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Article

A CRITICAL REVIEW OF ADVERSE EFFECTS TO THE KIDNEY: MECHANISMS, DATA SOURCES AND *IN SILICO* TOOLS TO ASSIST PREDICTION

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ABSTRACT

Introduction: The kidney is a major target for toxicity elicited by pharmaceuticals and environmental pollutants. Standard testing which often does not investigate underlying mechanisms has proven not to be an adequate hazard assessment approach. As such, there is an opportunity for the application of computational approaches that utilise multi-scale data based on the Adverse Outcome Pathway (AOP) paradigm, coupled with an understanding of the chemistry underpinning the molecular initiating event (MIE) to provide a deep understanding of how structural fragments of molecules relate to specific mechanisms of nephrotoxicity.

The aim of this investigation was to review the current scientific landscape related to computational methods, including mechanistic data, AOPs, publicly available knowledge bases and current *in silico* models, for the assessment of pharmaceuticals and other chemicals with regard to their potential to elicit nephrotoxicity. A list of over 250 nephrotoxicants enriched with, where possible, mechanistic and AOP-derived understanding was compiled.

Expert opinion: Whilst little mechanistic evidence has been translated into AOPs, this review identified a number of data sources of *in vitro*, *in vivo* and human data that may assist in the development of *in silico* models which in turn may shed light on the inter-relationships between nephrotoxicity mechanisms.

Key words: kidney, nephrotoxicity, *in silico*, computational models, (Q)SAR, mechanisms

1.0 <u>INTRODUCTION</u>

Acute renal failure in critically ill patients, as well as those with chronic kidney disease, was related to drug therapy in about 20% and 35% of cases reported respectively [1–3]. As a result of such toxicity, six prescription drugs (beta-ethoxy-lacetanilanide, bucetin, phenacetin, suprofen, thiobutabarbitone and zomepirac) were withdrawn from the market between 1983 and 1993, at great cost, due to renal adverse events, solely or in combination with other adverse effects [4]. Therefore, eliminating drug candidates which cause these adverse effects at early stages of drug design is extremely important to ensure patient safety. However, despite its importance for drug development and for many other industrial sectors, nephrotoxicity is a complex endpoint and often occurs gradually or as a complication related to other pathologies such as diabetes [5] and hypertension [6], thus making it difficult to identify even with sophisticated toxicity testing or clinical trials.

Established approaches to identify kidney toxicants have traditionally relied on extensive animal testing. However, the "Toxicity Testing in the 21st Century" paradigm calls for use of alternative testing strategies [7]. Computational approaches such as (quantitative) structure-activity relationships ((Q)SARs)[1] and structural alerts (SAs) are currently used to predict a variety of organ toxicities e.g. for hepatic toxicity [8]. In recent years, much emphasis has been placed on understanding the underlying mechanisms of liver toxicity which have led to the development of several Adverse Outcome Pathways (AOPs), many SAs and QSARs [8–10]. The relative successes with the development of alternatives for identifying liver toxicants has demonstrated that success can be achieved and it is possible to address other organ level toxicity in a similar manner. Thus, there is a growing movement to other important organs in the body in order to reach the ultimate goal of mapping the toxicological pathways of pharmaceuticals, cosmetics and other chemicals within humans [11].

The kidney is a major target for toxicity elicited by pharmaceuticals and environmental pollutants. Approximately 20% of acquired acute kidney injury (AKI) cases are associated with the use of drugs [12]. Being burdened with multiple comorbidities, the average patient tends to take several medications which may cause kidney injury [13]. Environmental chemicals including certain heavy metals, trichloroethylene, and bromobenzene have been known to cause nephrotoxic effects [14]. One of the reasons for the kidney being a key target of toxicity may be related to the kinetics of

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[1]In this paper, (Q)SAR will be mentioned if both SAR and QSAR are referred to while SAR and QSAR are stated to refer to either approach specifically.

many xenobiotic substances. High exposures are reached because of a high blood flow in the kidneys and extensive reabsorption, predominantly in the proximal tubule.

Considering that renal toxicity is a major drug safety issue, standard testing which often does not investigate underlying mechanisms has proven not to be an adequate assessment approach. As such, this is an opportunity for the application of computational approaches that utilise the AOP paradigm coupled with an understanding of the chemistry underpinning the MIE [10,15] to provide a deep understanding of how structural fragments of molecules relate to specific mechanisms of nephrotoxicity. In addition *in silico* approaches using multi-scale data have been demonstrated to provide valuable insight into hepatotoxicity pathways and the assessment of inter-individual variability [16,17]. Multi-scale models incorporate data which span various biological scales, i.e. population, individual whole body, tissue and multi-cellular, and sub-cellular metabolic and signalling pathways [18]. As multi-scale modelling answered some of the pressing questions regarding adverse events in the liver, it is likely to hold the same potential for kidney and bladder related toxicity.

The aim of this investigation was to review the current scientific landscape related to computational methods for the assessment of pharmaceuticals and other chemicals with regard to their potential to elicit nephrotoxicity and to provide a future perspective for this field of research. Here, the term ''nephrotoxicity'' includes both kidney toxicity and bladder disorders. To achieve the aim, the current data relating to this endpoint, which are accessible in publicly available knowledge bases and could aid the development of computational methods for this toxicity endpoint, were also reviewed. In addition, current *in silico* models (SAs, QSARS, mechanistic models) related to nephrotoxicity were examined and existing knowledge of relevant toxic mechanisms assessed in order to understand to which extent these have already been covered by existing approaches, including AOPs. Clinical manifestations of renal disease including oedema, uraemia, hyperphosphatemia, hyperkalaemia, hypocalcaemia, acidosis, hyperparathyroidism, and anaemia [19] go beyond the scope of this study and, therefore, were not considered. As uses of *in silico* toxicology approaches are ever increasing, this investigation also attempted to assess to what extent future models may inform hazard assessments and drug design, and what is needed to drive this field forward.

2.0 MECHANISMS OF KIDNEY AND BLADDER TOXICITY

In order to understand the highly specific adverse effects that may take place in the kidney and associated organs, it is essential to appreciate its function and physiology. The key function of the kidney is to eliminate endogenous waste products, control and maintain volume levels, endocrine function, electrolyte content and acid-base balance [20,21]. As major site of elimination of drugs and other chemical compounds, the kidney is a common target for toxicity. Since the kidney is highly vascularised, receiving about 25% of the resting cardiac output, it is exposed to exogenous compounds in large quantities through systemic circulation [20,22]. The functional units of the kidney are nephrons - each kidney contains around one million nephrons, which consist of the glomerulus – a ball of capillaries –, Bowman's capsule, and the tubular element (proximal tubule, Loop of Henle, distal tubule and collecting duct). When a substance reaches the glomerulus through the afferent arteriole it is likely to be filtered into the proximal tubules where the vast majority is reabsorbed back into the blood [23]. Compound accumulation and "local" toxic metabolite formation may occur, making the kidney vulnerable to toxicity via various and simultaneously occurring mechanisms [12,20,22].

As a result of the physiology, there are four main mechanisms of drug-induced renal toxicity which are most commonly manifested as acute kidney injury, namely haemodynamic alteration, (proximal and distal) tubular cell toxicity, (tubular, interstitial, tubulo-interstitial and glomerular) nephritis and tubular obstruction [24,25]. Comparatively little is known about bladder toxicity as a whole and less about its underlying mechanisms. However, an understanding of mechanisms, such as it is, will assist in the development of *in silico* models as well as the organisation of the associated data. Figure 1 shows the sites of the main mechanisms of chemical-induced kidney toxicity.

A consideration of mechanistic toxicology also provides the opportunity to link to relevant Adverse Outcome Pathways (AOPs). The AOP framework facilitates the organisation of mechanistic knowledge and grants validity and robustness to data included in the OECD-sponsored AOP Knowledge Base (AOP-KB), [26, http://aopkb.org]. Mechanistic data gathered and organised in the form of AOPs serve as a robust basis for the development of computational toxicology models [10,27]. If an MIE and/or Key Events (KEs) have been defined and respective data are available, a prediction approach to estimate a substance's potential to elicit one of more of these may be achieved using the knowledge in the AOP-KB and the public literature. Table 1 provides a starting point for *in silico* analyses based around the MIE in particular. The individual endpoints and apical effects are described in more detail in the remainder of Section 2. Additionally, AOPs may aid the grouping of chemicals for read-across [10]. Only a handful of kidney and bladder related AOPs have

been developed and proposed so far which implies that only a small amount of MIEs and KEs have been defined. Table 1 provides an overview of relevant AOPs that exist at the time of manuscript preparation, as sourced from the AOP Wiki [28, https://aopwiki.org], which is one key resource.

Figure 1: Sites and mechanisms of chemical-induced renal toxicity and respective substances potentially causing an effect at each [adapted from 25].

PROXIMAL TUBULAR CELL TOXICITY
aminoglycosides, cadmium, 4-aminophenols, cisplatin, cidofovir, stavudine, mercuric chloride, lead

HAEMODYNAMIC ALTERATION
ACE inhibitors, ARBs, NSAIDs, amphotericin B, diuretics, tacrolimus, radiocontrast agents, calcineurin inhibitors

GLOMERULAR, TUBULO- AND/OR INTERSTITIAL NEPHRITIS
allopurinol , aristolochic acid, cephalosporins, ciprofloxacin, diuretics, gold, interferon-alpha, lithium, macrolides, NSAIDs, pamidronate, penicillins, phenytoin, proton pump inhibitors,

DISTAL TUBULAR CELL TOXICITY
amphotericin B, calcineurin inhibitors , lithium, sulfadiazine

TUBULAR OBSTRUCTION
acyclovir, ciprofloxacin, indinavir, light chain antibodies, methotrexate, polyethylene glycol, sulfonamides,
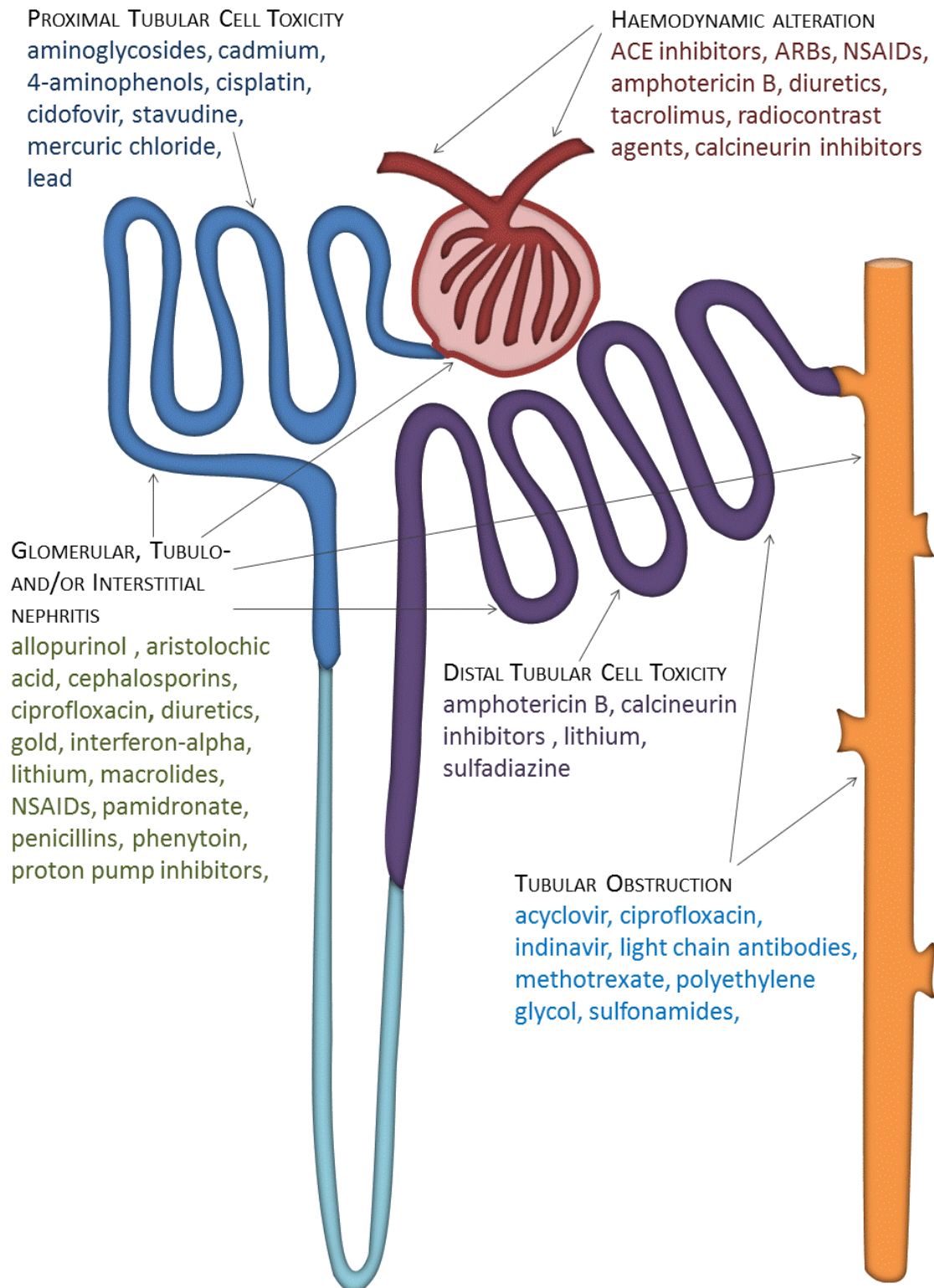
Table 1: Mechanisms of kidney toxicity, related (groups of) substances, and established and proposed MIEs and AOPs directly or indirectly associated with kidney toxicity

Mechanism	Overview	MIE	AOP	Compounds	Biomarkers
Haemodynamic alteration	Impaired autoregulatory capacity of the renal vasculature to vasodilate or vasoconstrict leading to a reduced GFR	COX-1 and/or COX-2 inhibition leading to reduced prostaglandin synthesis and uncontrolled renal vasoconstriction (aspirin, other NSAIDs, calcineurin inhibitors) [29,30]	AOP proposed by Lhasa Ltd. [29]	ACE inhibitors, ARBs, NSAIDs (e.g. aspirin), amphotericin B, tacrolimus, radiocontrast agents, calcineurin inhibitors (cyclosporine, tacrolimus) [12,25]	IL-18[I], lipocalin 2 (LCN-2 aka NGAL)[II] [31]
		Prevention of formation of angiotensin II (ACE inhibitors) [32]	No AOP found		
		Blockage of angiotensin II type 1 (AT1) receptors (ARBs) [33]	No AOP found		
		Increase endothelin and thromboxane and activation of the renin-angiotensin system (RAS) (vasoconstriction), and reduction prostacyclin, prostaglandin E2 and nitric oxide (NO) (vasodilation) (calcineurin inhibitors) [30]	No AOP found		
		Changing vascular smooth muscle cell permeability, cell depolarization with resultant opening of voltage-dependent calcium channels and muscle cell contraction (potential mechanism for amphotericin B) [34,35]	No AOP found		
		Metabolisation by oxidase in hepatocyte to benzoquinoneimine, followed by formation of GSH S-conjugates (4-aminophenol) [36]	OECD ENV/JM/MONO(2011)8: Nephrotoxicity induced by 4-aminophenols [36]		clusterin[III] , β2-
		Mitochondrial toxicity pathways: a) Mitochondrial DNA incorporation (stavudine,	AOP proposed by Lhasa Ltd. (stavudine, cidofovir) [37]		

Proximal and distal tubular cell toxicity	Extensive cellular uptake and intra-cellular accumulation inducing compromised mitochondrial respiration, oxidative stress, and the activation of intrinsic apoptotic and necrotic pathways	cidofovir) [37] b) Mitochondrial DNA polymerase gamma inhibition (stavudine, cidofovir) [37] c) Depletion of SH-groups leading to ROS induction (cisplatin) [44]		aminoglycoside antibiotics, amphotericin B, 4-aminophenols, cisplatin, nucleotide and nucleoside antivirals (stavudine, cidofovir) [25,36,37]	microglobulin[IV], cystatin C[V], heme oxygenase-1[VI], IL-18[I], lipocalin 2 (LCN-2 aka NGAL)[II], KIM-1[VII], miR-34a[IX] [31,38–43]
		Accumulation-induced lysosomal effects: a) accumulation induced lysosomal leakage leading to tubular dysfunction (aminoglycosides) [45] b) fusion of compound-containing pinocytic vacuoles and lysosomes causing osmotic nephrosis (contrast agents) [46]	No AOP found		
		After moving through cellular membrane, polyunsaturated region participates in auto-oxidation, lipid peroxidation and cell membrane damage; forming pores (amphotericin B) [34,35]	No AOP found		
Tubular, interstitial, tubulo-interstitial and glomerular nephritis	Inflammatory changes in the glomerulus, interstitial and tubular cells predominantly caused by immune	Interaction with hOAT1 and 3, accumulation within proximal tubule cells, followed by uncoupling/inhibition of mitochondrial oxidative phosphorylation and tubular/papillary necrosis (aspirin)* [29]	AOP proposed by Lhasa Ltd. [29]	NSAIDs (indomethacin, phenylbutazone, mefenamic acid, aspirin); antibiotics (cephalosporins, ciprofloxacin, ethambutol, isoniazid, macrolides, penicillins, rifampicin, tetracycline); loop (furosemide), potassium-sparing (triamterene) and thiazide diuretics; proton	IL-18[I]; lipocalin 2 (LCN-2 aka NGAL)[II];
		Production of inflammatory response triggering TNF-α (cisplatin) [44,53]	No AOP found		
		Formation of immune complex deposits (methicillin, rifampin, allopurinol, phenytoin) [47]	No AOP found		
		Formation of drug-protein hapten conjugates in renal tissue which elicit an immunogenic response	No AOP found		

	mechanisms resulting in fibrosis and renal scarring	(sulfamethoxazole metabolite = nitrososulfamethoxazole, methicillin) [47]		pump inhibitors (omeprazole); allopurinol, lithium, aristolochic acid, phenytoin, propylthiouracil, ranitidine [12,25,29,47–49]	osteopontin[VIII] [42,50–52]
		(Event 244 (AOP 38):Protein alkylation)** [28]	(AOP 38: Protein alkylation leading to liver fibrosis)** [28]		
Tubular obstruction	Crystal precipitation within the renal tubule depending on urinary pH and favoured by high concentrations in the urine	OAT interaction causing secretion via proximal tubule cells, accumulation and crystal formation in urine leading to concentration in renal tissue/tubule and obstructive nephropathy (acyclovir) [37]	AOP proposed by Lhasa Ltd. [37]	antibiotics (e.g. ampicillin, ciprofloxacin, vancomycin and sulphonamides), antivirals (e.g. indinavir and acyclovir), methotrexate [12,37,54]	Clusterin[III], lipocalin 2 (LCN-2 aka NGAL)[II], IL-18[I], KIM-1[VII] [52,55]

* Interstitial nephritis is not the adverse outcome of these AOPs. However, as NSAIDs have been associated with this mechanism of nephrotoxicity, and KEs, e.g. ROS production and necrosis, are part of this pathway, these AOPs were allocated here.

** This AOP is not directly related to nephrotoxicity but may be relevant for these pathways.

    I)   IL-18: inflammatory response, activating NFκB in response to ischemia-reperfusion injury of renal tubules (e.g. after contrast agent exposure)

    II)   LCN-2, NGAL: maximally expressed in kidney after early ischemic injury, in response to contrast agents; important mediator of innate immune responses

    III)   Clusterin: associated with membrane recycling, cell repair, ischemic injury in proximal and distal tubule

    IV)   β2-microglobulin: early marker of tubular injury

    V)   Cystatin C: related to ischemic injury in proximal tubule

    VI)   Heme oxygenase-1: changes in response to ischemic and cisplatin-induced injury

    VII)   KIM-1: found in urine after proximal tubular cell injury

    VIII)   Osteopontin: associated with accumulation of macrophages, expressed in the distal convoluted tubules, the thick ascending limbs of the loop of Henle and the proximal tubule

    IX)   miR-34: was upregulated following cisplatin induced acute kidney injury, may play a cytoprotective role for cell survival

Abbreviations:

ACE (angiotensin-converting enzyme) inhibitors; ARBs (angiotensin receptor blockers);

2.1 Haemodynamic alteration

The kidney auto-regulates the pressure within the glomerulus by adjusting the afferent and efferent arterial tone to maintain the glomerular filtration rate (GFR) and urine output [12]. GFR is one of the key parameters to assess intraglomerular haemodynamics as it estimates the volume of blood filtered through the glomeruli per minute [19]. The GFR value considered normal in a healthy adult – standardised for a body surface area of $1.73m^2$ – is around 100-120 mL per minute [19,56].

Patients with normal renal function who are being treated for hypertension generally do not encounter an increase in serum creatinine levels [33]. However, patients with chronic renal insufficiency and hypertension do when using antihypertensive drugs. A combination of factors related to chronic hypertension, such as structural and functional changes in small vessels of the kidney, contribute to a decrease in autoregulatory capacity of the renal vasculature leading to a reduced GFR and an increase in serum creatinine concentrations [33].

An excessive lowering of blood pressure through the use of medication may cause a decrease in intraglomerular pressure which may be exacerbated by a decline of efferent arteriole resistance due to vasodilation and/or afferent vasoconstriction [33,57]. The use of angiotensin-converting-enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) is associated with such effects, in particular with reducing efferent arteriolar tone. However, in patients with chronic kidney disease hypertension is common and a risk factor for the progression of renal damage [58]. Both, ACE inhibitors and ARBs are prescribed for their renoprotective effects in anti-hypertensive therapy, in combination to treat heart failure and CKD with proteinuria [59–62] even though this practice has been debated particularly for CKD patients aged 65 and older [63,64]. Also, careful dose titration is judged essential for ACE inhibitors [65] which indicates a narrow therapeutic index. ACE inhibitors prevent the formation of angiotensin II, a potent vasoconstrictor, which acts on vascular smooth muscle cells, with salt- and fluid-retentive properties [32].

Intravascular volume depletion may induce adverse effects of ACE inhibitors on the kidney [33]. ARBs also target the angiotensin II pathway by blocking angiotensin II type 1 (AT1) receptors while not acting on angiotensin II type 2 (AT2) receptors which are stimulated to a higher extent as a result of higher circulating angiotensin II concentrations [33]. AT1 receptors are primarily on efferent vessels increasing vasoconstriction if activated while AT2 receptors are predominantly found on afferent vessels [33,66–68]. AT2 receptor binding has been associated with antagonised renal vasoconstrictor response and natriuresis [69,70].

Even though ACE inhibitors and ARBs are considered renoprotective administered on their own and to counteract hypertension, they may aggravate nephrotoxic effects in combination with other drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and diuretics [71]. NSAIDs are known to cause alterations to intraglomerular haemodynamics by inhibiting either one or both isoenzymes of cyclooxygenase (COX-1 and COX-2) and, as a result, suppressing prostaglandin synthesis [24]. Prostaglandins mediate arteriolar vasodilation [72]. In certain conditions of decreased renal perfusion, e.g. cirrhosis and congestive heart failure, or volume depletion, renal function is increasingly dependent on prostaglandins [72,73]. In these instances, (selective and non-selective) NSAIDs used at high doses are associated with an increased risk of acute kidney failure [24]. An AOP was proposed describing this pathway [29].

A number of drugs induce renal dysfunction via more than one pathway. In the case of amphotericin B both haemodynamic and tubular adverse effects have been observed. The compound causes vasoconstriction of the renal arteriae, a subsequent decrease in renal blood flow and GFR, and polyuria [74]. On a cellular level, amphotericin B causes modifications in cell membrane integrity and increased influx of Ca^{2+} into the cytoplasm via newly formed pores [74–76]. These may lead to tubular cell toxicity as further described below.

2.2 Proximal and distal tubular cell toxicity

Renal tubular cells, especially proximal tubule cells, are vulnerable to the toxic effects of drugs. This is because their apical and basolateral transport systems facilitate extensive cellular uptake in their function of re-absorbing glomerular filtrate [20,23]. Thereby, proximal tubular cells are exposed to a high amount of circulating endogenous and exogenous compounds, including potential nephrotoxicants [12,20,23].

Tubular cell toxicity may be elicited via different pathways which are induced by therapeutic agents such as aminoglycoside antibiotics, cisplatin and amphotericin B [12,25]. For instance, aminoglycosides are cationically charged and therefore attracted to the anionic phospholipid-rich brush border located at the proximal tubular apical membrane [76]. Accumulation of the aminoglycosides in tubular cells leads to the disruption of endosomal and lysosomal membrane and activation of intrinsic apoptotic pathway [45,75,77]. This includes impaired mitochondrial respiration and induction of oxidative stress through increased free radical levels within the cell. The kidney is particularly vulnerable to reactive oxygen species (ROS) damage [78]. Several nephrotoxic

compounds, e.g. cisplatin, immunosuppressant drugs, NSAIDS and aminoglycosides, exert their toxic effects due to excess ROS production, and depletion of the antioxidant defence mechanism [78].

Oxidative injury, inflammation, apoptosis, acute tubular necrosis as well as vasoconstriction have been associated with aminoglycosides as well as exposure to cisplatin [53,75]. The extent of exposure is suggested to determine whether apoptotic or necrotic cell death is induced. High concentrations of cisplatin in the millimolar range were reported to result in necrosis while concentrations in the micromolar range provoked apoptosis – via the intrinsic mitochondrial, extrinsic death receptor and ER-stress pathways [53].

Experimental data suggested the intrinsic mitochondrial pathway to be the major pathway of cisplatin-induced apoptosis, likely to be induced by sulfhydryl group and mitochondrial glutathione (GSH) depletion [44]. Basolateral uptake by the organic cation transporter OCT2 has been demonstrated to be critical for cisplatin's toxic response to be elicited in the kidney [53]. Also, different segments of the nephron demonstrate diverse sensitivities to cisplatin which did not appear to be due to differences in uptake characteristics but intracellular effects [79]. S1 cells derived from the early portion of the proximal tubule expressed a considerably lower amount of the anti-apoptotic protein BCL-X_L than S3 cells derived from the late portion of the proximal tubule and distal convoluted tubular cells [79].

The mitochondria of proximal tubular cells also appear to be key targets of nucleotide and nucleoside antiviral drugs stavudine and cidofovir [37]. Mitochondrial toxicity induced via mitochondrial DNA incorporation or mitochondrial DNA polymerase gamma inhibition may lead to tubular cell necrosis and acute renal failure [37].

Amphotericin B is also commonly associated with acute tubular necrosis which may be secondary to changes in haemodynamics and cell membrane permeability as described above, and resulting renal tubular acidosis and hypokalemia [74,75,80]. Unlike aminoglycosides and cisplatin, amphotericin B appears to elicit cellular toxicity predominantly in distal tubular regions as opposed to the proximal tubules [24,25,53].

Another pathway leading to renal tubular necrosis is documented in an AOP related to 4-aminophenol exposure whereby 4-aminophenol cysteine S-conjugates reach and get concentrated in

proximal tubules [36]. There, cysteine S-conjugates are metabolized to benzoquinoneimines which cause oxidative stress and necrotic tubular cell death [36].

2.3 Tubular, interstitial, tubulo-interstitial and glomerular nephritis

Certain drugs, e.g. NSAIDs, antibiotics, loop and thiazide diuretics and proton pump inhibitors, induce kidney injury by producing inflammatory changes in the glomerulus, tubular cells and the interstitium, which can lead to fibrosis and renal scarring [12]. Many nephrologists consider these endpoints separately from each other due to differences in mechanisms leading to them. However, NSAID-induced nephritides may not be demarcated from each other but rather indicate a continuous spectrum of renal responses due to hypersensitivity against a drug influenced by the extent of drug exposure [81].

Another study showed that in all forms of progressive glomerulonephritis, a major tubulo-interstitial infiltrate of immune-competent cells was present [82]. Moreover, the outcome of different forms of progressive glomerulonephritis was found to be determined by the presence and severity of tubulo-interstitial changes rather than the degree of glomerular alteration [82]. For the purpose of this review, they will be discussed jointly.

Drug-induced acute interstitial nephritis occurs as a result of dose-dependent renal tubular cell damage (including necrosis) or from an immune reaction directed against endogenous antigens in the kidney, and develops in an idiosyncratic, non-dose-dependent fashion [12,22,47,57,83]. In immune reaction induced cases, the usual symptoms of hypersensitivity, e.g. fever and rash, may be lacking [47,83]. An immunological response may be initiated through the deposition of a drug acting as a hapten or a circulating antibody-drug-based immune complex within the interstitium where it gets targeted by a, mostly cell-mediated, immune response [47,83]. As neutrophils and macrophages are attracted to the site, ROS and inflammation mediators are released leading to phagocytosis, tubular cell and glomerular injury [83,84]. Common drugs that induce acute interstitial nephritis include allopurinol, NSAIDs, antibiotics, loop and thiazide diuretics and proton pump inhibitors [12,22,25,75].

Chronic interstitial nephritis tends to be less drug-induced, however, has been reported with lithium, NSAIDs and aristolochic acid [12,20]. The main characteristics of this mechanism of nephrotoxicity are interstitial fibrosis and interstitial damage by far exceeding any glomerular effects, which may

include periglomerular fibrosis [22]. Also, tubular atrophy and an inflammatory infiltrate of lymphocytes, plasma cells and macrophages are observed [22].

Glomerulonephritis has been reported to be induced by exposure to gold, interferon-α, cephalosporin, penicillin and pamidronate [12]. Its most common cause is IgA nephropathy, which is characterised by deposits of IgA-containing immune complexes in the kidney with proliferation of the glomerular mesangium [85]. Other forms of nephritis may be linked to autoimmune conditions, such as lupus nephritis.

The AOP suggested for aspirin describes the pathway from the MIE of uncoupling/inhibiting mitochondrial oxidative phosphorylation to acute renal failure following acute tubular necrosis [29]. As adverse outcome or KE, interstitial nephritis is not included even though aspirin and other NSAIDs are recognised to induce this endpoint.

2.4 Tubular obstruction

Tubular obstruction may be caused through crystal deposition within the renal tubules. Certain drugs such as antibiotics (e.g. ampicillin, ciprofloxacin and sulphonamides), antivirals such as indinavir and acyclovir, light chain antibodies, methotrexate and polyethylene glycol produce insoluble crystals in the body [12,75,76,86]. These crystals may precipitate within the distal tubule, and obstruct urine flow. The likelihood of crystal precipitation depends on the amount of drug in the urine, the solubility of the drug and on the pH of the urine which is altered in conditions of renal tubular acidosis, metabolic acidosis or alkalosis [76,87]. With acidic urine (pH <5.5), crystal precipitation is increased for sulfonamides and methotrexate, and with alkaline urine (pH > 6.0), it increases for indinavir and ciprofloxacin [76].

Renal hypoperfusion increases the chance of nephrotoxicity through this mechanism as renal tubules are exposed to high drug concentrations for longer than in a normally perfused kidney [20,87]. Low perfusion and a high intratubular drug concentration may lead to supersaturation within the distal tubules [88]. If the drug is administered at a high dose, mainly excreted via the kidney in its unchanged form and relatively insoluble in the urine, as in the case of acyclovir, crystal formation and intratubular precipitation is likely to occur [87].

A recent report documented a new mode of cast formation induced by vancomycin [54] which had previously been associated with acute tubular necrosis and acute interstitial nephritis [12,48,89].

These casts were described as atypical and non-crystalline consisting of vancomycin nanospheres entangled with uromodulin, an abundant protein in normal human urine, present in the tubular lumen and the Bowman's space suggesting tubular obstruction [54].

Little research appears to have been done to understand the formation of different shapes of crystals and their behaviour and pathomechanisms in different parts of the kidney [86].

2.5 Other mechanisms of nephrotoxicity

Rhabdomyolysis and thrombotic microangiopathy have been discussed as additional mechanisms of nephrotoxicity elsewhere [12,57] but may also be regarded as systemic causes of nephrotoxicity. Rhabdomyolysis also causes tubular obstruction and refers to a syndrome where disintegration of striated muscle leads to release of muscular cell constituents, predominantly myoglobin and creatinine kinase, into the plasma [90,91]. Normally, myoglobin is loosely bound to plasma globulins and only small amounts reach urine. However, in rhabdomyolysis, large amounts of myoglobin are released; significantly more than can be bound by plasma globulins. Myoglobin is then filtered by glomeruli and reaches the tubules, leading to renal obstruction and renal dysfunction [90,91]. Drugs that cause rhabdomyolysis include certain statins, sedative hypnotics and antidepressants, alcohol and agents of abuse such as cocaine, heroin, ketamine, and methadone [90,91]. As nephrotoxicity is not primarily induced by these drugs this pathway is not considered as key mechanism.

Thrombotic microangiopathy (TMA) is predominantly a vascular issue characterised by vessel wall thickening of arterioles or capillaries, and intraluminal platelet thrombosis, which leads to the obstruction of the vessel lumina. If these lesions prevail in the kidney, they are termed haemolytic uremic syndrome (HUS) and they are also, but less frequently, found in thrombotic thrombocytopenic purpura (TTP) which may be more associated with brain lesions [92,93]. Events leading to thrombotic microangiopathy are vascular injury - endothelial cells being the key target -, loss of endothelial thromboresistance, leukocyte adhesion to the damaged endothelium, complement consumption and enhanced vascular shear stress [92]. Drugs that cause nephrotoxicity through thrombotic microangiopathy include mitomycin C, clopidogrel, quinine, cyclosporine and tacrolimus [25,92,94,95]. The onset of general clinical manifestations such as microangiopathic haemolytic anaemia, and thrombocytopenia is often delayed [96]. TMA lesions have been reported in about 50 % of 128 patients diagnosed with IgA nephropathy [97], which is tightly linked to glomerulonephritis.

This, as well as a number of previously mentioned examples (e.g. tubular cell necrosis leading to interstitial nephritis in aspirin-induced nephrotoxicity) show very clearly that some of these mechanisms are interlinked as a number of substances elicit nephrotoxicity via more than one pathway. Mechanistic data describing molecular events initiating these toxicity pathways and effects further down the line are often lacking. Even fewer data are available which help to understand how these may be linked depending on dose and time.

2.6 Site-Selective Nephrotoxic Injury

Many drugs selectively cause nephrotoxicity through the above mechanisms on different segments of the nephron.

2.6.1 Glomerular Injury

The glomerulus is a primary site of chemical exposure and a number of drugs induce nephrotoxic effects there [74]. Glomerular ultrafiltration may be impaired by compounds acting on endothelial cells causing vasoconstriction of the renal arteriae (i.e. amphotericin B, gentamycin) or substances eliciting direct cytotoxic effect on glomerular epithelial cells (i.e. cyclosporine) [74]. By impairing GFR, the excretion of toxic metabolic waste is diminished. Glomerular injury may also result from circulating immune complexes getting trapped in the glomerulus and attracting neutrophils and macrophages which release ROS [74]. ROS greatly contribute to many glomerular diseases, including glomerulonephritis [98]. Heavy metals (e.g. $HgCl_2$), volatile hydrocarbons and organic solvents cause glomerular injury via the above mechanism, and can also cause an increase in membrane permeability in the glomerulus. This will allow larger molecules e.g. albumin and γ-globulin, which are normally prevented from entry, to pass to the ultra-filtrate and be excreted along with the urine, thus causing proteinuria [74,84].

2.6.2 Injury to Tubular Systems

The most common site of drug-induced renal toxicity is the proximal tubule due to significant accumulation of chemicals in the tubule, contributed by the high reabsorption rates [74,84]. The proximal tubular cells have a leaky epithelium, which enhances the flux of compounds into proximal tubular cells, unlike the distant tubule characterised by a tight epithelium with high electrical resistance [74]. The proximal tubules are the critical, if not exclusive, site of transport for organic anions and cations, low-molecular-weight proteins and peptides, GSH conjugates, and heavy metals [74]. Drugs that preferentially affect the proximal tubules include aminoglycosides, β-lactams

(including cephalosporins), haloalkane-S-conjugates and $\alpha 2\mu$-globulin bound chemicals i.e. cadmium, mercury and limonene [74,84,99]. For the cephalosporin antibiotic cephaloridine, the correlation between transport, accumulation, and nephrotoxicity is strong but this does not apply to other cephalosporins [74]. The intrinsic reactivity of the compound with molecular or subcellular targets within the proximal tubular cell is considered to be another decisive factor [74]. Chemical-induced injury is less common in the loop of Henle and the distal tubular system compared to the proximal tubules.

2.6.3 Papillary Injury

The renal papilla is also targeted for injury, mainly by excessive and abusive use of analgesics (analgesic nephropathy) [74,100]. This type of toxicity is characterised by renal papillary necrosis and chronic interstitial nephritis that leads to the onset of progressive kidney failure [100].

2.7 Bladder Toxicity

There is limited information on toxicity induced by compounds in the bladder. This could be because urine does not stay there for a long time. However, some carcinogenic compounds are known to target the bladder. One of the earliest examples of bladder cancer due to occupational exposure is 2-napthylamine [74]. Other aromatic amines are also known to be carcinogenic to the bladder. Metabolism of these compounds in the kidney and bladder has been recognised to play a vital role in this toxicity pathway [74]. Similarly, a metabolite of both cytotoxic drugs cyclophosphamide and ifosfamide, i.e. acrolein, is predominantly responsible for urothelial cell toxicity [101–104]. As stable urinary metabolite, acrolein reaches the bladder epithelial lining via the urine where toxicity is believed to be caused by ROS and nitric oxide (NO) production leading to lipid peroxidation, DNA damage and consequently necrotic cell death [103,104]. Other bladder related adverse events following drug exposure in humans include urolithiasis, blood in urine, and bladder disorders such as dysuria (i.e. painful urination), urinary incontinence, urinary retention and polyuria [49]. However, these may be additional symptoms of systemic toxicity and not be adverse effects primarily observed in the bladder.

Overall, each of these key mechanisms outlined above includes a number of sub-mechanisms which typically have not been defined conclusively on a molecular or level. An aspect which adds to the level of complexity already encountered when trying to establish models on these (sub-)mechanisms is the fact that some of these (sub-)mechanisms manifest jointly and therefore, appear to be interlinked, e.g. necrosis is part of the tubular cell toxicity mechanism but also appears to be present

in tubulo-interstitial nephritis. In Table 1, an AOP proposed by Lhasa Ltd. for the nephrotoxicity of NSAIDs is included, which denotes acute tubular necrosis and renal papillary necrosis as post-MIE KEs [29]. NSAIDs have been predominantly associated with interstitial nephritis and hemodynamic alteration [12,25] and not with tubular cell toxicity, which indicates that tubular necrosis is a pre-stage of interstitial nephritis.

In addition, the sensitivity and specificity of biomarkers, which have been used to a vast extent, e.g. serum creatinine and blood urea nitrogen, have been criticised over the last years [42,105,106]. In 2008, the FDA designated seven biomarkers of nephrotoxicity for use in animals and, on a case-by-case basis, in humans. These included urinary KIM-1, β2-microglobulin, cystatin C, clusterin, trefoil factor-3, albumin, and total protein. These markers and others (e.g., urinary NGAL, urinary IL-18, and the liver fatty acid binding protein (L-FABP)) have been studied in a range of conditions [31,39,42,106,107]. Certain biomarkers, which have been proposed, are specific to particular segments of the nephron but a signal in the proximal tubules may indicate various nephrotoxicity mechanisms. Knowledge is partly available suggesting that they may be attributed to a mechanism of toxicity [39,42,106] but a lot more research is needed to allow for a more refined mechanistic understanding.

3.0 <u>SOURCES OF DATA / INFORMATION ON KIDNEY TOXICITY SUITABLE FOR COMPUTATIONAL MODELLING</u>

Physiological, physico-chemical and toxicological data are the bedrock of the development of *in silico* models for toxicology. Some of the general issues related to data procurement for modelling purposes have been discussed elsewhere [108–113]. If the development of AOPs and multi-scale models is currently considered to be the panacea of 21st century toxicology, data spanning molecular to population levels are necessary to generate multi-scale models resembling the structure of AOPs.

For the registration of many chemicals and pharmaceuticals, adverse effects to the kidney and bladder are currently assessed through traditional toxicological approaches, involving *in vitro* and *in vivo* animal studies [114]. However, a standardised test specifically designed to investigate a substance's potential and mechanisms to elicit nephrotoxicity does not exist to date, as this would normally be assessed in repeat dose toxicity testing. In drug development, whilst safety pharmacology studies on the kidney are not part of the core required animal study battery, supplemental studies on the renal and urinary system may be performed if there is cause for

concern [115]. Furthermore, clinical studies of drug compounds in humans cover endpoints related to renal toxicity but their efficacy to assess this pathology adequately has been challenged by the high number of drug-induced acute renal failure cases in critically ill and chronic kidney disease patients.

A list of over 250 potential nephrotoxicants has been compiled including, where available, information on a putative or confirmed MIE and AOP, using current knowledge from the literature [12,20,25,28–30,34–39,44–49,53,54,73,86,89,101–104,116–188]. This list can be accessed via the supplemental information and provides a comprehensive, publicly available compilation of nephrotoxicity data. For modelling purposes, this list needs to be enriched with chemistry-, activity- and toxicity-related data. There are over 400 databases available of which over 200 are publicly available geared towards chemistry, toxicology, Absorption, Distribution, Metabolism and Excretion (ADME) properties, as well as molecular biology (-omics) and pathways, which may be accessed for model generation. The following review of databases sheds some light on how well publicly available data may inform future nephrotoxicity modelling. This review is by no means complete but covers the most significant resources currently available for modelling. Searches were performed at timepoints recorded in the references' section.

In reviewing data sources, it must be remembered that several different types of data and data compilations are required and ideally these should be suitable for modelling. Traditional QSAR modelling requires datasets of consistent information for a group of compounds, this could include the presence or absence of nephrotoxicity or quantitative estimates of potency – providing the data have been measured in a consistent manner (i.e. the same test protocol). Read-across can be attempted on smaller data sets – even a one-to-one approach using a potentially wider variety of data – non-standard data from multiple and different sources can be used to build up a weight of evidence. However, physiologically based kinetic (PBK) and multiscale models focus on several parameters (clearance and absorption rates, volume of distribution, partition coefficient) for a single compound. Thus, there may be different uses of the data resources covered in this section.

3.1 Chemical and biological data

In order to relate adverse effects to structural components, properties such as solubility, receptor binding, or enzyme inhibition, data on chemical structures, physico-chemical and functional properties and potency are needed.

A reliable source of information on chemical structures is ChemID*plus* Advanced [189, https://chem.nlm.nih.gov/chemidplus/]. A search in the toxicity field for "kidney, ureter, and bladder" effects revealed 139,289 records for 1,352 structures. However, for each record, it is not immediately obvious why a compound is associated with the above mentioned endpoint. ChemID*plus* is based on more than 100 sources, including the Comparative Toxicogenomics Database (CTD), the Hazardous Substances Data Bank (HSDB®), the Integrated Risk Information System (IRIS), and the International Toxicity Estimates for Risk (ITER) [190,191] such that information – which is often replicated many times, may be drawn from any of these resources.

Other reliable sources of chemical information include, but are not limited to, the following. Chemical structures and physico-chemical properties can be sourced per chemical and downloaded from the U.S. EPA Chemistry Dashboard [192, https://comptox.epa.gov/dashboard/]. Elsewhere, ChEMBL [193,194, https://www.ebi.ac.uk/chembl/] is a database of bioactive compounds which allows access to compound-specific ADME and bioactivity information (e.g. binding measurements, functional assay data) including specifics on the mechanism of action, and (non-)molecular targets. Pharmacological, biological and chemical data of pharmaceuticals and other substances can be found in DrugBank [195,196, https://www.drugbank.ca/]. ChEMBL and DrugBank data may be searched for jointly and in parallel to information from other databases through UniChem [197,198, https://www.ebi.ac.uk/unichem/]. Compound-specific physico-chemical properties may also be sourced from PubChem [199,200, https://pubchem.ncbi.nlm.nih.gov/]. The Online Chemical Modelling Environment [201,202, https://ochem.eu/home/show.do] offers pharmacological and physico-chemical data along with an interface for calculating and selecting a number of molecular descriptors.

3.2 Molecular Biology (-Omics) Data

High-throughput biology data including genomics, proteomics, transcriptomics and metabolomics in various tissues have been used to identify relevant molecular mechanisms, toxicity pathways, and biomarkers. A number of kidney tissue specific databases discussed below have been established to provide gene, peptide and protein expression data.

The Renal Gene Expression Database (RGED) [203,204, http://rged.wall-eva.net/] and Nephroseq™ [205, https://www.nephroseq.org/resource/login.html] are platforms providing free access to gene expression information in specific renal diseases. Within RGED, only searches by gene are possible

while on Nephroseq™ a more refined analysis may be done on molecular characteristics of disease phenotypes, markers of disease progression and to a very limited extent treatment response.

Data on protein expression in healthy and diseased kidney tissue and urine can be accessed through the Human Kidney & Urine Proteome Project (HKUPP) [206, http://www.hkupp.org/index.htm]. Search options for data established in the glomerulus, proximal and distal tubules and the collecting ducts from three samples of three kidney cancer patients (two males and one female, aged between 71 and 77) are under construction at the time of this review.

The Urinary Peptidomics and Peak-maps database (UPdb) [207,208, http://www.padb.org/updb/] gathers information on urinary peptides modified in disease. Of relevance to nephrotoxicity modelling are entries related to exposure to mixtures of arsenic and lead [sourced from 209], membranous glomerulonephritis [sourced from 210], IgA nephropathy [sourced from 211] and healthy volunteers as controls [various sources including 212,213].

Via the Kidney & Urinary Pathway Knowledge Base (KUPKB) [214,215, http://www.kupkb.org/#tab0], a collection of human and animal derived urine and kidney tissue based miRNA, mRNA, protein and metabolite expression data can be accessed. KUPKB can be searched for information related to specific locations, cells or fluids within the kidney and bladder or a specific condition or disease model. The extent of molecular expression is reported as 'up', 'down', 'present', 'absent', 'medium' and 'strong', which requires accessing the original data source if numerical measures are necessary for further analysis.

Sources of genomics data with a clear focus on toxicity endpoints are the Comparative Toxicogenomics Database (CTD) [216,217, http://ctdbase.org/] and the ToxicoGenomics Project-Genomics Assisted Toxicity Evaluation system (Open TG-GATEs) [218,219, http://toxico.nibiohn.go.jp/english/]. A search of the CTD for the keyword "kidney" in all sections shows matches with 3 chemicals, 291 genes, 68 gene ontologies, 51 diseases, but 0 pathways. The Open TG-GATEs may be browsed for chemicals or kidney pathologies, which may not be unambiguously assigned to one of the key toxicity mechanisms discussed in more detail below.

3.3 *In vitro*
In vitro data in this section include information from receptor binding and single cell assays up to the sophistication of 3D tissue cultures, bioreactors and organoids. The cheaper and more rapid the

assay, the greater is the likelihood of a proliferation of data for modelling. A major platform providing high-throughput *in vitro* screening data is the U.S. Environmental Protection Agency's (EPA) ToxCast[TM] programme. On the ToxCast[TM] Dashboard website [220, https://actor.epa.gov/dashboard/], when the database was filtered for assays on the kidney, data for 70 assays are shown to be present (according to assay component endpoint names), 1 of which is based on rat kidney membranes, 2 on pig tissues, and 67 on human cell lines. These assays vary to a great extent, for instance in terms of their statistics (e.g. the total number of samples tested, and percent of active samples) or their biological process target (e.g. regulation of catalytic activity, receptor binding, or protein stabilisation). An overview of these assays can be found in Appendix A contained in the supplementary information. When assessing whether a substance is active in an assay, the effect concentrations need to be compared to the compound's burst concentration which denotes a cytotoxic effect at that level [221]. The utility of *in vitro* ToxCast[TM] data has been widely debated [222–229], and a more detailed analysis is required to confirm to which extent these targets are related to currently known KEs of nephrotoxicity pathways. In addition to kidney tissue assays, the ToxCast[TM] database contains a broad variety of assays, which may be relevant to *in silico* models for nephrotoxicity, e.g. cytotoxicity or oxidative stress. Lin and Will [120] found that HepG2 (hepatocellular carcinoma), H9c2 (embryonic myocardium), and NRK-52E (kidney proximal tubule) cells equally serve to screen for general, non-organ-specific cytotoxicity. Therefore, liver, heart or other tissue cells may be suitable for the prediction of nephrotoxicity endpoints.

The Hazardous Substances Data Bank (HSDB®) [230, https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm] contains 393 records for the search term "nephrotoxic*" and 2865 records for "kidney". When selecting "download records", general, i.e. non-nephrotoxicity-specific, *in vitro* and *in vivo* animal toxicity, metabolism and pharmacokinetic data may be downloaded as txt file. Renal transporter expression levels, their localisation, substrates and inhibitors may be found on the UCSF-FDA TransPortal [231,232, http://transportal.compbio.ucsf.edu/]. *In vitro* models of particular interest due to their enhanced complexity and physiological relevance are spheroid and kidney slice assays. Whilst research with spheroids has been conducted to investigate kidney disease and treatment options [233–235], no or few relevant toxicological studies with kidney spheroids were identified. Conversely, toxicity and kinetic studies have been performed in animal and human kidney slices for years [236–239] but no database was identified, in which kidney slice data can specifically be searched for.

3.4 *In vivo*

In vivo data provide valuable insights into mechanistic pathways of kidney and bladder toxicants and the most meaningful are likely to be gained from long-term, repeated dose, low concentration tests. As with all endpoints, the relevance of inter-species differences must, however, be appreciated if there is an expectation to extrapolate animal data to humans. For instance, amongst many well known issues, rat data cannot be used to establish dose-response relationships of effects dependent on proximal tubular transporter activity as significant differences in transporter clearance between humans and rats exist [56,240].

From the U.S. EPA's Toxicity Reference Database (ToxRefDB), lowest effect levels (LELs) can be retrieved on 30 kidney related toxicity endpoints and 13 urinary bladder related toxicity endpoints [126]. Appendix B contained in the supplementary information lists all these endpoints. However, only 12 compounds were identified with the lowest LEL associated with urinary or kidney endpoints. Other compounds, for which urinary or kidney related LELs were recorded, had LELs associated with other toxicity endpoints lower than those associated with nephrotoxicity.

As mentioned above, *in vitro* and *in vivo* animal toxicity, metabolism and pharmacokinetic data may be downloaded in txt file format from the Hazardous Substances Data Bank (HSDB®). In addition, the COSMOS DB [241,242, http://www.cosmostox.eu/what/COSMOSdb/] contains *in vivo* toxicity data including highest no effect level (HNEL) and lowest effect level (LEL) information. When performing a database search with a toxicity query for the endpoint "chronic toxicity" and the site "kidney" for all species/strains, routes of exposure, effects, assays and both sexes, 68 hits, i.e. compounds inducing nephrotoxic effects in animals, are presented. For the vast majority of these substances, i.e. 53, one study was recorded; for 13 compounds, 2 studies were recorded and for two substances 4 and 5 studies were presented. A toxicity query for the endpoints "special toxicology study" and "subchronic toxicity" and the site "kidney", resulted in 202 hits with mostly 1 and up to 11 studies recorded. More specific sites can be queried for, e.g. "kidney > renal tubule > epithelial" or "kidney > interstitial cells" which facilitates the search for mechanism-specific toxicity information.

Fraunhofer ITEM created a commercially accessible database on high-quality subacute to chronic toxicity studies called RepDose [243]. A subset of approximately 200 subacute studies is made available for free on http://fraunhofer-repdose.de/ [244]. The database query for "kidney" as organ/target parameter found 113 entries. However, these data were not displayed and query conditions needed to be restricted in order for results to be displayed. After specifying the effect

"nephropathy", 8 entries were found and displayed, i.e. the CAS numbers and names of 8 substances. 9 entries were identified for "kidney" and "necrosis". These results indicate that RepDose may be used to identify potential nephrotoxicants but does not provide *in vivo* data for further analysis.

A Urinary Protein Biomarker (UPB) database in Chinese language may be accessed via http://bmicc.cn/web/share/search/hupd [245].

3.5 Human data - clinical and post-marketing

For known nephrotoxicants, clinical and post-marketing data as well as case reports in the public domain give insight into toxic effects observed in humans at a given dose. Most valuable are clinical data as doses and effects are clearly recorded. With the use of more refined biomarkers, more information will be gained from these studies in the future.

From the Hazardous Substances Data Bank (HSDB®), epidemiological data and case reports can be retrieved in the form of a txt file. However, data gathered from post-marketing reports often do not include (reliable) information on the dose taken by the patient. If dosing information is available – as in the case of clinical studies –, it may be challenging to compare dose-response data of investigations which adhered to differing classifications as different definitions and criteria to classify the severity of certain nephropathy outcomes exist [24].

The U.S. National Library of Medicine provides clinical trial data on ClinicalTrials.gov [246, https://clinicaltrials.gov/]. Queries can be performed for substances, however, not for adverse events. Dosing information and adverse events are published for completed studies but these data do not appear in the downloadable record.

The U.S. Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS), formerly AERS, can be accessed via http://www.fdable.com/basic_query/aers [247] and was searched for kidney related specific event descriptions such as kidney fibrosis or focal glomerulonephritis, or renal failure in general. Among the information given are the primary suspect drug, patient outcome, age and gender of the patient. Adverse event data existing in FAERS may have been submitted to the FDA by drug and therapeutic biological product manufacturers, healthcare professionals or consumers. When using these data, their limitations need to be taken into account, i.e. no proof of a causal relationship between exposure to a drug and adverse event, duplicate reports and inherent

incompleteness of the database [248]. Ursem et al. [49] grouped adverse effects reported to FDA's previously established Spontaneous Reporting System (SRS) and the AERS into a renal disorder cluster, a nephropathies cluster, a kidney function tests cluster and a bladder disorder cluster and through a weight of evidence approach identified substances most likely to induce these cluster endpoints.

When accessing EudraVigilance, the European database of suspected adverse drug reaction reports [249, http://www.adrreports.eu/], the number of individual cases of adverse events following the administration of a drug can be queried . Searches are performed for drug products or substances, and occurrences on "Renal and urinary disorders" per age group may be retrieved on the section "Number of Individual Cases by Reaction Groups". Displayed graphs clearly show whether renal and urinary disorders are predominant adverse events for a particular drug but no information are immediately evident on comorbidities of the patient population and exact doses administered.

3.6 Relevance of existing sources to *in silico* modelling

Section 3 demonstrates considerable resources for *in silico* modelling of nephrotoxicity. However, no comprehensive database exists with multi-scale information that can be used for *in silico* nephrotoxicity model development. Databases focussing on kidney tissue data (predominantly in the omics field) are principally geared towards the diagnostics, understanding pathological pathways and treatment of kidney disease as opposed to chemical induced kidney toxicity. In order to establish reliable dose-response relationships, *in vivo* data are highly desirable. The AOP paradigm and means of organising information provides a possible structure to multi-scale models. Table 2 summarises which type of data informs the various parts of an AOP-structured multi-scale *in silico* model. The use of (novel and existing) data sources to support *in silico* modelling is taken up in more detail in Section 5 (Expert Opinion).

Table 2: Data informing all stages of a multi-scale *in silico* model

Level	Mechanism	Cellular	Apical toxicity	Human toxicity
Examples of data and what they may be used for	Molecular biology (-omics) data on MIEs or, if unavailable, other molecular mechanisms	*In vitro* data on receptor binding, cellular toxicity, cellular uptake, enzymatic activity	*In vivo* data on mechanistic pathways and their association with biomarkers	Clinical trials / post-marketing data on human-specific effects, dose-response-relationships,

				sensitivity/ specificity of biomarkers
Comparative databases	RGED, Nephroseq™, HKUPP, UPdb, KUPKB, CTD	ToxCast™, HSDB®, UCSF-FDA TransPortal	ToxRefDB, COSMOS DB, RepDose, UPB	HSDB®, ClinicalTrials.gov, FAERS, EudraVigilance

4.0 (Q)SARs AND MATHEMATICAL MODELS TO SIMULATE KINETICS AND TOXICITY: CURRENT STATE-OF-THE-ART AND NEXT STEPS

As noted above, as part of the hazard identification process it is important to be able to predict accurately human nephrotoxicity. The traditional approach for determining safety and toxicity of drug candidates is through histopathological observation from *in vivo* animal studies [42,250–252] or, more recently, from targeted *in vitro* testing. However, in recent decades, alternative methods for hazard assessment without the need for testing, such as *in silico* approaches, have been increasingly applied, particularly for the prioritisation of data requirements and identification of chemicals that may require more detailed risk assessment. Computational toxicology also allows for the possibility to link molecular pathways to cellular processes and a toxicity endpoint at the tissue level.

In this review, two fundamentally different *in silico* toxicology methods are discussed, i.e. chemistry driven (Q)SARs and physiologically-based mathematical models. The former identify relationships between a structure of a molecule and its toxicity while the latter simulate the physiologically-based toxicokinetics of a compound which allows predictions of whether (and which) effects may be elicited.

4.1 (Q)SAR models

In silico methods include chemical structure driven SARs and physico-chemical property and molecular descriptor driven QSARs to predict and profile toxicities [253]. These provide a correlation between an effect, often a regulatory endpoint, and properties of a molecule. SARs are often developed into SAs which relate qualitatively a particular biological effect or toxicity endpoint to a specific fragment of a molecule [253,254]. SAs have been developed to aid identification of

chemicals that can bind to proteins [255,256] or induce mitochondrial toxicity [257]. These chemical-biological interactions have been identified as potential mechanisms of eliciting renal (and other) toxicities. SAs associated with potential toxicity have been compiled and encoded into predictive software, for example ToxAlerts – a freely available screening tool available within the online chemical modelling environment (https://ochem.eu/home/show.do) and the alerts incorporated in DEREK Nexus (Lhasa Ltd, Leeds). For certain compounds, toxicity may be elicited via the formation of reactive metabolites, rather than inherent toxicity of the parent molecule. Claesson and Minidis [258] have collated and organised publicly available SAs that may be associated with reactive metabolite formation and idiosyncratic adverse drug reactions. QSARs provide a statistical relationship between the structure of a chemical, its physico-chemical properties and its effects [253]. QSAR models have demonstrated good predictive ability, especially for simple end points [250,259].

The primary objective of (Q)SARs is to distinguish between toxicologically inactive or active compounds and, where possible, provide a quantitative estimate of potency or relative effect. Frequently, several mechanisms elicit the same toxicological endpoint. Thus, predictive models must be able to distinguish all fragments corresponding to all relevant mechanisms from inactive fragments. Two main types of commercial systems have been developed: knowledge-based systems (e.g, DEREK Nexus and OncoLogic) and statistically-based systems (e.g. TOPKAT and MultiCASE) [260] although there is a trend for hybrid systems which may link a quantitative estimate to an SA e.g. ChemTunes [261, https://www.mn-am.com/products/chemtunes]. Knowledge-based systems use rules derived from human expert opinion and interpretation of toxicology data to define the relationship between a structure and its activity. These rules are utilised to predict potential toxicity of known and novel chemical compounds. Statistically-based systems use calculated parameters and statistical methods to derive mathematical relationships for a training set of compounds [260].

(Q)SAR models developed so far for nephrotoxicity, specifically, are summarised in Table 3 with details on the exact endpoint, number and type of molecules the model is based on, the method used, results, as well as strengths and weaknesses of the approach below.

Table 3: Summary of (Q)SAR models associated with kidney and bladder toxicity

Endpoint	Number and type of molecules	Method	Results: QSAR / SAR	Strengths	Weaknesses	Model reference
Rat nephrotoxicity	16 derivatives or 1,2- and 1,4-naphthoquinones	SA	Whilst preliminary SAs were presented, no definitive SAs were defined	Changes in the extent of nephrotoxicity due to structural alterations of 2-hydroxy-1,4-naphthoquinone and 4-amino-1,2,-naphthoquinone were determined	No SAs identified; mechanism of toxicity was not determined, small applicability domain	Munday et al. [262]
Toxicity to the kidney and urinary tract based on repeat-dose toxicity study-derived NOAELs and LOAELs in rats	503 chemicals	SAs based on likelihood ratio and percentage of true positives	6 SAs	Mechanistic information available for some SAs based on literature	Mode of action data is generally basic and not available for all SAs	Pizzo et al. [263]
Nephrotoxicity	Confidential database amalgamated from multiple industries	Knowledge-based expert system using SARs	SAs	SAs are in some cases associated with mechanistic information	Certain substances, e.g. inorganic compounds, cannot be analysed	Derek Nexus [264]
"Nephrotoxicity" (NT) and subcategory endpoints "kidney necrosis" (KN), "kidney relative weight gain" (KWG) and "nephron injury" (NI)	Training set: NT: 847; KN: 221; KWG: 240; NI: 598; Test set: NT: 154; KN: 42; KWG: 49; NI: 109	QSAR models and toxicophores	192 SAs for all kidney-related endpoints	QSAR models demonstrate good performance overall; QSARs and toxicophores were based on the same compound sets; comparatively broad applicability domain	Endpoints "kidney weight gain", "nephron injury" and "nephrotoxicity" cannot be specifically attributed to a nephrotoxicity mechanism	Myshkin et al. [265]
Competency of rat liver MGST1 to catalyse	9 haloalkenes; as no or low GSH	QSAR model	Linear relationship between E_{LUMO} values between -1.14	Findings may be valuable for understanding and predicting the	Small applicability domain; linear relationship does not exist for	Jolivette and

bioactivation of molecules to toxic metabolites	transferase activity was detected for 4 molecules, QSAR was investigated for 5 haloalkenes		to -0.73 eV and the natural logarithms of activities for GSH conjugation reaction	route of metabolism of haloalkenes and their associated toxicities; haloalkenes are widely used, so results may help to make chemical design amendments for many applications	ELUMO values outside of -1.14 to -0.73 eV range	Anders [266]
α2μ-globulin nephropathy in male rats	Not specifically known, dataset includes 43 43 aliphatic and alicyclic hydrocarbon structures	Combination of two QSARs based on multiple regression analysis and principal component analysis	Incorporating electro-negativity properties, size and shape dependent fit of binding site: log IC$_{50}$ = 4.525 (-ve charge density) - 0.044 (Mol Vol) = 2.545; r$_2$ = 0.836; cross-validated r$_2$ = 0.601	To identify whether kidney lesions observed in *in vivo* studies in male rats may be caused by this pathway	Mechanism specific to male rats, not relevant to humans; applicability domain was not discussed	Barratt [99]
Tubular necrosis, interstitial nephritis and tubulo-interstitial nephritis in humans	Parent compounds (251 nephrotoxicants and 387 non-nephrotoxicants) and their urinary metabolites (307 nephrotoxicants and 233 non-nephrotoxicants)	Binary classification QSAR models	Eight substructure fragments common to both datasets for all of the above three nephrotoxicity mechanisms	Metabolism-dependent toxicity; may help to understand to which extent and how the three mechanisms are interlinked; consideration of metabolites; based on human data	Model could not be accessed; data on molecules were not published	Lee et al. [252]
"Nephropathies", "acute renal disorders", "bladder disorders",	Not specifically known	QSAR models	Four QSAR programmes were used, i.e. MC4PC, BioEpisteme, MDL QSAR	Based on human data and four QSAR programmes with different prediction programmes; high	Non-uniform reporting of adverse events post marketing approval; patient population with multi-drug	Matthews et al. [267]

"blood in urine", "urolithiases" and "kidney function tests" in humans			and Leadscope Predictive Data Miner	applicability domain among pharmaceuticals	exposure and co-morbidities; certain drugs (inorganic chemicals, high molecular weight substances (>5000 Da), organometallic chemicals, gases) and industrial chemicals not covered; while specificity was set at > 80 %, sensitivity was in some cases very low (< 20 %)	
Pathways associated with renal tubular degeneration based on rat kidney data	88 chemicals, including 22 molecules inducing renal tubular degeneration	QSAR linking physico-chemical features to transcriptional activity	Nephrotoxic substances are associated with high polarisability, low electronegativity, and low symmetry	Multi-scale modelling linking chemical data to gene expression and a nephropathological outcome	Focus on agents which directly act on proximal tubular cells; applicability domain is unclear	Antczak et al. [268]
Renal adverse drug reactions	507 drugs (126 active, 208 inactive, 173 of undetermined activity)	QSAR based on decision tree inference analysis using CART and CHAID	CART model highlights influence of amine functions, sulphur, and carboaromatic ring structures. For substances less toxic to the kidney, CHAID model found few aromatic atoms (<19), a basic pK_a <10.71, van der Waals surface area <1,014.5 Å^2, and logP values >2.43	Both models performed well, with CART and CHAID model CCRs of 88.6 and 84.7%, respectively; based on human data	Only valid for drug-like molecules, not necessarily suitable for other compounds	Hammann et al. [269]
Urinary tract toxicity (LD_{50} in kidney, ureter,	258 organic compounds	Classification and regression QSAR models based on	SVMBoost based on the RBF kernel accomplishes the best quantitative and	Reliable prediction is achieved by both regression and classification	Adequacy of mouse intraperitoneal LD_{50} values as endpoint is unclear	Lei et al. [270]

| and bladder) in mice | | eight machine learning approaches | qualitative predictions for the test set (MCC of 0.787, AUC of 0.893, sensitivity of 89.6%, specificity of 94.1%, and global accuracy of 90.8%) | models based on the SVMBoost approach; all tested chemicals are within the application domain coverage | | |

Abbreviations: CART = classification and regression tree; CHAID = chi-squared automatic interaction detector; CCRs = corrected classification rates; LD_{50} = dose which is lethal to half of total treated animals

More detailed information on these QSAR models are provided in Appendix C [87,99,252,262–276] of the supplementary information. It is also noted that QSAR models have been developed to predict renal clearance, which were examined in more detail elsewhere [56].

4.2 Mathematical (mechanism-based) models

In contrast to (Q)SAR methods, mathematical mechanistic and physiologically-based models can be used to simulate the kinetics of a compound through the body and at the site of toxicity. As a vast number and quantity of substances are moving through the kidney, and considering the key principle of toxicology – the dose makes the poison (Paracelsus) – an understanding of a compound's movement and its potential for accumulation at specific sections of the kidney are considered critical. In section 2, accumulation is described to play an important role in certain nephrotoxicity pathways.

Some of the early mechanistic models to predict renal clearance include passive reabsorption and urine flow [277], which are supplemented by protein binding and glomerular filtration [278–281] and active secretion [282–285]. Subsequently, Felmlee et al. [286] develop a hybrid physiological, mechanistic toxicokinetic (TK) model to simulate the saturable renal reabsorption and capacity-limited metabolic clearance of γ-hydroxybutyric acid (GHB) with two ultrafiltrate compartments representing the proximal and distal tubules, and active renal reabsorption from the first ultrafiltrate compartment only. Two (fast and slow) tissue distribution compartments best described plasma GHB concentration. As in earlier models, urine flow and the glomerular filtration rate (GFR) are included, while passive reabsorption, active secretion and protein binding are not incorporated, as they do not seem to play a vital role in the renal elimination of GHB.

Felmlee et al. [287] extended their work by adding active tubular secretion to their investigations into active tubular reabsorption. The authors evaluate previously published compartmental and semi-physiologically based models pharmacokinetic (PK) models of active tubular reabsorption and secretion. By merging some of these approaches, they establish a universal mechanistic model predicting renal clearance of substances being subject to active secretion, active reabsorption or both of these processes, for a broad applicability domain. Metabolism and passive reabsorption are not considered in this study.

Independent of models to predict the kinetics of therapeutic or other chemical compounds, Layton [288] review mathematical models to describe physiological and pathophysiological processes of the kidney. These processes include the regulation of glomerular filtration and renal blood flow by the tubulo-glomerular feedback and myogenic mechanism, epithelial and renal oxygen transport, and the urine concentrating mechanism. A mechanistic understanding of intrarenal oxygen transport and consumption may be a valuable component to add to a toxicokinetics related kidney model as renal tissue hypoxia has been argued to drive kidney disease [289,290].

One of the most detailed mechanistic kidney models is Mech KiM which predicts renal elimination by accounting for glomerular filtration, active and passive reabsorption, active and passive secretion, renal metabolism, bypass of parts of the renal blood flow, transporter scaling factors and population variability [56]. The nephron is divided into eight segments representing the glomerulus, proximal and distal tubules, Loop of Henle and collecting ducts. Each segment encompasses three compartments, illustrating the blood space, tubular fluid and cellular mass. While applying the law of conservation of mass, ordinary differential equations describe the movement of a compound between compartments. Limitations of this model revolve around missing data, e.g. on proximal tubular cells per gram of kidney (PTCPGK) and absolute renal transporter abundances at different parts of the nephron.

Overall, there are limited computational toxicity methods available for more complex endpoints such as nephrotoxicity – likely to be due to the highly complex mechanisms of toxicity [250,252,262] or limitations of the availability of structured, high quality data. Most models presented above focus only on very small groups of compounds, meaning that the applicability domain is limited to the specific groups of compounds used to develop the model. Some models, i.e. Lee et al. [252], Myshkin et al. [265], Matthews et al. [267] and DEREK, have been generated on the basis of larger datasets or commercial software which are not publicly available. Mechanistic models to compute the biokinetics of compounds have limitations due to missing data and are in part not publically available either. To date, there is no multi-scale nephrotoxicity model available which may be due to the lack of high quality data connecting molecular and cellular mechanisms to tissue and organ processes leading to an adverse individual outcome while understanding uncertainties related to inter-individual variability. In the following section, knowledge of the main mechanisms of nephrotoxicity as well as missing information vital to modelling purposes will be summarised.

5.0 <u>EXPERT OPINION</u>

As a major organ of elimination and therefore subject to high exposure of compounds, the kidney has been recognised as a significant target for drug and chemical induced toxicity. This, and the lack of comprehensive test data for many chemicals in sectors other than pharma, clearly show that screening for, and assessment of, nephrotoxicity is an important area for improvement. *In silico* methods have been evolving steadily and have the potential to contribute immensely to the field of toxicology in general. Although several *in silico* models currently exist for other organ-level toxicities e.g. hepatotoxicity, this review has found that *in silico* models associated with nephrotoxicity are very limited, and even fewer differentiate between key mechanisms or incorporate mechanistic data.

In order to improve and expedite the development of *in silico* models for kidney toxicity, at least three – highly interrelated - problems have to be overcome. These are:

i)   The identification and definition of effects to the kidney that may be brought about by chemical exposure.

ii)   A full description of relevant mechanisms of toxic action relevant to kidney toxicity.

iii)   Access to appropriate data ranging from *in vivo* through to molecular responses.

The identification of effects to the kidney requires an ontology to be developed that will unify existing knowledge. This may grow out of networked AOPs although would benefit from a systematic evaluation of current knowledge and effects. Once an ontology has been developed it would be the ideal starting point for a framework to underpin data and models. With regard to the data from which to develop the models, no assay exists which is specifically targeted towards renal toxicity endpoints and interspecies variability between rats and humans is known to be relevant for certain pathways (i.e. certain transporter-driven and α2μ-globulin related nephrotoxicity). Therefore, the usefulness of *in vivo* data in the area needs to be assessed carefully. Human and *in vitro* assay data are widely available but need to be utilised with care due to challenges related to relevance for one of the discussed nephrotoxicity mechanisms, comparability of studies, pre-existing comorbidities in patient populations and other factors related to data interpretation. A strategic for utilising existing data at different levels of AOPs is shown in Table 2. Such an approach to organising information, as defined within an appropriate ontology, may prove to be an extremely effective, and not requiring full testing of every compound, solution to model development. In addition, as the lack

of readily available data is considered to be a key limiting factor when it comes to the generation of future computational models in this field, therefore the following recommendations may help to drive future modelling efforts forward:

(i) Improved understanding of how novel, recently proposed, biomarkers relate to the mechanisms of nephrotoxicity, discussed earlier, and how they are related quantitatively to each other. This may result in alternative sources of data and could be facilitated by statistical approaches.

(ii) A global review of the quality of currently available kidney toxicity data is needed as well as an assessment of how these data relate to each other (e.g. cellular vs. tissue vs. organ-level effects). Information would be leveraged more readily if databases allowed for searches on both compounds and mechanistic data (including dosing information) enabling discrimination between the various nephrotoxicity endpoints.

(iii) Generation of more AOPs for nephrotoxicity with MIE and KE related data being searchable in a central database linked to respective mechanistic toxicological data would also assist the development of more computational models.

The linkages between *in silico* models have been defined clearly, with data related to the MIE being able to contribute to the development of a model [10]. While a binary interpretation of an MIE may be used to understand whether a substance has the potential to elicit such a molecular event, a more quantitative understanding of MIEs and KEs may also be achieved with predictive models [291]. For this, potency measurements, i.e. dose response relationships, of substances inducing MIEs and biological events further down the pathway are necessary in order to establish the relationship between these biological events [291]. Once more nephrotoxicity-related AOPs have been developed, an AOP-derived *in silico* framework would provide the robustness needed for a model to reliably inform screening of new drug candidates, and efforts to prioritise substances for further testing.

With regard to developing better *in silic*o modelling frameworks it is the development of SAs which is the logical starting point. These need to be refined and extended in order to facilitate the prediction of the hazard potential of a more comprehensive array of compounds. For instance, much existing knowledge on SAs has been compiled [292]. The existing knowledge needs to be rationalised such that a robust set of SAs can be established. Such an "*in silico* profiler" will assist in the designing-out of toxicity as well as grouping, allowing for read-across, especially to estimate the chronic toxicity of data-poor substances. A potentially rich source of information to develop further structural information are the data resultant from ToxCast. It has been shown that fingerprints using

available ToxCast data on kidney tissue cell lines may be developed [293]. Such fingerprints could consist of a defined number of *in vitro* assays reflecting the toxic mechanism of a specific group of known nephrotoxicants. If a new chemical is shown to generate hits according to one of the defined fingerprints, the likelihood of the chemical to cause nephrotoxic effects is considered to be high.

Of the *in silico* models developed so far, there is always a place for QSARs, but only where suitable data allow. These may be based, for instance, around *in vitro* data for specific effects as opposed to the more ambitious modelling of whole organism toxicity. To facilitate better QSARs, besides incorporating data related to the toxicodynamics of a compound in the kidney, accounting for toxicokinetics and the potential of a substance to accumulate at a specific site within the kidney is considered to be equally important as an adverse effect may be caused due to supersaturation of parts of renal system. In vitro analyses indicate that many chemicals elicit toxicity via unspecific cytotoxicity [223,225,294]. Therefore, a mechanistic model simulating the toxicokinetics of compounds in the different parts of a nephron would help to understand whether a substance may be accumulating or not. Overall, it is clear that experimental and computational efforts have to go hand in hand, along with development of mechanistic knowledge, to achieve much-needed progress in this area.

Methods:

The scientific literature available via Google Scholar, ScienceDirect, PubMed, Web of Science and Scopus was reviewed for nephrotoxic substances, information related to mechanisms of nephrotoxicity and existing computational models to predict nephrotoxicity. Nephrotoxicants and mechanistic data were identified by searching the terms "drug-induced nephrotoxicity" OR "drug-induced kidney toxicity" OR "drug-induced kidney injury" OR "drug-induced kidney damage" OR "drug-induced urinary tract toxicity", and "chemical-induced nephrotoxicity" OR "chemical-induced kidney toxicity" OR "chemical-induced kidney injury" OR "chemical-induced kidney damage" OR "chemical-induced urinary tract toxicity". Nephrotoxicants were also identified from the combined COSMOS and Munro dataset and the Toxicity Reference Database (ToxRefDB). The combined COSMOS and Munro dataset was exported from the COSMOS Database [118, https://cosmosdb.eu/cosmosdb.v2/accounts/login/?next=/cosmosdb.v2/ > Computational Methods > TTC Export], and only molecules for which "kidney" or "urinalysis parameters" are recorded first as critical effects were added to the list of nephrotoxicants. Besides information on critical effects stated in the combined COSMOS and Munro dataset, the species and, if available, kidney-related critical effect details were added. The ToxRefDB, which contains lowest effect levels (LELs) of over 800 molecules for over 1'000 endpoints (e.g.

SUB_rat_SystemicCarcinogenic_adult_Urinary_UrinaryBladder, where SUB stands for subchronic), was downloaded from the U.S. EPA Toxicity ForeCaster (ToxCast[TM]) Data website [126, https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data]. A compound was added to the nephrotoxicants' list if its lowest LEL was only associated with kidney or urinary bladder related endpoints. SMILES codes of identified nephrotoxicants were sourced from Drugbank [195,196, https://www.drugbank.ca/], PubChem [199,200, https://pubchem.ncbi.nlm.nih.gov/search/], and ChemID*plus* [189, https://chem.nlm.nih.gov/chemidplus/]. *In silico* models were queried by using the terms ''structural alerts'' OR "structural fragments" OR "*in silico*" OR "QSAR" OR "mechanistic model" OR "mathematical model" AND ''nephrotoxicity'' OR "kidney toxicity" OR "kidney injury" OR "kidney damage" OR ''urinary tract toxicity''.

Appendix A: Kidney assay data available in the ToxCast™ Dashboard

AssayComponentEndpointName	BiologicalProcessTarget	AssayFunctionType	GeneName	IntendedTargetFamily	Organism
NVS_ENZ_pMTHFR	regulation of catalytic activity	enzymatic activity	methylenetetrahydrofolate reductase (NAD(P)H)	oxidoreductase	pig
NVS_ENZ_pMTHFR_Activator	regulation of catalytic activity	enzymatic activity	methylenetetrahydrofolate reductase (NAD(P)H)	oxidoreductase	pig
NVS_MP_rPBR	receptor binding	binding	translocator protein	transporter	rat
OT_AR_ARSRC1_0480	protein stabilization	binding	androgen receptor	nuclear receptor	human
OT_AR_ARSRC1_0960	protein stabilization	binding	androgen receptor	nuclear receptor	human
OT_ER_ERaERa_0480	protein stabilization	binding	estrogen receptor 1	nuclear receptor	human
OT_ER_ERaERa_1440	protein stabilization	binding	estrogen receptor 1	nuclear receptor	human
OT_ER_ERaERb_0480	protein stabilization	binding	estrogen receptor 1	nuclear receptor	human
OT_ER_ERaERb_1440	protein stabilization	binding	estrogen receptor 1	nuclear receptor	human
OT_ER_ERbERb_0480	protein stabilization	binding	estrogen receptor 2 (ER beta)	nuclear receptor	human

OT_ER_ERbERb_1440	protein stabilization	binding	estrogen receptor 2 (ER beta)	nuclear receptor	human
OT_FXR_FXRSRC1_0480	protein stabilization	binding	nuclear receptor subfamily 1, group H, member 4	nuclear receptor	human
OT_FXR_FXRSRC1_1440	protein stabilization	binding	nuclear receptor subfamily 1, group H, member 4	nuclear receptor	human
OT_NURR1_NURR1RXRa_0480	protein stabilization	binding	retinoid X receptor, alpha	nuclear receptor	human
OT_NURR1_NURR1RXRa_1440	protein stabilization	binding	retinoid X receptor, alpha	nuclear receptor	human
OT_PPARg_PPARgSRC1_0480	protein stabilization	binding	peroxisome proliferator-activated receptor gamma	nuclear receptor	human
OT_PPARg_PPARgSRC1_1440	protein stabilization	binding	peroxisome proliferator-activated receptor gamma	nuclear receptor	human
TOX21_AR_BLA_Agonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_AR_BLA_Agonist_ch2	regulation of transcription factor activity	reporter gene	null	background measurement	human
TOX21_AR_BLA_Agonist_ratio	regulation of transcription factor activity	reporter gene	androgen receptor	nuclear receptor	human
TOX21_AR_BLA_Antagonist_ratio	regulation of transcription factor activity	reporter gene	androgen receptor	nuclear receptor	human

TOX21_AR_BLA_Antagonist_viability	cell proliferation	viability	null	cell cycle	human
TOX21_AutoFluor_HEK293_Cell_blue	NA	background control	null	background measurement	human
TOX21_AutoFluor_HEK293_Cell_green	NA	background control	null	background measurement	human
TOX21_AutoFluor_HEK293_Cell_red	NA	background control	null	background measurement	human
TOX21_AutoFluor_HEK293_Media_blue	NA	background control	null	background measurement	human
TOX21_AutoFluor_HEK293_Media_green	NA	background control	null	background measurement	human
TOX21_AutoFluor_HEK293_Media_red	NA	background control	null	background measurement	human
TOX21_ELG1_LUC_Agonist	regulation of transcription factor activity	reporter gene	ATPase family, AAA domain containing 5	hydrolase	human
TOX21_ERa_BLA_Agonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_ERa_BLA_Agonist_ch2	regulation of transcription factor activity	reporter gene	null	background measurement	human
TOX21_ERa_BLA_Agonist_ratio	regulation of transcription factor activity	reporter gene	estrogen receptor 1	nuclear receptor	human
TOX21_ERa_BLA_Antagonist_ratio	regulation of transcription factor activity	reporter gene	estrogen receptor 1	nuclear receptor	human

TOX21_ERa_BLA_Antagonist_viability	cell proliferation	viability	null	cell cycle	human
TOX21_PPARg_BLA_Agonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_PPARg_BLA_Agonist_ch2	regulation of transcription factor activity	reporter gene	null	background measurement	human
TOX21_PPARg_BLA_Agonist_ratio	regulation of transcription factor activity	reporter gene	peroxisome proliferator-activated receptor gamma	nuclear receptor	human
TOX21_FXR_BLA_agonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_FXR_BLA_agonist_ch2	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_FXR_BLA_agonist_ratio	regulation of transcription factor activity	reporter gene	nuclear receptor subfamily 1, group H, member 4	nuclear receptor	human
TOX21_FXR_BLA_antagonist_ratio	regulation of transcription factor activity	reporter gene	nuclear receptor subfamily 1, group H, member 4	nuclear receptor	human
TOX21_FXR_BLA_antagonist_viability	cell proliferation	viability	null	cell cycle	human
TOX21_PPARd_BLA_agonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human

TOX21_PPARd_BLA_agonist_ch2	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_PPARd_BLA_agonist_ratio	regulation of transcription factor activity	reporter gene	peroxisome proliferator-activated receptor delta	nuclear receptor	human
TOX21_PPARd_BLA_antagonist_ratio	regulation of transcription factor activity	reporter gene	peroxisome proliferator-activated receptor delta	nuclear receptor	human
TOX21_PPARd_BLA_antagonist_viability	cell proliferation	viability	null	cell cycle	human
TOX21_PPARg_BLA_antagonist_ratio	regulation of transcription factor activity	reporter gene	peroxisome proliferator-activated receptor gamma	nuclear receptor	human
TOX21_PPARg_BLA_antagonist_viability	cell proliferation	viability	null	cell cycle	human
TOX21_VDR_BLA_agonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_VDR_BLA_agonist_ch2	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_VDR_BLA_agonist_ratio	regulation of transcription factor activity	reporter gene	cytochrome P450, family 24, subfamily A, polypeptide 1	cyp	human
TOX21_VDR_BLA_antagonist_ratio	regulation of transcription factor activity	reporter gene	cytochrome P450, family 24, subfamily A, polypeptide 1	cyp	human

TOX21_VDR_BLA_antagonist_viability	cell proliferation	viability	null	cell cycle	human
TOX21_FXR_BLA_agonist_viability	cell proliferation	viability	null	cell cycle	human
TOX21_ERa_BLA_Antagonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_ERa_BLA_Antagonist_ch2	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_FXR_BLA_Antagonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_FXR_BLA_Antagonist_ch2	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_PPARd_BLA_Agonist_viability	cell proliferation	viability	null	cell cycle	human
TOX21_PPARd_BLA_Antagonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_PPARd_BLA_Antagonist_ch2	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_PPARg_BLA_Antagonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_PPARg_BLA_Antagonist_ch2	regulation of transcription	background control	null	background measurement	human

	factor activity				
TOX21_VDR_BLA_Antagonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_VDR_BLA_Antagonist_ch2	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_AR_BLA_Antagonist_ch1	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_AR_BLA_Antagonist_ch2	regulation of transcription factor activity	background control	null	background measurement	human
TOX21_VDR_BLA_Agonist_viability	cell proliferation	viability	null	cell cycle	human
NCCT_HEK293T_CellTiterGLO	cytotoxicity	viability	null	cell cycle	human

For additional information on assays, please reference the ToxCast^{TM} dashboard.

Appendix B: Kidney and urinary bladder related toxicity endpoints available in ToxRefDB

SUB_dog_SystemicCarcinogenic_adult_PathologyNonProliferative_Urinary_Kidney
CHR_dog_SystemicCarcinogenic_adult_PathologyNonProliferative_Urinary_Kidney
CHR_mouse_SystemicCarcinogenic_adult_PathologyNonProliferative_Urinary_Kidney
CHR_mouse_SystemicCarcinogenic_adult_PathologyProliferative_Urinary_Kidney
CHR_mouse_SystemicCarcinogenic_adult_PathologyNeoplastic_Urinary_Kidney
CHR_mouse_SystemicCarcinogenic_adult_PathologyGross_Urinary_Kidney
CHR_rat_SystemicCarcinogenic_adult_PathologyNonProliferative_Urinary_Kidney
CHR_rat_SystemicCarcinogenic_adult_PathologyProliferative_Urinary_Kidney
CHR_rat_SystemicCarcinogenic_adult_PathologyGross_Urinary_Kidney
CHR_rat_SystemicCarcinogenic_adult_PathologyNeoplastic_Urinary_Kidney
SUB_rat_SystemicCarcinogenic_adult_PathologyNonProliferative_Urinary_Kidney
SUB_rat_SystemicCarcinogenic_adult_PathologyGross_Urinary_Kidney
SUB_rat_SystemicCarcinogenic_adult_PathologyProliferative_Urinary_Kidney
DEV_rat_SystemicCarcinogenic_adult_PathologyGross_Urinary_Kidney
DEV_rabbit_SystemicCarcinogenic_adult_PathologyGross_Urinary_Kidney
MGR_rat_SystemicCarcinogenic_adult_PathologyNonProliferative_Urinary_Kidney
MGR_rat_SystemicCarcinogenic_adult_PathologyProliferative_Urinary_Kidney
MGR_rat_SystemicCarcinogenic_adult_PathologyGross_Urinary_Kidney
SUB_mouse_SystemicCarcinogenic_adult_PathologyNonProliferative_Urinary_Kidney
SUB_mouse_SystemicCarcinogenic_adult_PathologyProliferative_Urinary_Kidney
SUB_dog_SystemicCarcinogenic_adult_Urinary_Kidney
CHR_dog_SystemicCarcinogenic_adult_Urinary_Kidney
CHR_mouse_SystemicCarcinogenic_adult_Urinary_Kidney
CHR_rat_SystemicCarcinogenic_adult_Urinary_Kidney
SUB_rat_SystemicCarcinogenic_adult_Urinary_Kidney
DEV_rat_SystemicCarcinogenic_adult_Urinary_Kidney
DEV_rabbit_SystemicCarcinogenic_adult_Urinary_Kidney
MGR_rat_SystemicCarcinogenic_adult_Urinary_Kidney
MGR_rat_SystemicCarcinogenic_juvenile_Urinary_Kidney

SUB_mouse_SystemicCarcinogenic_adult_Urinary_Kidney
CHR_mouse_SystemicCarcinogenic_adult_PathologyNonProliferative_Urinary_UrinaryBladder
CHR_mouse_SystemicCarcinogenic_adult_PathologyProliferative_Urinary_UrinaryBladder
CHR_rat_SystemicCarcinogenic_adult_PathologyNonProliferative_Urinary_UrinaryBladder
CHR_rat_SystemicCarcinogenic_adult_PathologyProliferative_Urinary_UrinaryBladder
CHR_rat_SystemicCarcinogenic_adult_PathologyNeoplastic_Urinary_UrinaryBladder
SUB_rat_SystemicCarcinogenic_adult_PathologyProliferative_Urinary_UrinaryBladder
SUB_rat_SystemicCarcinogenic_adult_PathologyNonProliferative_Urinary_UrinaryBladder
SUB_rat_SystemicCarcinogenic_adult_PathologyGross_Urinary_UrinaryBladder
CHR_dog_SystemicCarcinogenic_adult_Urinary_UrinaryBladder
CHR_mouse_SystemicCarcinogenic_adult_Urinary_UrinaryBladder
CHR_rat_SystemicCarcinogenic_adult_Urinary_UrinaryBladder
SUB_rat_SystemicCarcinogenic_adult_Urinary_UrinaryBladder
SUB_mouse_SystemicCarcinogenic_adult_Urinary_UrinaryBladder

CHR=chronic/cancer; MGR=multigenerational reproductive; DEV=prenatal developmental; SUB=subchronic;

Appendix C: Description of (Q)SAR models associated with kidney and bladder toxicity

C.1 Munday et al. [262]

Munday et al. [262] aimed to derive structural alerts for rat nephrotoxicity from 16 1,2- and 1,4-naphthoquinones. Some naphthoquinones, e.g. 2-hydroxy-1,4-naphthoquinone and 2-amino-1,4-naphthoquinone, had been found to be nephrotoxic, mainly causing renal tubular necrosis in rats associated with presence of casts in the tubules [262,271,272]. 2-hydroxy-1,4-naphthoquinone, the active component in henna, produced fatal renal tubular necrosis in a child who was cutaneously treated with henna [273]. The mechanism of renal tubular necrosis induced by the 1,4-naphthoquinones was, and still appears to be, unknown. Munday et al. [262,272] hypothesised that nephrotoxicity was a result of tautomerism of hydroxyl or amino 1,4-quinolones to strongly reactive 1,2-naphthoquinones or 1,2-naphthoquinoneimines. However, the results of the study suggest that this hypothesis is wrong. The results did not allow for the definitive identification of structural features associated with naphthoquinone-induced nephrotoxicity or the conclusive determination of

the mechanism. However, this study confirmed that 2-hydroxy-1,4-naphthoquinone and 4-amino-1,2,-naphthoquinone were extremely toxic to the kidney. Several alterations in structure of the above compounds caused the following changes in their toxic effect to the kidney: methylation of the amino group substantially increased nephrotoxicity, whilst methylation of the hydroxyl group, arylation of the amino-group and substitution with a chloro or amino group in the 3-position of the quinone ring eliminated nephrotoxicity. It was also found that substitution with small substituents i.e. methyl or ethyl decreases the extent of renal tubular damage – the larger the size of alkyl group, the greater the reduction of renal damage.

C.2 Pizzo et al. [263]

Pizzo et al. [263] conducted a study to evaluate, identify and group substructures into six structural alerts recognised as being toxic to the kidney and / or urinary tract using repeated-dose toxicity studies on rats taken from the Hazard Evaluation Support System (HESS) database. SAs were selected based on their likelihood ratio (LR) and percentage of true positives. These alerts were encoded as SMARTS (SMiles ARbitrary Target Specification). A plausible mechanistic explanation for these substructures to cause nephrotoxicity was only provided for the 2nd, 3rd, 5th and 6th SAs.

The 2nd SA identified is sulphanilamide, which belongs to the class of sulphonamides. According to the literature, sulphonamides can cause obstructive nephropathy as they are insoluble in acidic urine, which causes them to precipitate as crystals in the tubules [87]. SA 3 is found in benzonitriles which have been reported to be harmful to the kidney due to adverse effects leading to cytotoxicity in the human embryonic renal cell line HEK293T [274]. SA 5 is chloroform, which is also reported as a structural alert in other studies, including Myshkin et al. [265]. Chloroform is believed to cause nephrotoxicity via metabolism by P450 enzymes into toxic metabolites which may induce renal cancer through cytotoxicity eliciting regenerative cell proliferation [274]. SA 6 is biphenyl; according to Pizzo et al. [263], biphenyl was identified seven times in the dataset of 89 active kidney toxicants, out of which five cases were found in nephrotoxic molecules. Biphenyls cause haematuria, increased urinary pH, formation of calculi inducing urinary tract tumours [263,275]. According to ChemDraw Professional (version:16.0.1.4 (77)), SA 1 and 4 are benzenesulfonic acid and hepta-1,5-diene, respectively. Most of the above alerts were reproduced in the study by Myshkin et al. [265].

C.3 Derek Nexus

Derek Nexus is a knowledge-based expert system that uses SAs to provide *in silico* prediction of toxicity [264]. As well as the key mechanisms mentioned earlier, Derek Nexus may identify

compounds eliciting nephrotoxicity via additional mechanisms. Examples of these mechanisms include: necrosis and fibrosis of the renal medullary interstitium; drug-induced ureteral obstruction (when drug causes blockage in one or both of the ureters leading from kidneys to the bladder) and drug-induced formation of cholesterol emboli. Also, Derek Nexus EREK may trigger a warning for α2μ-globulin nephropathy. Any structural alerts are identified in the original structure as highlighted toxicophores. A list of key literature references is provided, along with, in some cases, a proposed mechanism.

C.4 Myshkin et al. [265]

Myshkin et al. [265] described the construction and validation of QSAR models based on a database of organ-level toxicity and the identification of toxicophores. QSAR models were generated to predict organ toxicity endpoints, including "nephrotoxicity" with subcategories for "kidney necrosis", "kidney relative weight gain" and "nephron injury", using a recursive partitioning algorithm. According to the authors, the models demonstrated good predictive performance overall. When developing the compound sets, chemicals that were known to cause the toxicity (positives) and chemicals known not to cause toxicity (negatives) were included. The positives that were correctly predicted by the models were then clustered based on common toxicophore substructures by the JKlustor 5.9.0 utility from ChemAxon.

A total of 192 toxicophores were identified for all nephrotoxicity endpoints. However, the endpoints "kidney weight gain", "nephron injury" and "nephrotoxicity" are unspecific toxicity endpoints with regard to attempting to identify a mechanistic explanation for the nephrotoxic effect. These three endpoints could be associated with all of the nephrotoxicity pathways discussed in more detail below. Some of the substructures identified by Myskin et al. [265] were also identified in the other studies mentioned above; for example, chloroform, providing further justification and confirmation. In addition, some of the substructures identified are also relatively unspecific, e.g. cyclohexane or chlorobenzene. In the case of chlorobenzene, some more specific related substructures have been proposed, such as 1,2-dichlorobenzene, 1,3-dichlorobenzene or 4-chlorophenol. For this reason, it may be useful to study these substructures in more detail and find more defined alerts relating to nephrotoxicity.

C.5 Jolivette and Anders [266]

Jolivette and Anders [266] developed a linear QSAR model to predict the nephrotoxicity of 9 haloalkenes. Haloalkenes are high-volume chemicals and common environmental pollutants.

Bioactivation of haloalkanes occurs to form reactive intermediates. This process involves the formation of hepatic glutathione S-conjugates, which are then hydrolysed by peptidases into cysteine S-conjugates. Cysteine S-conjugates subsequently undergo bioactivation, by renal cysteine conjugate lyases, to form the nephrotoxic intermediates. The reaction of glutathione with haloalkenes is catalysed by microsomal (MGST1) and cytosolic glutathione transferases (cGST) [266]. In this study, a computational chemistry approach was used to test the hypothesis that an SAR exists and that E_{LUMO} (energies of the lowest unoccupied molecular orbitals) values can be used to predict the ability of rat liver MGST1 to catalyse the reaction of glutathione with haloalkenes. No, or a low level of, conjugate formation was detected for four of the nine molecules investigated. A linear relationship was found between the natural logarithms of the specific activities for the glutathione conjugation reaction catalysed by rat liver MGST1 and E_{LUMO} values for hexafluoropropene, 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene, 1,1,2-trichloro-3,3,3-trifluoro-1-propene and 1,1-dibromo-2,2-difluoroethene. This linear relationship corresponds only to four data points and cannot be extrapolated to E_{LUMO} values outside of the range -1.14 to -0.73 eV. When the E_{LUMO} value for tetrachloroethene was added to those of the previously mentioned four compounds and compared with the natural logarithms, a linear relationship was not seen. It was found that haloalkenes with more negative E_{LUMO} values demonstrate a greater specific activity for the enzyme-catalysed reaction of glutathione conjugation than haloalkenes with less negative E_{LUMO} values. This indicates that the chemical reactivity of the substrate plays a crucial role in the rate at which the glutathione-dependent biotransformation of the haloalkenes takes place.

C.6 Barratt [99]

α2μ-globulin nephropathy occurs in male rats as a result of a compound's binding affinity to $\alpha_{2\mu}$-globulin, which causes the chemical-protein complex to accumulate in renal lysosomes [99]. This leads to tubular necrosis and eventually cell death. Barratt [99] derived two QSAR models to screen for the potential of a compound to induce α2μ-globulin nephropathy by multiple regression analysis and principal components analysis using the following properties: negative charge density of the binding molecule, molecular volume, and inertial axis lengths. The QSAR model based on multiple regression analysis alone did not accommodate all of the molecular features that allowed accurate predictions of chemicals that may cause α2μ-globulin nephropathy. Therefore, a previous dataset by Bomhard et al. [276] consisting of 43 aliphatic and alicyclic hydrocarbon structures was added and subjected to a principal components analysis. The toxicological endpoint assessed in Bomhard et al. [276] was α2μ-accumulation in renal lysosomes in male rats. It was found that for a molecule to cause α2μ-globulin nephropathy, its size and shape should be so that it fits with the binding site on

α2μ-globulin. It should also have a hydrogen bond acceptor. Lastly, hydrophobic interactions were found to be a contributing factor but not to a significant extent when included in either QSAR. The shape, size and electronegativity elements related to active compounds were incorporated in both QSARs.

Barratt [99] concluded that the combination of the two QSARs is useful in identifying molecular structures with a potential to cause α2μ-globulin nephropathy. The applicability domain of both QSARs was not discussed. Furthermore, α2μ-globulin nephropathy has been acknowledged as an adverse effect specific to male rats and little relevant to human health as α2μ-globulin is not synthesised in the human liver [99]. Hence, these models may only be applied to assess whether compounds which have been linked to nephrotoxicity in male rats induce these effects via the α2μ-globulin pathway.

C.7 Lee et al. [252]

Lee et al. [252] developed binary classification QSAR prediction models for three nephrotoxicity mechanisms: tubular necrosis, interstitial nephritis, and tubulo-interstitial nephritis. These models were built using two data sets, i.e. parent compounds (251 nephrotoxicants and 387 non-nephrotoxicants) and their urinary metabolites (307 nephrotoxicants and 233 non-nephrotoxicants) based on clinical trials and post-marketing surveillance reports. A list of the parent and metabolite compounds was not published. Models were computed using a support vector machine and 20 descriptors with highest information gains in the form of eight different fingerprints for parent and metabolite sets and each of the three mechanisms. According to the authors, the predicted accuracies of the models for each type of kidney injury were better than 83% for external validation sets. This indicates that the models used could prove to be useful in identifying potentially nephrotoxic compounds.

Substructural fragments were analysed and were documented alongside the frequency of fragment enrichment factors. The selected eight substructures were common to both datasets for all of the above three nephrotoxicity mechanisms. Lee et al. [252] concluded that consideration of the metabolism of a chemical is important to predict its nephrotoxicity potential. When comparing the number of nephrotoxicants and non-nephrotoxicants with a specified fragment of the metabolite set to respective numbers of the parent compound set, the number of nephrotoxicant metabolites increased dramatically. This is due to the bioactivation of a potentially non-nephrotoxic compound into a toxic metabolite. Also, when these alerts were compared approximately to those found by

Pizzo et al. [263], similarities were not observed at first sight. Lee et al. [252] have provided a link that offers free access to the software on their website. However, this link does not appear to be working at this time: http://bmdrc.org/DemoDownload.

C.8 Matthews et al. [267]

Matthews et al. [267] created QSAR models to predict drug-induced urinary tract toxicity in humans on the basis of adverse events reported post marketing approval. Six endpoints of urinary tract injury were considered, namely nephropathies, acute renal disorders, bladder disorders, blood in urine, urolithiases and kidney function tests. Four QSAR approaches were applied using the following software: MC4PC, BioEpisteme, MDL QSAR and Leadscope Predictive Data Miner. The best predictive performance was achieved for the endpoints kidney function test with 87.7% specificity, 43.3% sensitivity and 91.6% coverage, and nephropathies with of 81.5% specificity, 49.5% sensitivity and 91.4% coverage. One of the limitations of these models is the variable quality of the post-marketing reported adverse event data used which may be compromised through non-uniform reporting of an adverse effect and its occurrence in patients who are often subject to multi-drug therapy.

C.9 Antczak et al. [268]

This study aimed to link physico-chemical features to drug-induced transcriptional responses and phenotypic outcome using a multivariate statistical approach. A number of KEGG pathways, which were reported to be significantly perturbed by nephrotoxic compounds under consideration in this study, may be specifically associated with nephrotoxicity [268]. The model showed that nephrotoxic substances are characterised by high polarisability, low electronegativity and low molecular symmetry.

C.10 Hammann et al. [269]

For this study, Hammann et al. [269] use decision tree inference analysis, a machine learning method, based on the chemical, physical, and structural properties and adverse drug reactions of 507 drugs. As decision tree inference algorithms, classification and regression tree (CART) and chi-squared automatic interaction detector (CHAID) were selected. Both models performed well, with CART and CHAID model corrected classification rates (CCRs) of 88.6 and 84.7%, respectively. The CART model highlights that amine functions, sulphur, and carboaromatic ring structures have an influence on a drug's potential to cause nephrotoxicity. For substances safer to the kidney, CHAID

model found few aromatic atoms (<19), a basic pK_a <10.71, van der Waals surface area <1,014.5 $Å^2$, and logP values >2.43.

C.11 Lei et al. [270]

Lei et al. [270] used a mouse intraperitoneal urinary tract toxicity data set of 258 chemicals from the ChemID*plus* public database to develop eight qualitative and quantitative structure-activity relationship (QSAR), i.e. classification and regression, models to predict urinary tract toxicity. The recursive feature elimination method incorporated with random forests (RFE-RF) was applied for dimension reduction, followed by the utilisation of eight machine learning approaches for QSAR modelling, i.e., relevance vector machine (RVM), support vector machine (SVM), regularized random forest (RRF), C5.0 trees, eXtreme gradient boosting (XGBoost), AdaBoost.M1, SVM boosting (SVMBoost), and RVM boosting (RVMBoost). Among these, RVMBoost based on the RBF kernel accomplishes the best quantitative and qualitative predictions for the test set (MCC of 0.787, AUC of 0.893, sensitivity of 89.6%, specificity of 94.1%, and global accuracy of 90.8%). All chemicals included in this study are within the application domain coverage. Overall, a reliable prediction is achieved by both regression and classification models developed by the SVMBoost approach.

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