](AOP 27)	• Inhibition of bile salt export pump (ABCB11) • Activation of specific nuclear receptors, transcriptional change • Bile accumulation • Cytokine release • Increased inflammation • Reactive oxygen species production • Peptide oxidation		Vinken et al., 2013
Liver inflammation [1](AOP 144)	• Lysosome disruption • Peptide oxidation • Mitochondrial dysfunction 1 • Cell injury/death • Cytokine release • Inflammatory cells	• Iron compounds • ROS • o-Methyl-serine dodecylamide hydrochloride (MSDH) • alpha-Tocopheryl succinate • 3-Aminopropanal • Artesunate • Naphtharazine • Fluoroquinolones	
Decrease lung function [1](AOP 148)	• EGFR activation • Trans differentiation of ciliated epithelial cells • Metaplasia of goblet cells • Goblet cell hyperplasia • Proliferation of goblet cells • SP1 activation • Apoptosis of ciliated epithelial cells • Increased mucin production • Chronic mucus hypersecretion	• ROS	
Increased mortality (Impaired heart function) [1](AOP 104)	• Impaired ion channels • Altered action potential • Increased cardiac arrythmia		
Hypertension [1](AOP 149)	• Peptide oxidation • S-Glutathionylation, eNOS • Decrease in GTPCH-1 • Decrease in tetrahydrobiopterin	• ROS	

Adverse outcome	Molecular initiating event (MIE) / Key events (KEs)	Stressor	References
	• Uncoupling of eNOS • Depletion of nitric oxide • Impaired vasodilation • Increase in vascular resistance • Decrease in AKT/eNOS activity		
Learning and impaired memory [4](AOP 48)	• Binding of agonist to ionotropic glutamate receptors • Mitochondrial dysfunction 1 • Cell injury/death • Neurodegeneration • Overactivation of NMDARs • Intracellular calcium overload • Decrease in neuronal network function in adult brain • Neuroinflammation	• Domoic acid	
Epileptic seizures [2](AOP 10)	• Binding at picrotoxin site of iGABAR chloride channel • Reduction in ionotropic GABA receptor chloride channel conductance • Reduction of neuronal synaptic inhibition • Generation of amplified excitatory postsynaptic potential (EPSP) • Occurrence of a paroxysmal depolarizing shift	• Lindane • Endosulfan • Picrotoxin • Dieldrin • Heptachlor • RDX • Fipronil	
Parkinsonian motor deficits [3](AOP 3)	• Binding of inhibitor to NADH-ubiquinone oxidoreductase (complex I) • Inhibition of NADH-ubiquinone oxidoreductase (complex I) • Mitochondrial dysfunction 1 • Impaired proteostasis • Neuroinflammation • Degeneration of dopaminergic neurons of the nigrostriatal pathway	• MPP+	
Acute mortality [1](AOP 16)	• Inhibition of acetylcholinesterase (AchE) • Accumulation of acetylcholine in synapses • Increased atrioventricular block and bradycardia • Increased respiratory distress/arrest • Induction of ataxia, paralysis, or hyperactivity	• Organophosphates • Carbamates	Russom et al., 2014
Acute mortality [1](AOP 96)	• Modulation of sodium channel • Prolonged depolarization of neuronal membrane • Neurotransmitter release • Muscle contraction • Increased ataxia, paralysis, or hyperactivity	• Cypermethrin • Permethrin • Esfenvalerate • Tralomethrin • Bifenthrin • Cyfluthrin • lambda-Cyhalothrin	
Neurodegeneration [1](AOP 17)	• Binding to SH/seleno proteins • Oxidative stress • Glutamate dyshomeostasis • Cell injury/death • Neuroinflammation • Tissue resident cell activation • Increase of pro-inflammatory mediators • Decrease of neuronal network function	• Mercuric chloride • Acrylamide • Acrolein • Methylmercuric(II) chloride • Thiomersal	
The acute neurotoxicity induced by binding of pyrethroid insecticides to voltage-gated sodium channels	• Binding to the alpha subunit of voltage-gated sodium channels • Changes in the kinetics of channel opening and closing • Alterations in excitability of neuronal membranes	• Pyrethroid	Bal-Price et al., 2017

Adverse outcome	Molecular initiating event (MIE) / Key events (KEs)	Stressor	References
(not in AOP-Wiki)	• Dysregulation of neural networks		
Delayed neuropathy	• Binding to neuropathy target esterase (NTE)	• Organophosphates	Bal-Price et al., 2015
Colony loss/failure [1](AOP 84)	• MIE unknown • Suppression of the immune system • Increased viral susceptibility • Impaired development		
Colony loss/failure [1](AOP 85)	• MIE unknown • Suppression of the immune system • Increased viral susceptibility • Abnormal foraging activity and behavior		
Inhibition/activation of gastric ulcer formation [1](AOP 217)	• MIE unknown • Inhibition of PTGS-1 (Prostaglandin-endoperoxide synthase 1) • Activation of PTGS-1 • Activation of PTGS-2 (Prostaglandin-endoperoxide synthase 2) • Inhibition of PTGS-2 • Increase in bicarbonate • Increased mucous production • Increased mucosal blood flow • Increased platelet aggregation • Increased angiogenesis • Decreased leukocyte adherence • Increased leukocyte adherence • Activation of leukocytes • Activation of mucosal defense • Increased surfactant production • Decreased surfactant production • Activation of phospholipase • Increase of ammonium ($NH4^+$)	• Nonsteroidal anti-inflammatory drug • *Helicobacter pylori*	
Gastric ulcer formation [1](AOP 227)	• Reduced PTGS1 function • Reduced platelet aggregation • Decreased mucosal blood flow • Reduced mucosal defense • Decreased mucous production • Decrease in bicarbonate production	• Nonsteroidal anti-inflammatory drug	
Gastric ulcer [1](AOP 228)	• Reduced PTGS2 function • Decreased angiogenesis • Increased leukocyte adherence • Increased leukocyte activation	• Nonsteroidal anti-inflammatory drug	
Kidney toxicity [1](AOP 33)	• Activation of 5HT2c • Key events unknown		
Renal failure and mortality [1](AOP 177)	• Inhibition of cyclooxygenase 1 activity • Decrease of prostaglandin F2alpha concentration in plasma • Renal ischemia • Renal proximal tubular necrosis • Increased potassium concentration in blood • Cardiac arrhythmia • Increased oxidative stress • Increased uric acid concentration in blood • Occurrence of tophi (urate) deposition	• Flunixin meglumine • Ketoprofen • Diclofenac sodium • Clofibrate • Indomethacin • Ibuprofen • Meloxicam • Celecoxib • Nimesulide • Phenylbutazone • Carprofen	
Renal failure and mortality [1](AOP 138)	• Inhibition of organic anion transporter 1 (OAT1) • Increased uric acid concentration in blood • Renal proximal tubular necrosis • Increased potassium concentration in blood • Occurrence of tophi (urate) deposition	• Ketoprofen • Diclofenac sodium • Indomethacin • Ibuprofen • Phenylbutazone • Amlexanox • Oxaprozin • Nitazoxanide	

Adverse outcome	Molecular initiating event (MIE) / Key events (KEs)	Stressor	References
	• Cardiac arrhythmia • Increased oxidative stress	• Ketorolac • Tromethamine • Telmisartan • Diflunisal • Mefenamic acid	
Kidney toxicity [1](AOP 256)	• Inhibition of mitochondrial DNA polymerase gamma (Pol gamma) • Mitochondrial DNA depletion • Mitochondrial dysfunction • Increased cytotoxicity (renal tubular cell)	• Tenofovir • Tenofovir disoproxil fumarate • Adefovir • Adefovir dipivoxil • Cidofovir	
Kidney toxicity [1](AOP 257)	• Binding of substrate to endocytic receptor • Disturbance of lysosomal function • Disruption of lysosome • Increased cytotoxicity (renal tubular cell)	• Cadmium • Aminoglycosides (high) • Gentamicin • Tobramycin • Vancomycin (moderate) • Polymyxin B • Colistin • Albumin • Low molecular weight proteins	
Kidney toxicity [1](AOP 258)	• Protein alkylation • Mitochondrial dysfunction • Decrease in mitochondrial ATP production • Increased cytotoxicity (renal tubular cell)	• Allyl alcohol • Carbon tetrachloride • Retinol • Dimethyl nitrosamine • Thioacetamide	
Renal failure and mortality [1](AOP 186)	• MIE unknown • Increase of reactive oxygen species production • Increased oxidative stress • Renal proximal tubular necrosis • Increased in blood potassium concentration • Cardiac arrhythmia	• Diclofenac sodium	

Hyperlinks direct to the specific AOP in the AOPWiki; Status of AOP in AOPwiki: 1= under development, 2= under review; 3 = approved; 4 = endorsed; Under certain exposure conditions (concentration and time) the above KEs defined in the outlined AOPs could contribute to the mechanisms of acute toxicity. For instance, antagonists binding at the picrotoxin site of iGABAR chloride channel channels, leads to reduction of ionotropic GABA receptor function, causing epileptic seizures. However, in some cases, the same mechanisms also will be linked to chronic toxicity.

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