Development of an adverse outcome pathway for kidney tubular necrosis

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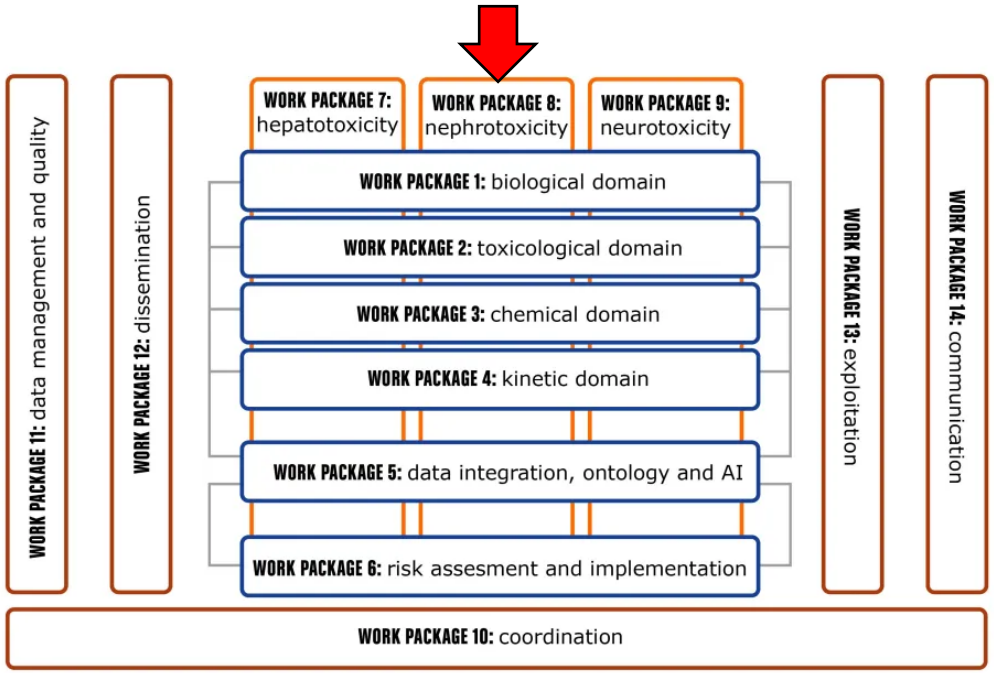
***I**nstitute for*
***P**harmaceutical*
***S**ciences*

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 963845

The ONTOX Project



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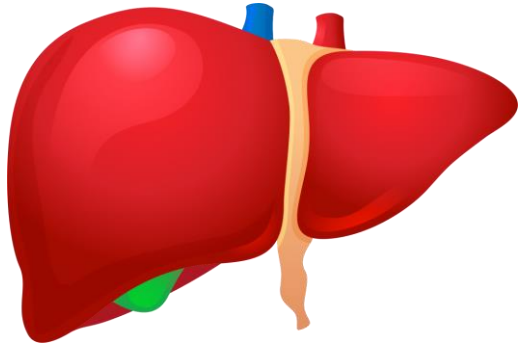
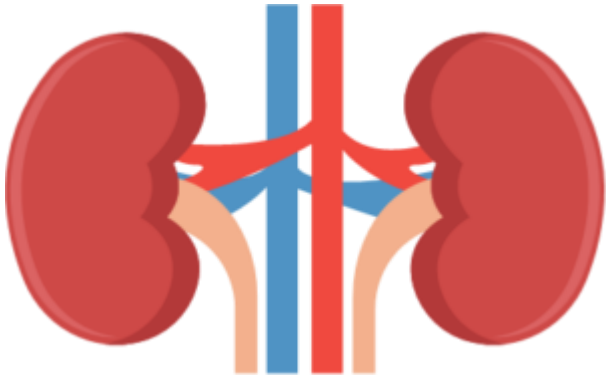
The ONTOX Project

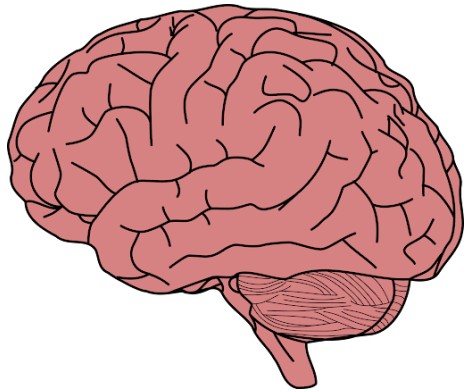
For proof-of-concept purposes, focus will be on 6 specific NAMs addressing adversities in 3 organs:



Liver
Steatosis
Cholestasis



Kidneys
Tubular necrosis
Crystallopathy



Brain
Neural tube closure
Cognitive function defects

Data generated will be integrated in physiological maps, quantitative adverse outcome pathway networks and ontology frameworks.

Kidney Tubular Necrosis

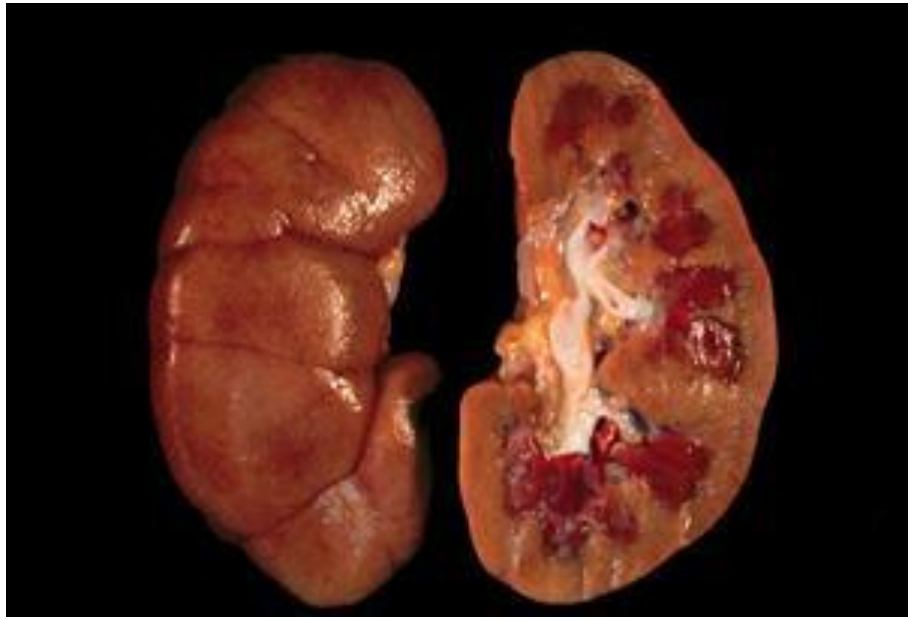
The most common form of drug-induced kidney injury.

Incidence approximately 88 per 100 000 individuals.

Tubular injury leads to reduction in kidney function.

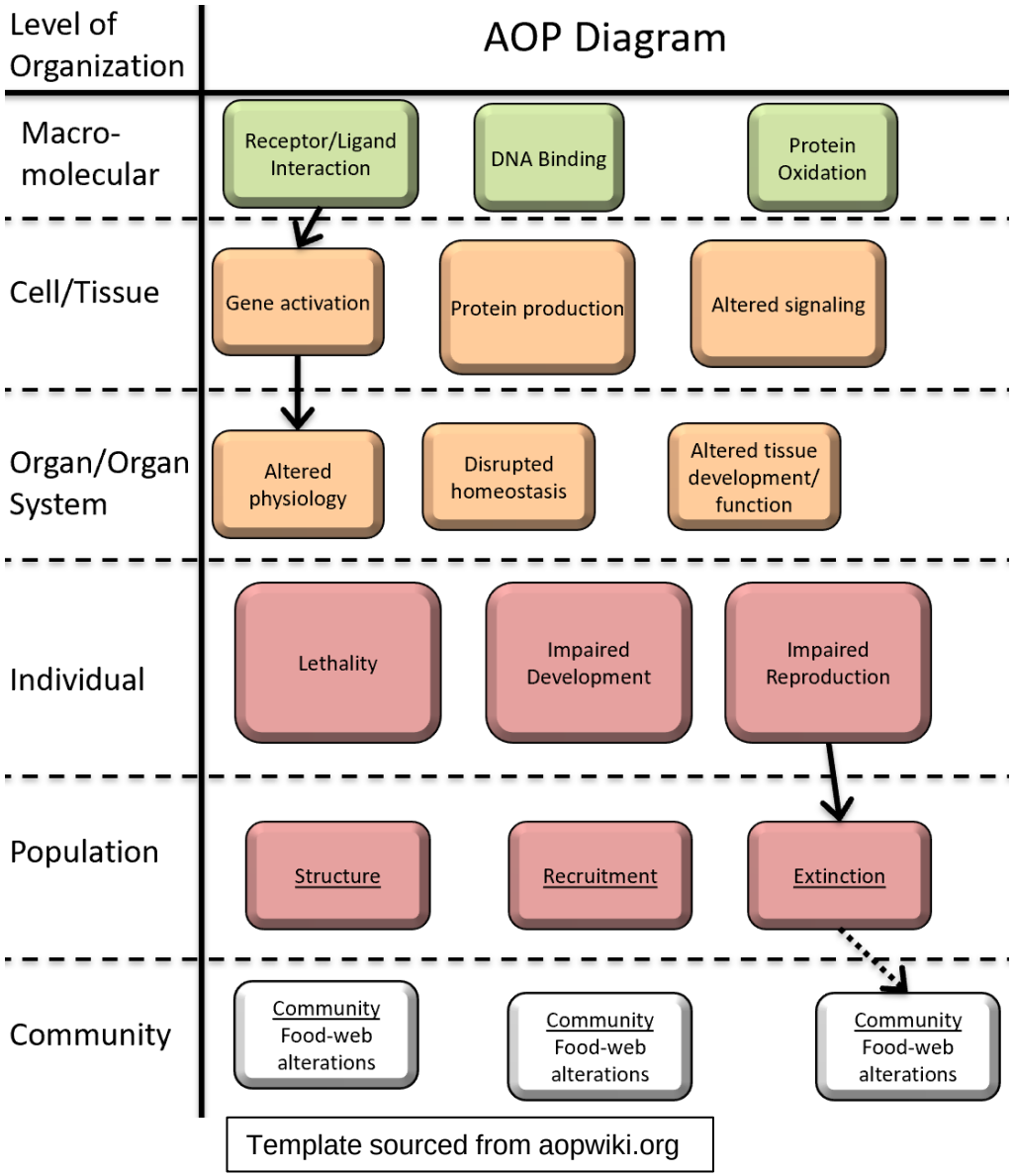
Tubular cell damage/death causes:

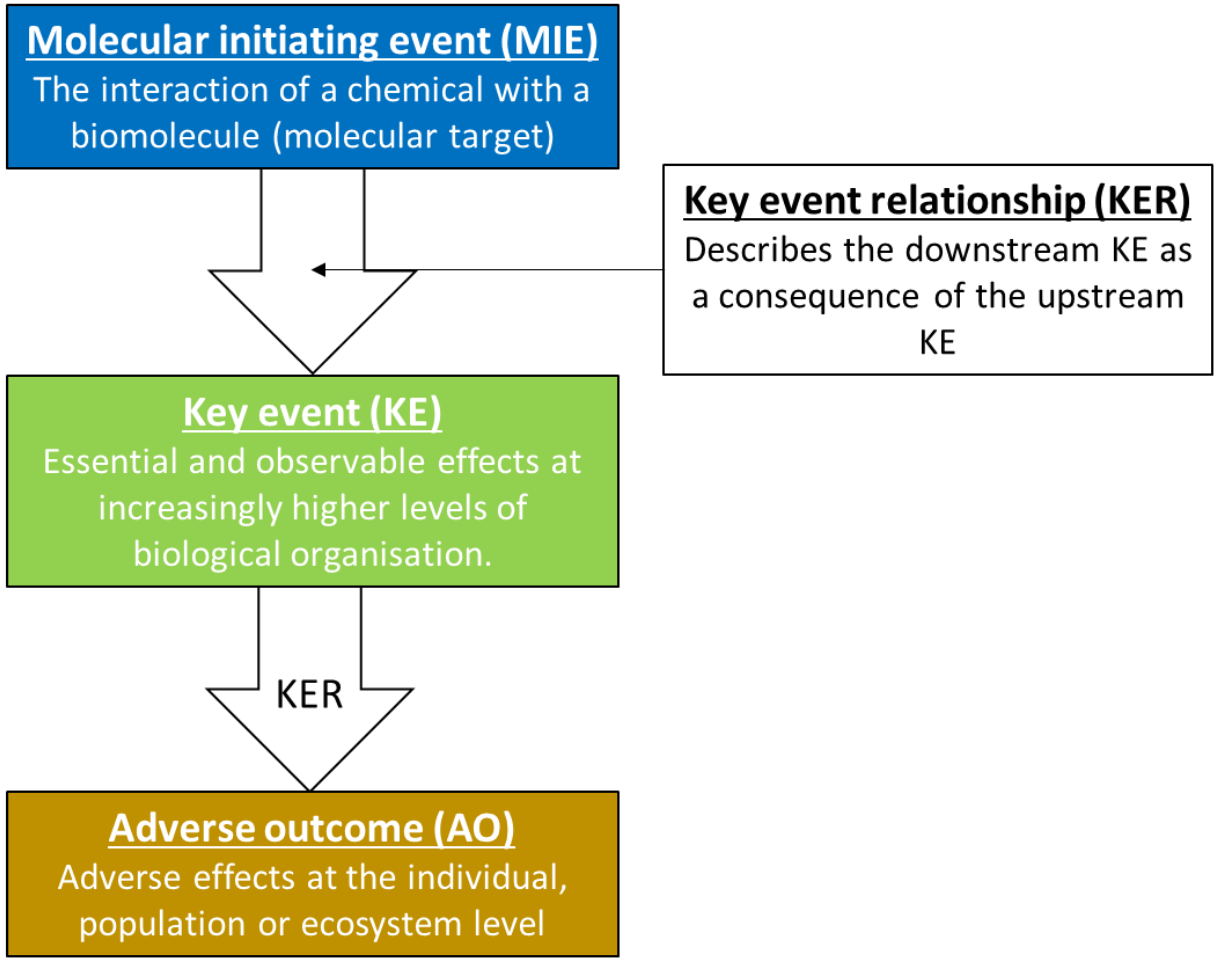
- Spilling of cellular components
- Tubular obstruction
- Impaired tubular reabsorption



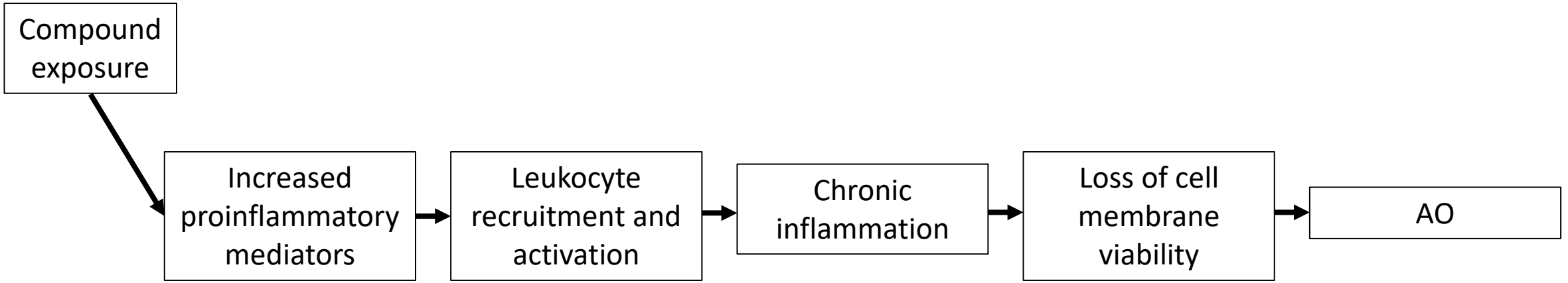
How can adverse outcome pathways help?

Adverse Outcome Pathways (AOPs) Explained





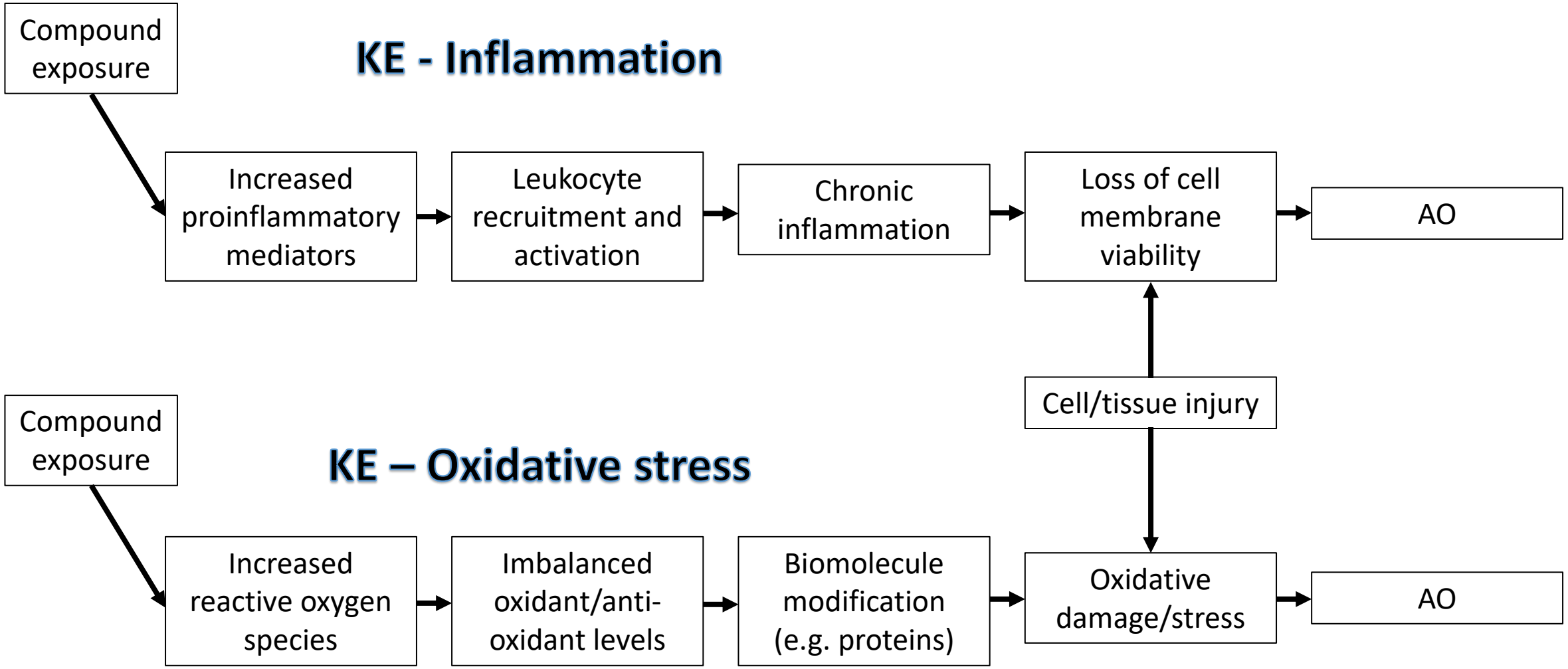
Key Event – Inflammation

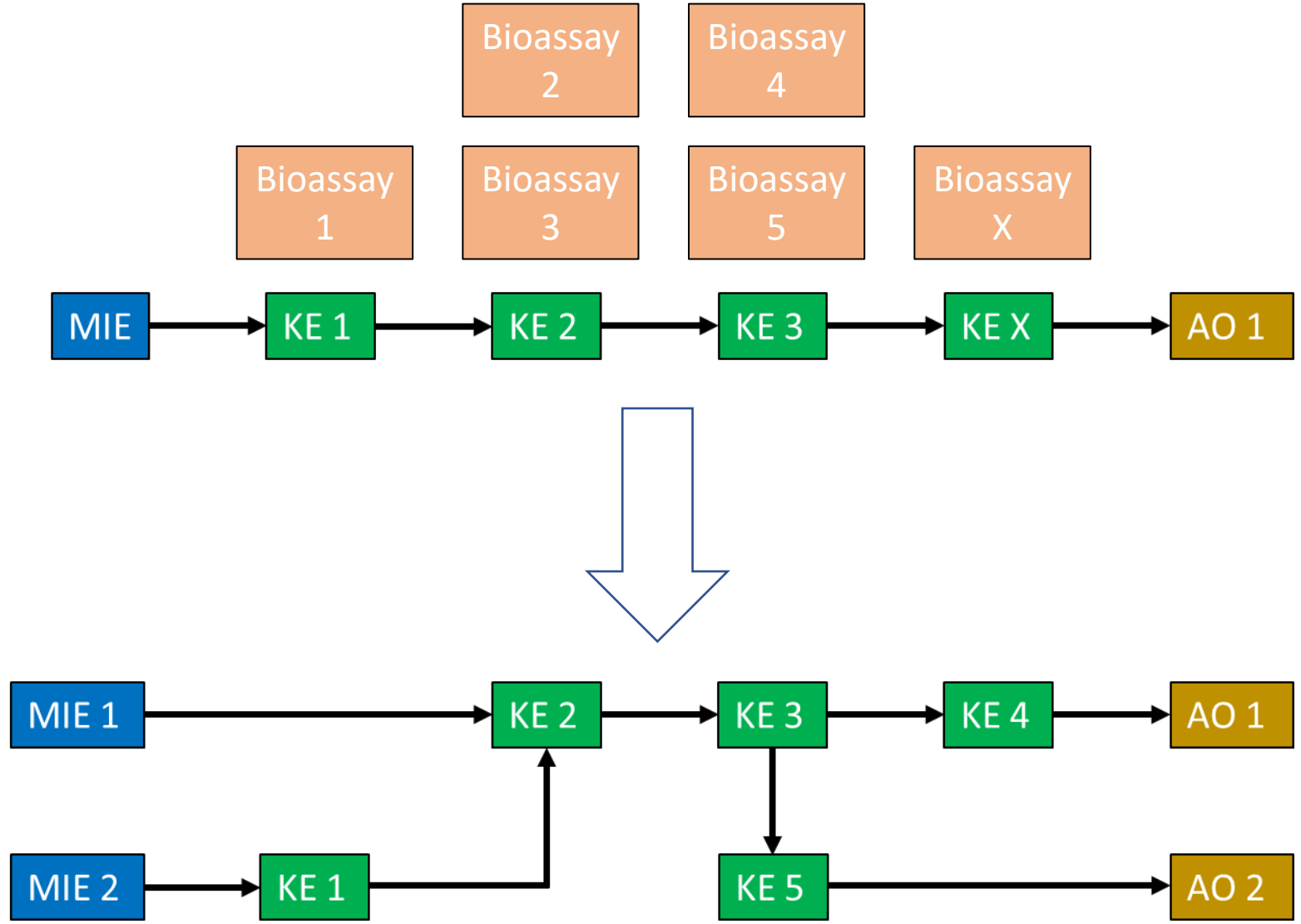


Biological/toxicological assay	**Measurements**	**KE associated with measurements**	**AO associated with KE**
ELISA assay	Inflammatory cytokines (e.g. IL-x, TNF-α, TGFβ, NFκβ, MIF, IFN-γ, CYP1A)	Increased pro-inflammatory mediators	Kidney injury
Quantitative RT-PCR	Targeted gene expression	Increased pro-inflammatory mediators	Kidney injury

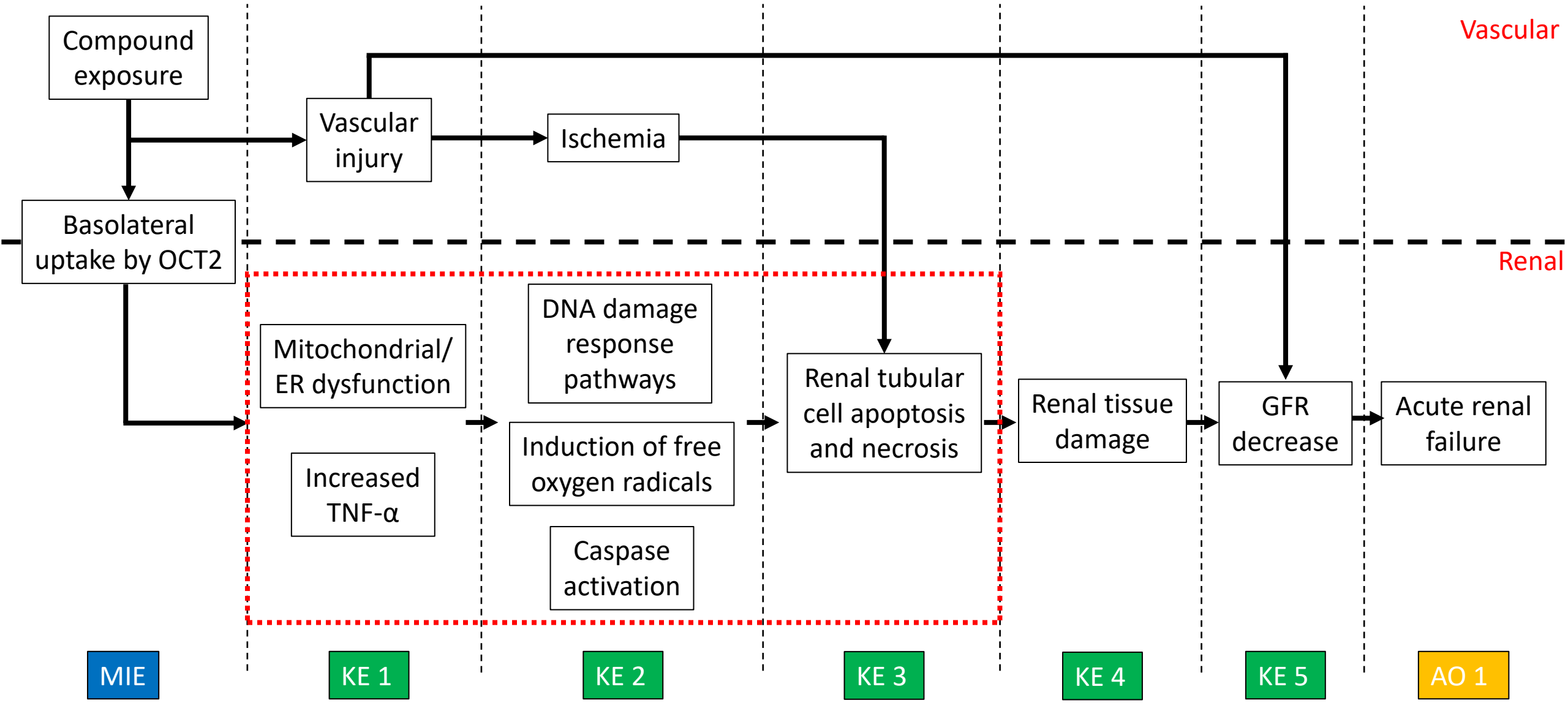
IL = interleukin, TNF = tumour necrosis factor, TGF = transforming growth factor, NF = nucleic factor, MIF = macrophage migration inhibitory factor, IFN = interferon, CYP = cytochrome p450

Key Events Detailed




Bioassay 2
Bioassay 4
Bioassay 1
Bioassay 3
Bioassay 5
Bioassay X
MIE
KE 1
KE 2
KE 3
KE X
AO 1
MIE 1
KE 2
KE 3
KE 4
AO 1
MIE 2
KE 1
KE 5
AO 2

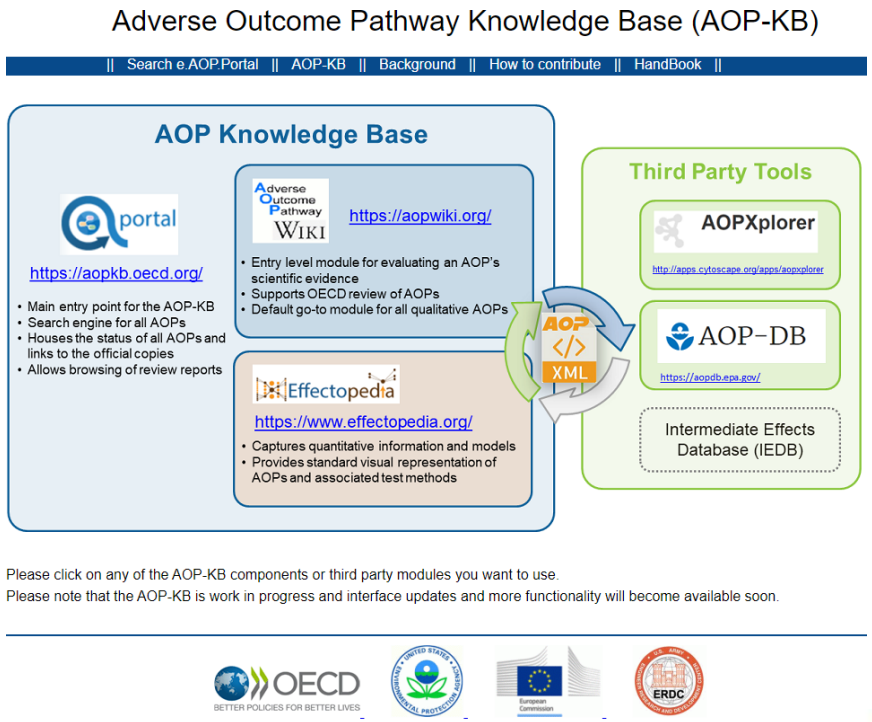
If Cisplatin-induced nephrotoxicity were an AOP….



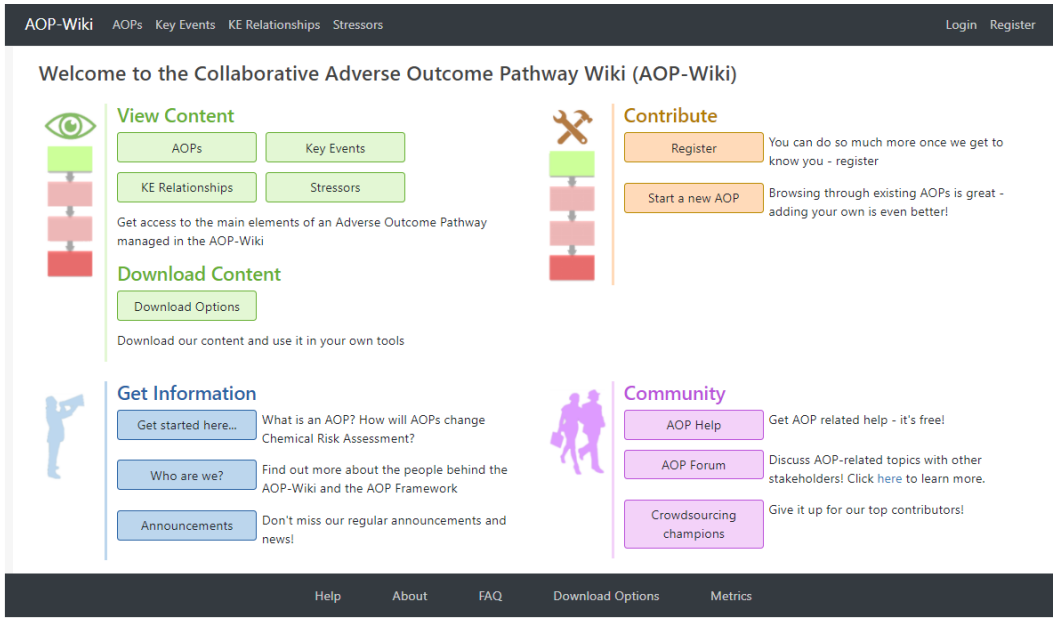
Adapted from WALKER, R. & ENDRE, Z. Cellular Mechanisms of Drug Nephrotoxicity. 2013.

AOP Knowledge Base

In 2012, the Organisation for Economic Cooperation and Development (OECD) proposed the development of a database that focuses on AOP development to standardise the generation and review of user submitted AOPs.



https://aopkb.oecd.org/index.html



https://aopwiki.org/

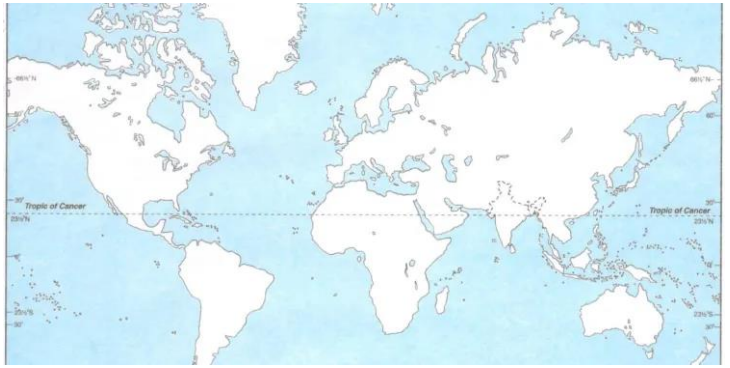
ONTOX ⟷ AOP-Wiki

Project Aims - Tubular Necrosis AOP Development

1. Literature search for kidney tubular necrosis identified existing research utilizing terms relevant to clinical biochemistry, urinary biomarkers, histology, and clinical presentations.

2. Physiological maps of the kidney were designed to establish physiological mechanisms contributing to tubular necrosis.

3. Systematic mapping of nephrotoxicity AOPs to form networks and identify relevant MIEs and KEs using existing AOPs from the AOP Wiki.



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Development of the AOP network for nephrotoxicity

Published AOPs from the AOP-Wiki were used to develop an AOP network for nephrotoxicity.

ID	Title	Molecular initiating event	Adverse outcome	Author status	SAAOP status	OECD status	OECD project	Reference
33	Kidney toxicity induced by activation of 5HT2C	Activation, 5HT2c	Increased, Kidney Failure	Open for adoption	Under development			https://aopwiki.org/aops/33
53	ER agonism leading to reduced survival due to renal failure	Agonism, Estrogen receptor	Increased, nephropathy	Under Development: Contributions and Comments Welcome	Under development		1.29	https://aopwiki.org/aops/53
105	Alpha2u-microglobulin cytotoxicity leading to renal tubular adenomas and carcinomas (in male rat)	Increased, Binding of chemicals to 2u (serum)	Increase, Adenomas/carcinomas (renal tubular)	Under Development: Contributions and Comments Welcome	Under development		1.29	https://aopwiki.org/aops/105
116	Cytotoxicity leading to renal tubular adenomas and carcinomas (in male rat)	Increase, Cytotoxicity (tubular epithelial cells)	Increase, Adenomas/carcinomas (renal tubular)	Under Development: Contributions and Comments Welcome	Under development		1.29	https://aopwiki.org/aops/116
128	Kidney dysfunction by decreased thyroid hormone	Thyroid hormone synthesis, Decreased	Occurrence, Kidney toxicity	Under development: Not open for comment. Do not cite	Included in OECD work plan	Under Development	1.40	https://aopwiki.org/aops/128
138	Organic anion transporter (OAT1) inhibition leading to renal failure and mortality	Inhibition, organic anion transporter 1 (OAT1)	Increased Mortality and Decline, Population	Under Development: Contributions and Comments Welcome	Under development		1.29	https://aopwiki.org/aops/138
177	Cyclooxygenase 1 (COX1) inhibition leading to renal failure and mortality	Inhibition, Cyclooxygenase 1 activity	Increased Mortality and Decline, Population	Under Development: Contributions and Comments Welcome	Under development		1.29	https://aopwiki.org/aops/177
186	Unknown MIE leading to renal failure and mortality	Unknown, MIE	Increased Mortality	Under Development: Contributions and Comments Welcome	Under development		1.29	https://aopwiki.org/aops/186
256	Inhibition of mitochondrial DNA polymerase gamma leading to kidney toxicity	Inhibition of mitochondrial DNA polymerase gamma (Pol gamma)	Occurrence, Kidney toxicity	Under development: Not open for comment. Do not cite	Included in OECD work plan	Under Development	1.43	https://aopwiki.org/aops/256
257	Receptor mediated endocytosis and lysosomal overload leading to kidney toxicity	Binding of substrate, endocytic receptor	Occurrence, Kidney toxicity	Under development: Not open for comment. Do not cite	Included in OECD work plan	Under Development	1.43	https://aopwiki.org/aops/257
258	Renal protein alkylation leading to kidney toxicity	Alkylation, Protein	Occurrence, Kidney toxicity	Not under active development	Included in OECD work plan	Under Development	1.43	https://aopwiki.org/aops/258
276	Inhibition of complex I of the electron transport chain leading to chemical induced Fanconi syndrome	Binding of inhibitor, NADH-ubiquinone oxidoreductase (complex I)	Chemical induced Fanconi syndrome	Under development: Not open for comment. Do not cite				https://aopwiki.org/aops/276
284	Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress leads to chronic kidney disease	Binding, Thiol/seleno-proteins involved in protection against oxidative stress	Chronic Kidney disease	Under development: Not open for comment. Do not cite				https://aopwiki.org/aops/284
377	Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Acute Respiratory Distress Syndrome (ARDS) and Multiple Organ Dysfunction (MOD)	Prolonged TLR9 activation	Acute Respiratory Distress Syndrome and Multiple Organ Dysfunction/ Increased mortality	Under development: Not open for comment. Do not cite				https://aopwiki.org/aops/377
384	Hyperactivation of ACE/Ang-II/AT1R axis leading to chronic kidney disease	Hyperactivation of ACE/Ang-II/AT1R axis	Chronic kidney disease	Under development: Not open for comment. Do not cite				https://aopwiki.org/aops/384
413	Oxidation and antagonism of reduced glutathione leading to mortality via acute renal failure	Oxidation, glutathione	Increased Kidney failure and mortality	Open for citation and comment				https://aopwiki.org/aops/413
437	Inhibition of mitochondrial electron transport chain (ETC) complexes leading to kidney toxicity	Inhibition, Mitochondrial Electron Transport Chain Complexes	Occurrence, Kidney toxicity	Under development: Not open for comment. Do not cite				https://aopwiki.org/aops/437
447	Kidney failure induced by inhibition of mitochondrial electron transfer chain through apoptosis, inflammation and oxidative stress pathways	Inhibition, Mitochondrial Electron Transport Chain Complexes	Increased, Kidney Failure	Under development: Not open for comment. Do not cite				https://aopwiki.org/aops/447

Details of the selected AOPs and their developmental stage at the time of retrieval.

Barnes *et al.* unpublished

Selection process highlighted AOPs for exclusion

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33	Kidney toxicity induced by activation of 5HT2C	Activation, 5HT2c	Increased, Kidney Failure	Open for adoption	Under development			https://aopwiki.org/aops/33	No KEs linking MIE to AO
53	ER agonism leading to reduced survival due to renal failure	Agonism, Estrogen receptor	Increased, nephropathy	Under Development: Contributions and Comments Welcome	Under development		1.29	https://aopwiki.org/aops/53	Specific focus on proposed AOP
105	Alpha2u-microglobulin cytotoxicity leading to renal tubular adenomas and carcinomas (in male rat)	Increased, Binding of chemicals to 2u (serum)	Increase, Adenomas/carcinomas (renal tubular)	Under Development: Contributions and Comments Welcome	Under development		1.29	https://aopwiki.org/aops/105	
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138	Organic anion transporter (OAT1) inhibition leading to renal failure and mortality	Inhibition, organic anion transporter 1 (OAT1)	Increased Mortality and Decline, Population	Under Development: Contributions and Comments Welcome	Under development		1.29	https://aopwiki.org/aops/138	
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257	Receptor mediated endocytosis and lysosomal overload leading to kidney toxicity	Binding of substrate, endocytic receptor	Occurrence, Kidney toxicity	Under development: Not open for comment. Do not cite	Included in OECD work plan	Under Development	1.43	https://aopwiki.org/aops/257	
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384	Hyperactivation of ACE/Ang-II/AT1R axis leading to chronic kidney disease	Hyperactivation of ACE/Ang-II/AT1R axis	Chronic kidney disease	Under development: Not open for comment. Do not cite				https://aopwiki.org/aops/384	
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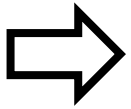
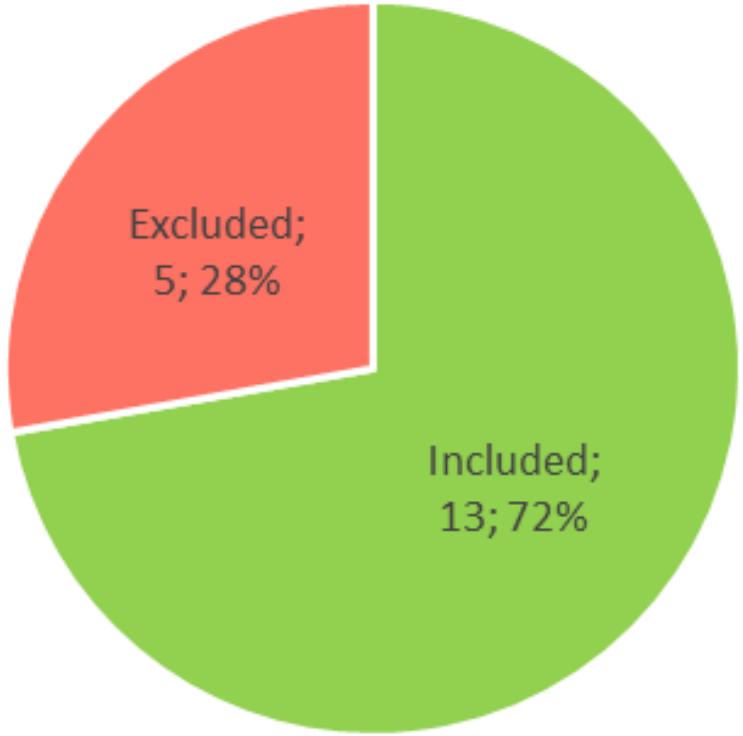
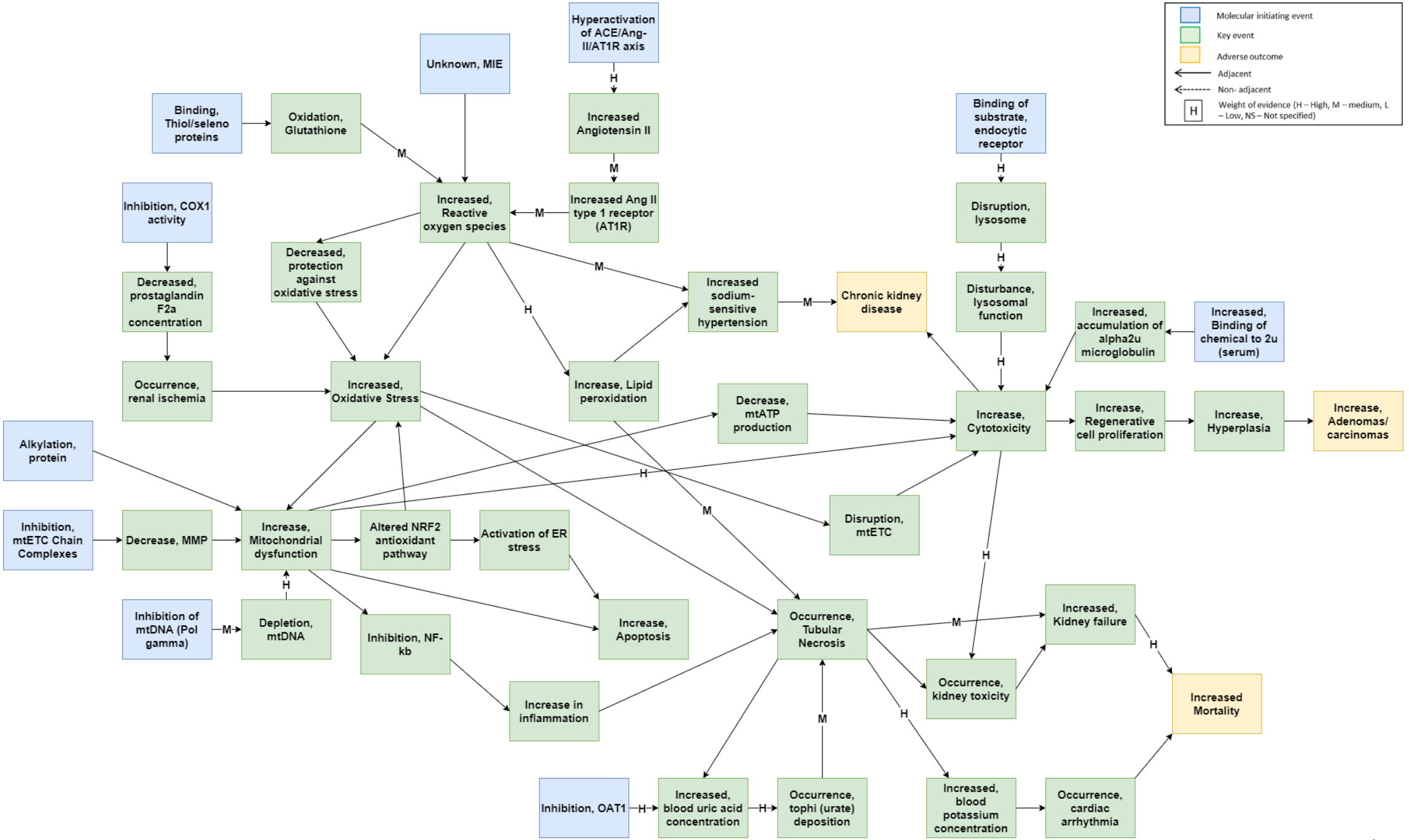




Reasons for exclusion include:

- No adjacency metrics reported (n=2)
- Specific focus on reported AOP (n=2)
- Ill-defined AOP (n=1)

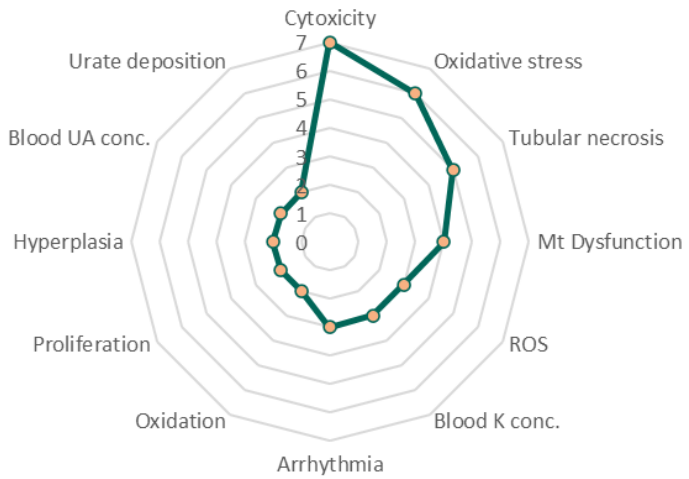
Barnes *et al.* unpublished



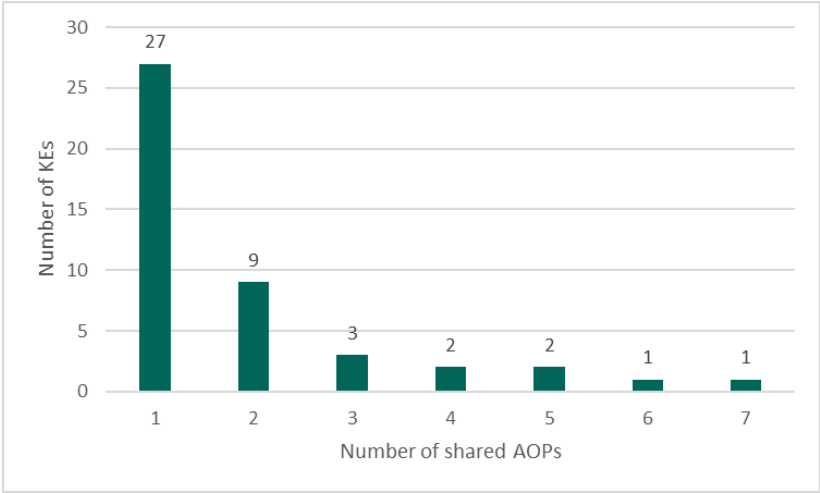
Barnes *et al.* unpublished

Nephrotoxicity AOP network analytics

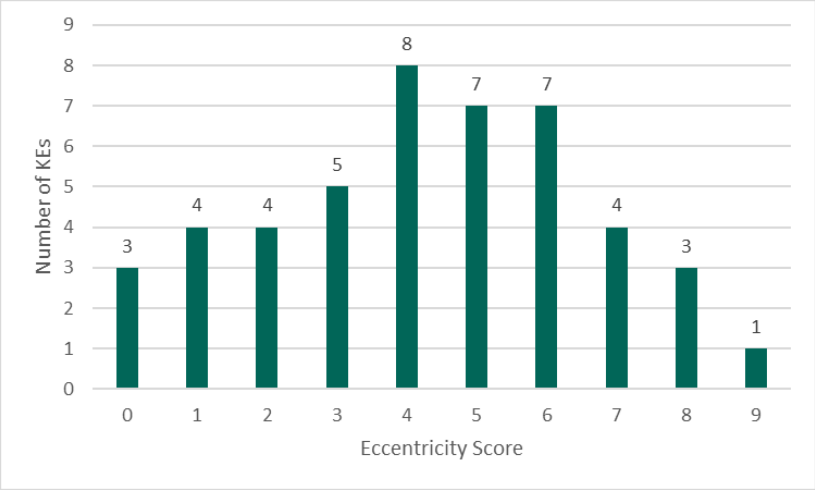
Scoring & Distribution



Interconnectivity



Eccentricity



Convergence & Divergence

Convergent KEs		Divergent KEs	
KE type	**KE name**	**KE type**	**KE name**
KE	Occurrence, Tubular necrosis	KE	Increase, Mt dysfunction
MIE/KE	Increase, Cytotoxicity	KE	Increased, ROS
KE	Increase, Oxidative stress	KE	Increase, Lipid peroxidation
KE	Increased Sodium-sensitive hypertension	KE	Altered NRF2 antioxidant pathway
KE/AO	Increased, Kidney Failure	MIE	Inhibition, mtETC complexes
KE/AO	Occurrence, Kidney toxicity	MIE	Alkylation, Protein
KE	Increase, Apoptosis	MIE	Increased, Binding of chemicals to 2u
KE	Increased, blood uric acid conc.	MIE	Inhibition, OAT1
AO	Chronic kidney disease	MIE	Binding of substrate, endocytic receptor
AO	Increased Mortality	MIE	Inhibition of mtDNA (Pol gamma)
AO	Increase, Adenomas/carcinomas	MIE	Unknown, MIE
		MIE	Binding, Thiol/seleno-proteins
		MIE	Inhibition, COX1 activity
		MIE	Hyperactivation of ACE/Ang-II/AT1R axis

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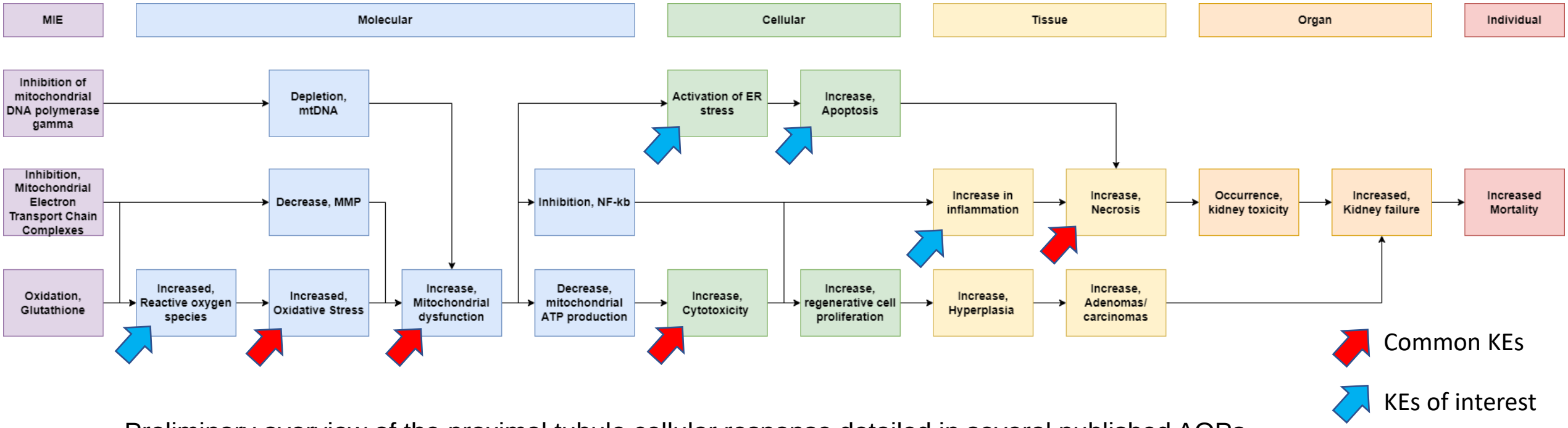
Network Overview

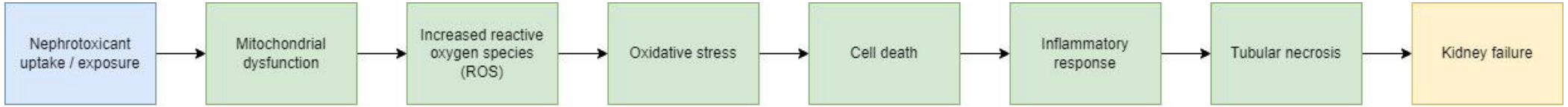


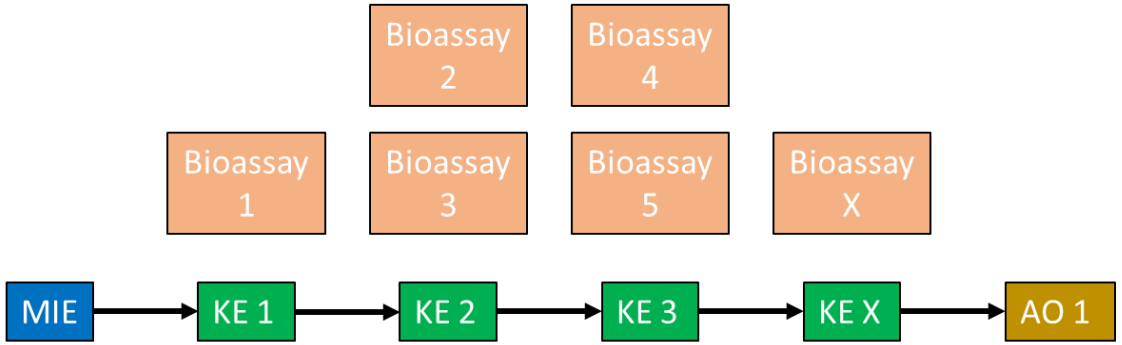
- Preliminary overview of the proximal tubule cellular response detailed in several published AOPs.
- Developed **linear network** to help identify sequence of adversity for the AOP network.
- Proposed sequence of **generic key events** to assay for initial profiling of proposed compounds.
  - *Highlighted a few additional KEs of interest for consideration.*
- Further investigation into **molecular mechanisms** involved for **each nephrotoxic agent** should be considered.

Development of *in vitro* test batteries

Tubular Necrosis

Kidney disorder involving damage to the kidney tubule cells, often leading to kidney failure.



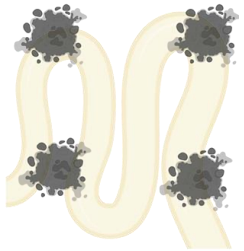


Key Event	Bioassay
Cytotoxicity	Cell viability assays – membrane integrity (NAG/WST/LDH assay), mitochondrial function (LDH, ATP), caspase activation etc.
Cell barrier/membrane permeability	Loss of gap or tight junctions, barrier integrity (TRITC/FITC), TEER (multi-cell-type cultures)
Inflammation	Expression of pro-inflammatory and pro-fibrotic mediators e.g. TNF-α, IL-x, IFNγ (e.g. protein ELISA, mRNA analysis by RT-PCR)
Oxidative stress	Fluorescence assays measuring ROS synthesis, glutathione depletion etc.
Mitochondrial dysfunction	MMP assay, MTT assay

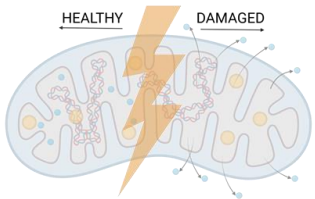
ATP – adenosine triphosphate, FITC – fluorescein isothiocyanate, IFN – interferon, LDH – lactate dehydrogenase, MMP – mitochondrial membrane potential, NAG – N-acetylβ-D-glucosaminidase, ROS – reactive oxygen species, TEER – transepithelial electrical resistance, TRITC – tetramethylrhodamine, WST – water soluble tetrazolium salts

**Preliminary model subject to change*

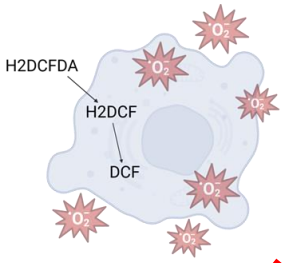
In vitro assay optimization – Tubular Necrosis



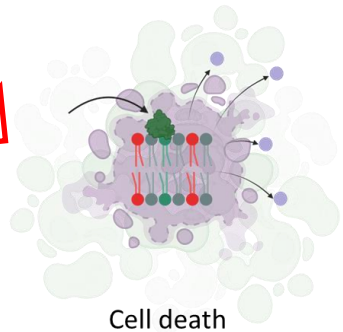
Cytotoxicity
- *Prestoblue*
- *LDH activity*



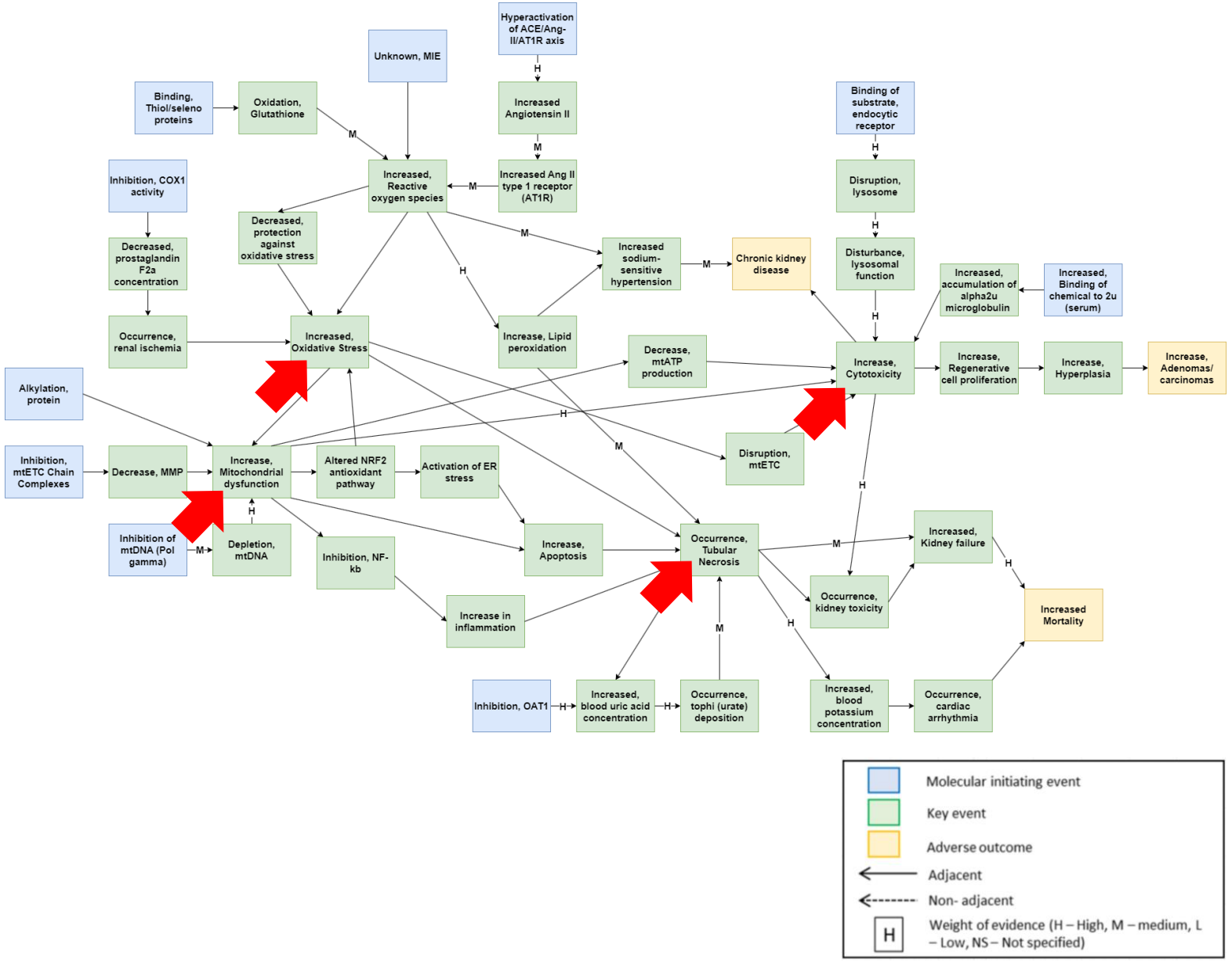
Mitochondrial dysfunction
- *JC-10 MMP*



Oxidative stress
- *ROS detection*



Cell death
- *Annexin V FITC/PI*



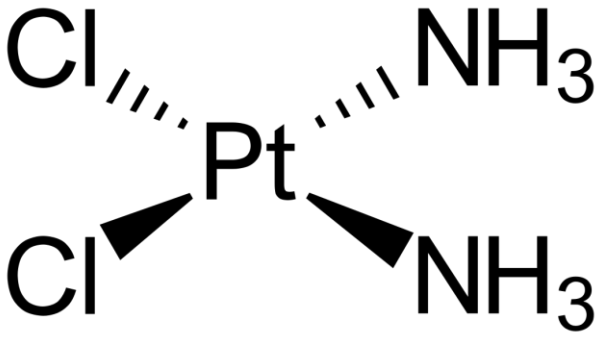
Platinum Chemotherapeutics

Cisplatin will be used to help build the *in vitro* test battery to investigate kidney tubular necrosis.

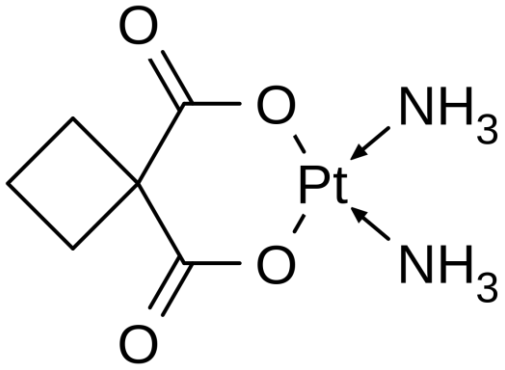
Evokes a cascade of negative cellular responses following uptake into kidney proximal tubules.

Widely utilized drug, reported to induce all highlighted KEs of interest.

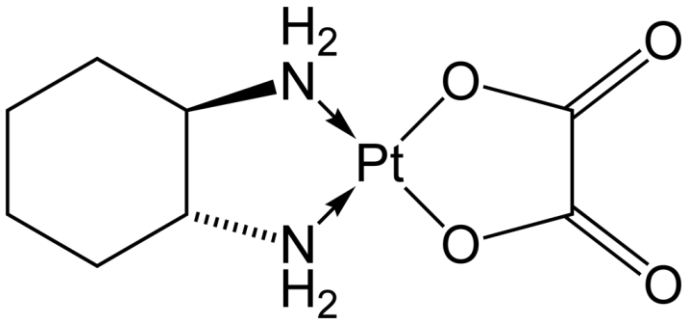
Will also use alternative platinum derivatives for test battery development and assay optimization.



Cisplatin

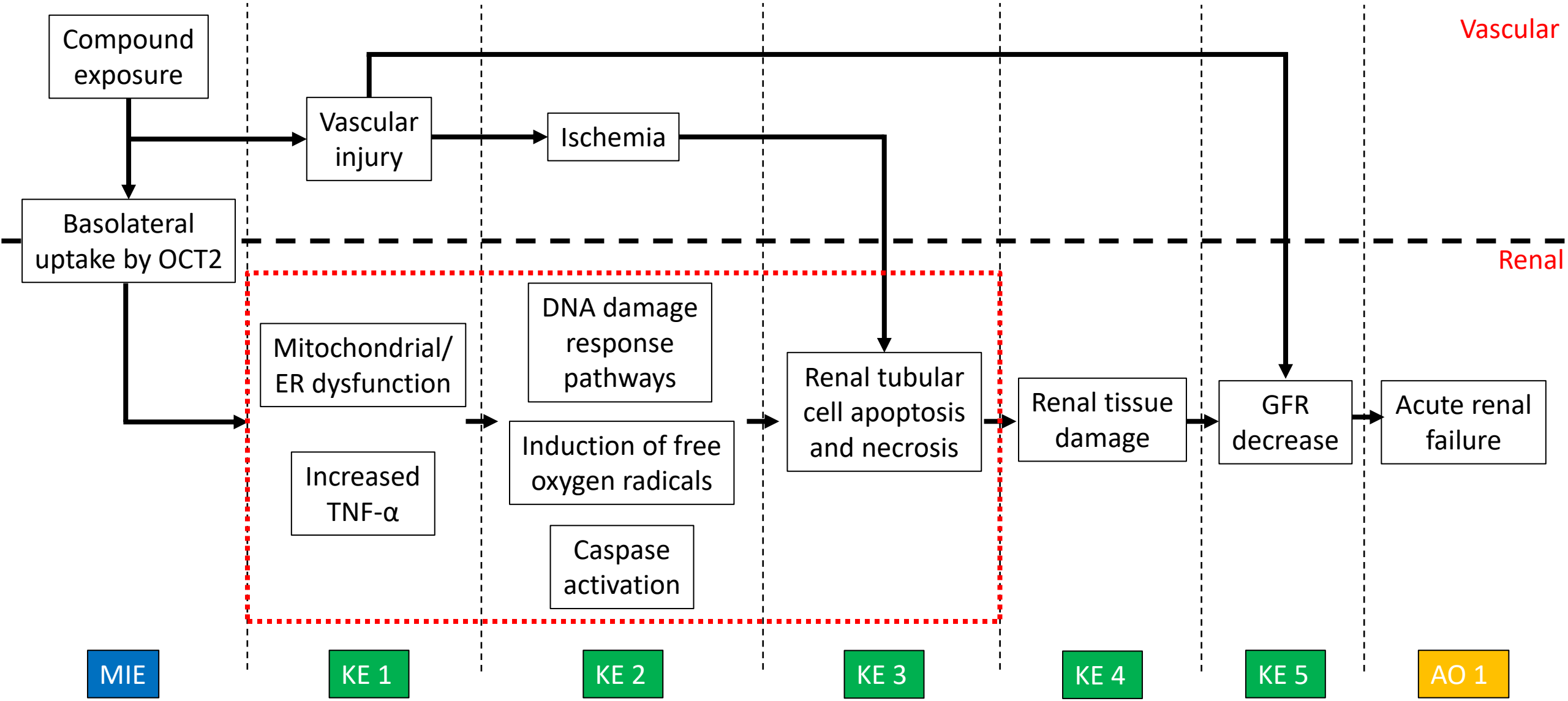


Carboplatin



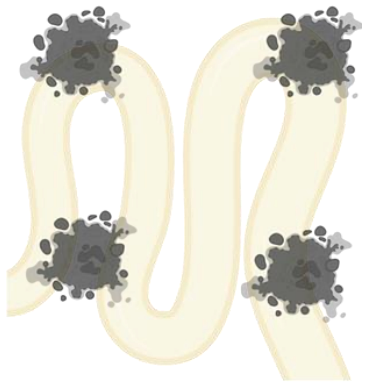
Oxaliplatin

Cisplatin

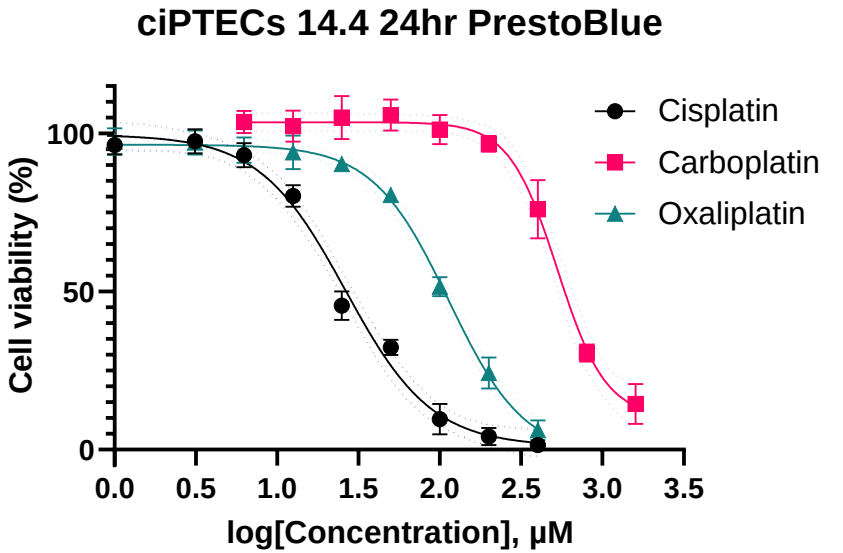


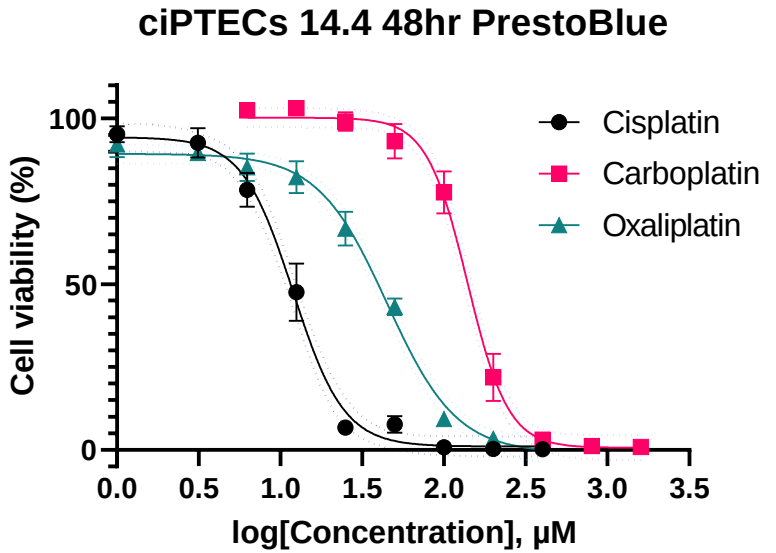
Adapted from WALKER, R. & ENDRE, Z. Cellular Mechanisms of Drug Nephrotoxicity. 2013.

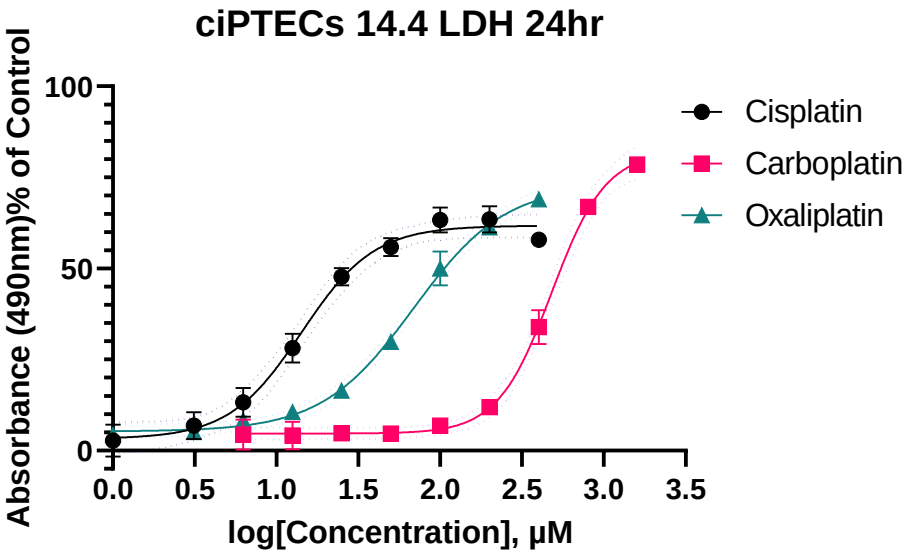
In vitro assay optimization – Tubular Necrosis

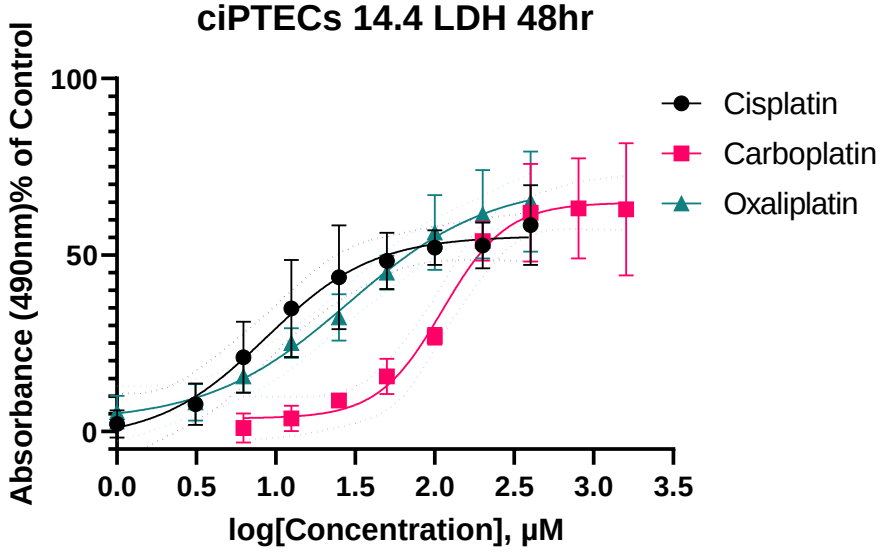


Cytotoxicity
- *Prestoblue*
- *LDH activity*

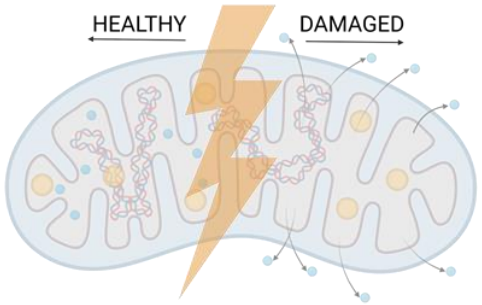




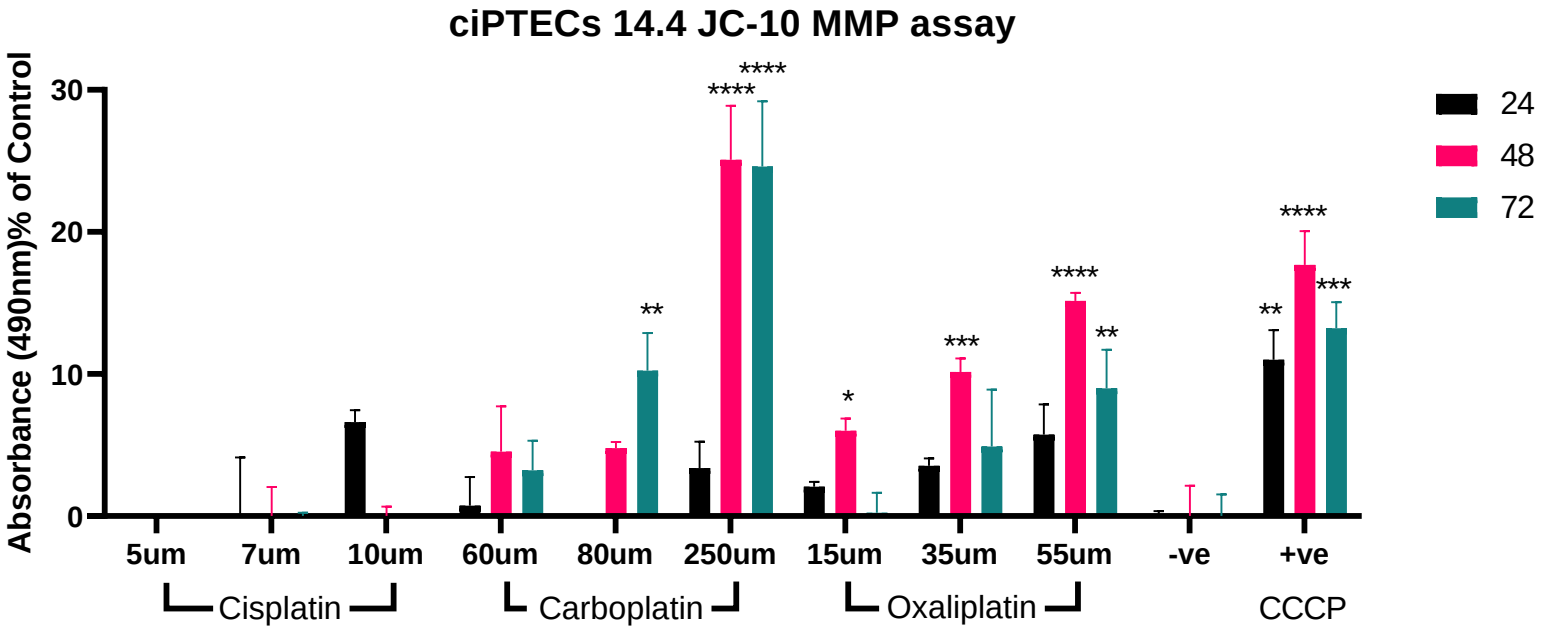




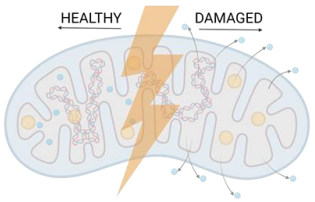
In vitro assay optimization – Tubular Necrosis



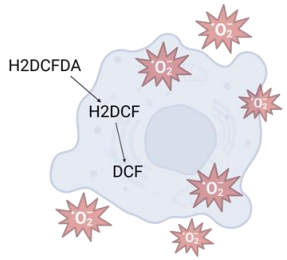
Mitochondrial dysfunction
- JC-10 MMP



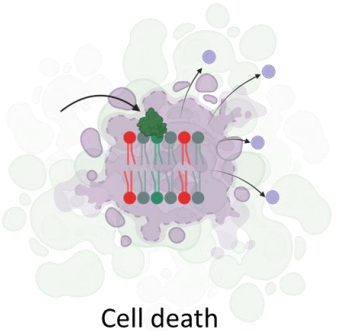
Cytotoxicity
- *Prestoblue*
- *LDH activity*



Mitochondrial dysfunction
- JC-10 MMP



Oxidative stress
- ROS detection



Cell death
- Annexin V FITC/PI

AOP 472: DNA adduct formation leading to kidney failure

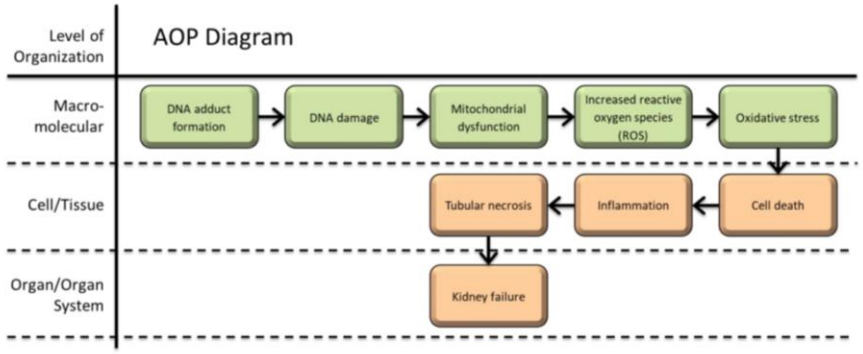
1. Title

Pt-DNA adduct formation leading to kidney failure

1.1 Short name

Pt-DNA adduct formation leading to kidney failure

2. Graphical representation



3. Authors

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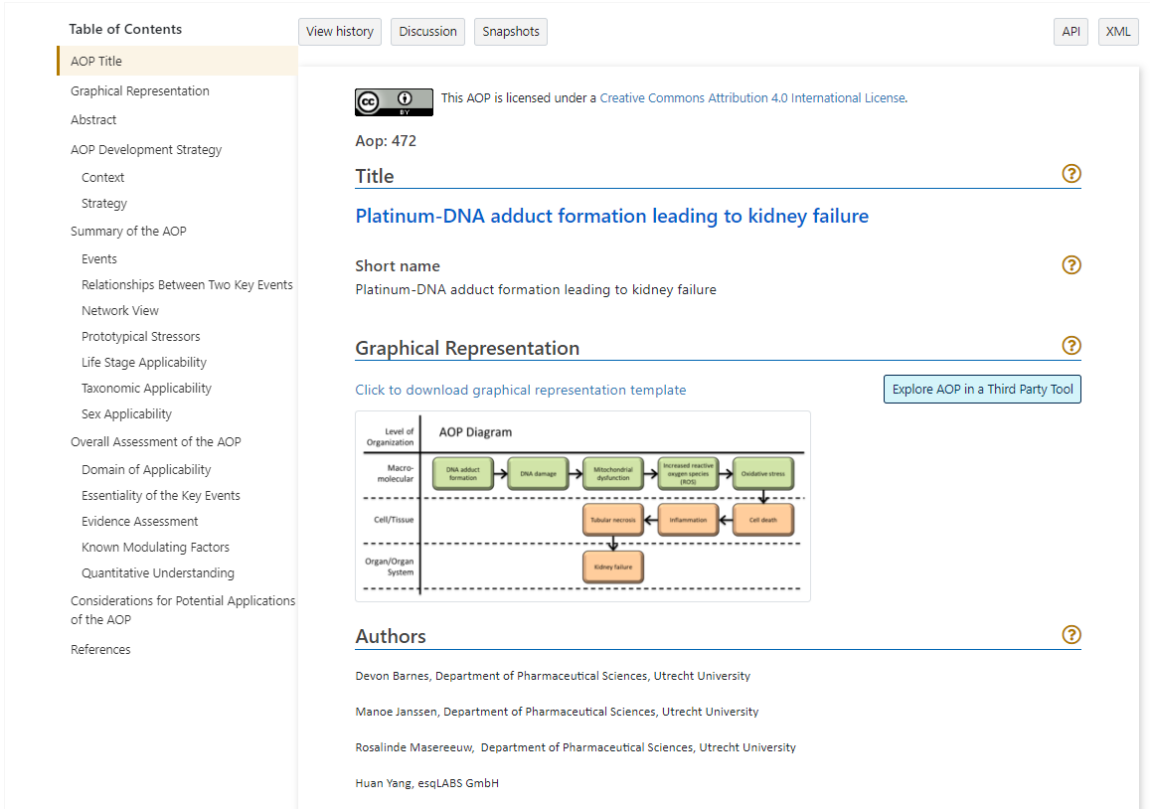
Rosalinde Masereeuw, Department of Pharmaceutical Sciences, Utrecht University
Huan Yang, esqLABS GmbH



https://aopwiki.org/aops/472

- ‘DNA adduct formation leading to kidney failure’
  - AOP identified and supported with literature.
  - Specification sheet finalized and preliminarily version uploaded to AOP Wiki.

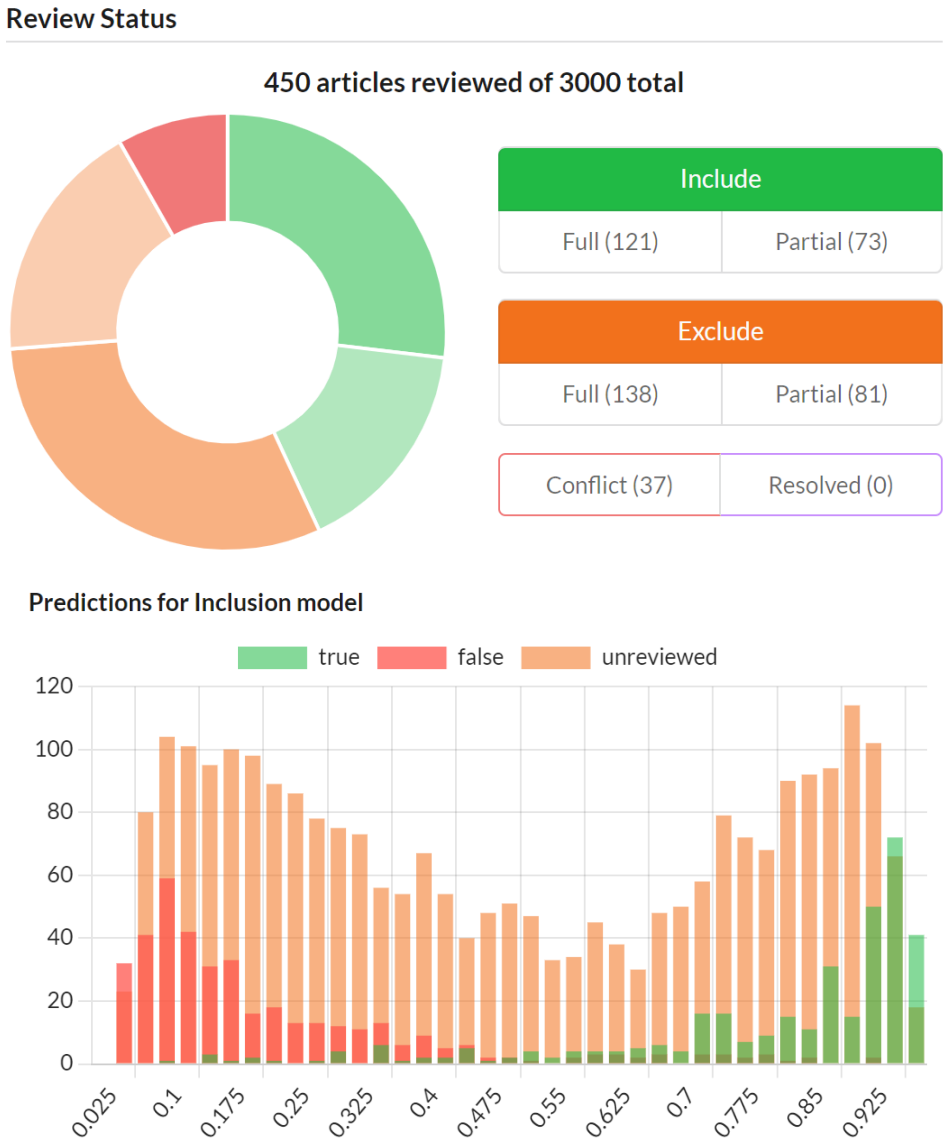
Project Aims - Tubular Necrosis AOP Development

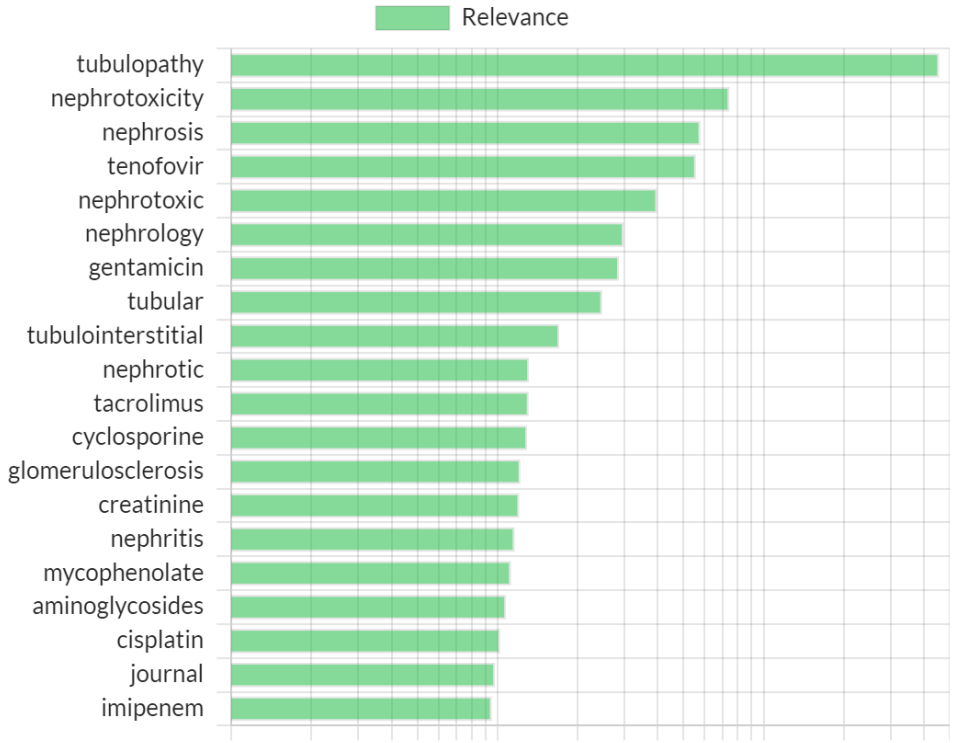
1. Literature search for kidney tubular necrosis identified existing research utilizing terms relevant to clinical biochemistry, urinary biomarkers, histology, and clinical presentations.

2. Physiological maps of the kidney were designed to establish physiological mechanisms contributing to tubular necrosis.

3. Systematic mapping of nephrotoxicity AOPs to form networks and identify relevant MIEs and KEs using existing AOPs from the AOP Wiki.

Literature Screening - Sysrev





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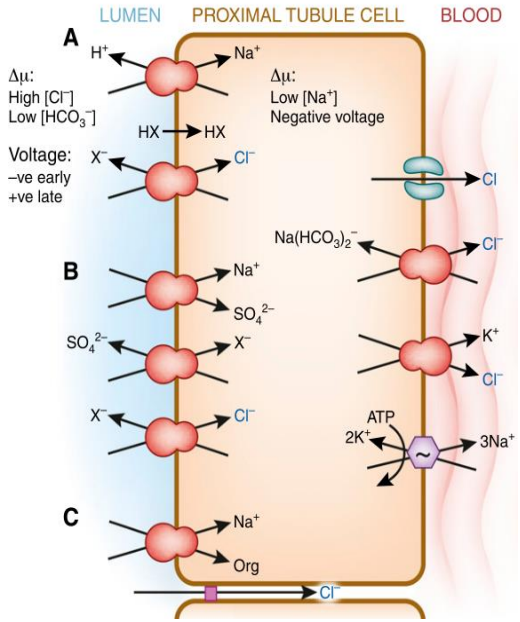
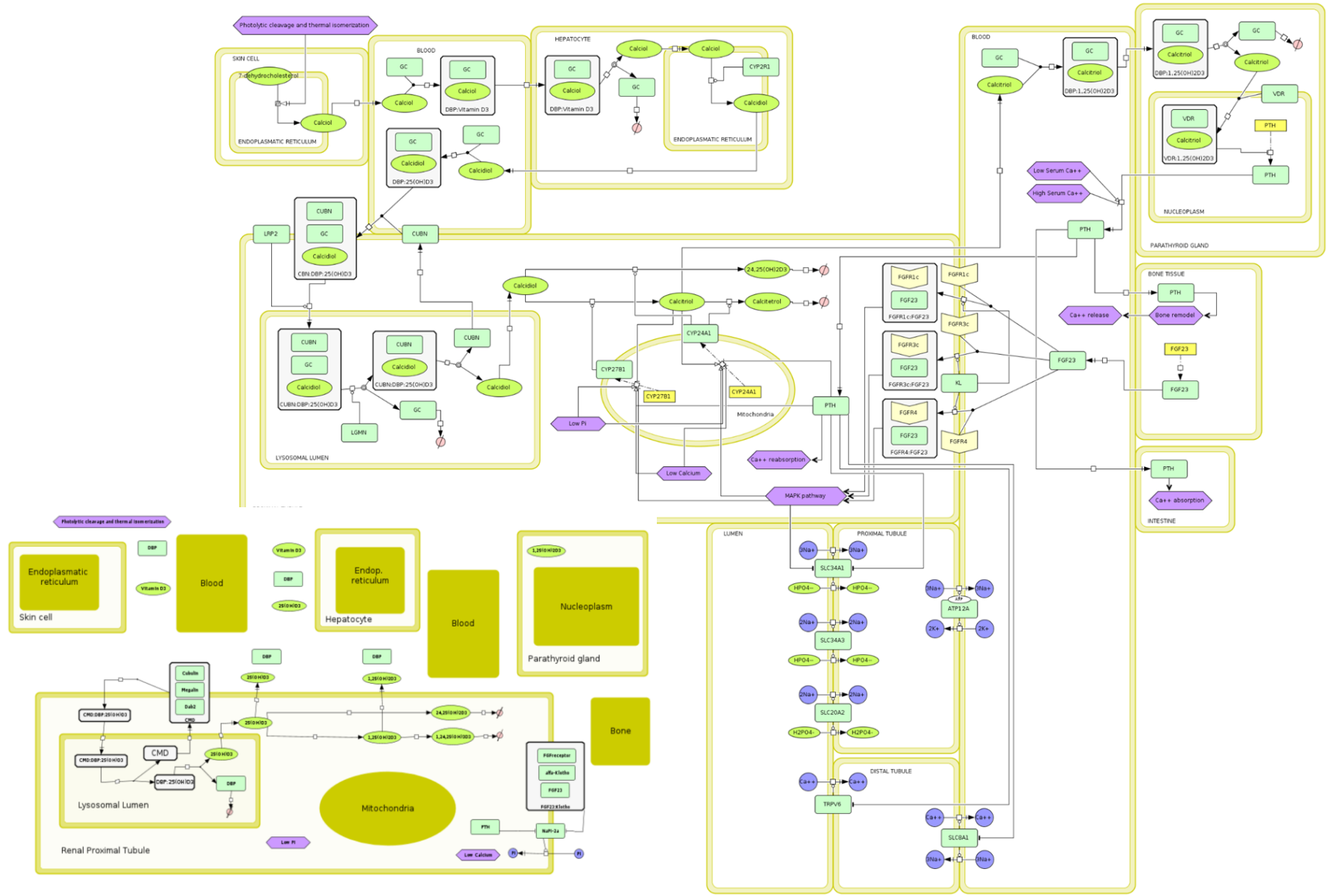
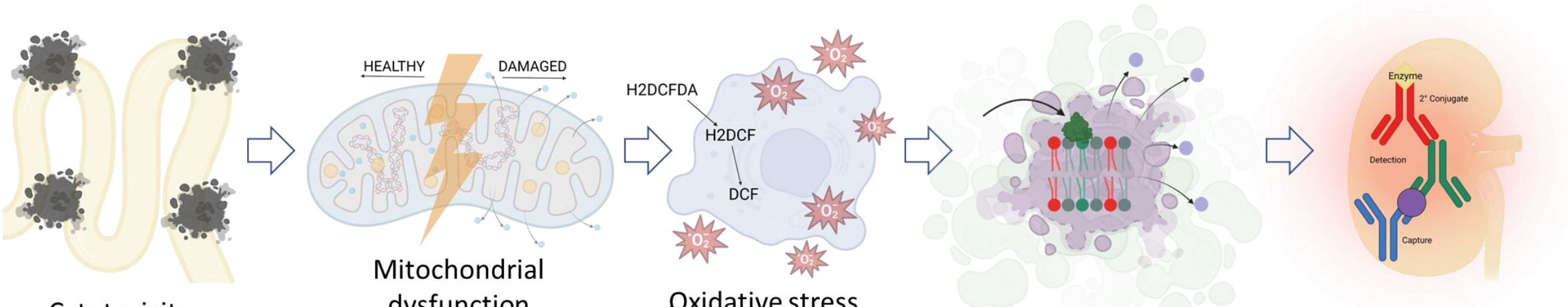
Physiological Maps - Nephron





Conclusions

- Introduced multi-faceted approach for creating kidney ontologies.
- Nephrotoxicity AOP network identified key events of interest.
- First steps toward development of *in vitro* test battery for kidney tubular necrosis.



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UIPS Utrecht Institute for Pharmaceutical Sciences

Utrecht University

ASPIS

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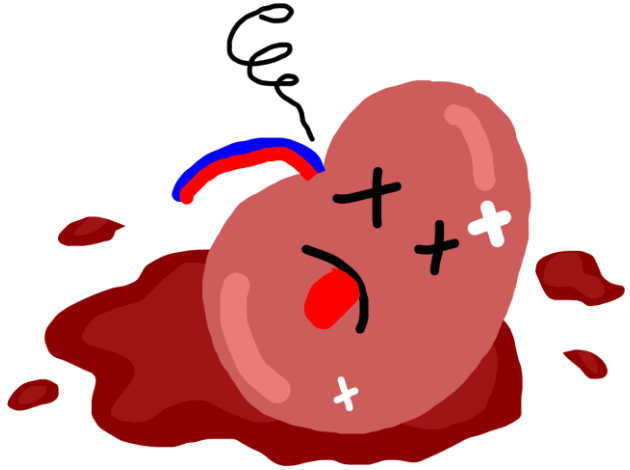

Thank you for your attention

d.a.barnes@uu.nl