Nephrotoxic Biomarkers with Specific Indications for Metallic Pollutants: Implications for Environmental Health

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ABSTRACT: Environmental and occupational exposure to heavy metals and metalloids is a major global health risk. The kidney is often a site of early damage. Nephrotoxicity is both a major consequence of heavy metal exposure and potentially an early warning of greater damage. A paradigm shift occurred at the beginning of the 21st century in the field of renal medicine. The medical model of kidney failure and treatment began to give way to a social model of risk factors and prevention with important implications for environmental health. This development threw into focus the need for better biomarkers: markers of exposure to known nephrotoxins; markers of early damage for diagnosis and prevention; markers of disease development for intervention and choice of therapy. Constituents of electronic waste, e-waste or e-pollution, such as cadmium (Cd), lead (Pb), mercury (HG), arsenic (As) and silica (SiO_2) are all potential nephrotoxins; they target the renal proximal tubules through distinct pathways. Different nephrotoxic biomarkers offer the possibility of identifying exposure to individual pollutants. In this review, a selection of prominent urinary markers of tubule damage is considered as potential tools for identifying environmental exposure to some key metallic pollutants.

KEYWORDS: Heavy metals, nephrotoxicity, kidney injury, urinary biomarkers, environmental and occupational exposure

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Introduction

It is predicted that electronic waste (e-waste)[1] will reach 74 million metric tonnes by 2030, making this the world's fastest-growing domestic waste stream. In addition to the growing threat of e-wastes, urbanisation and industrialisation have contributed to metal contamination in the environment in low-income countries adversely affecting human health.[2] Among the possible sources of contamination are soil, dust and food matrices.[2-4] Drinking water contaminated with heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) is a major health concern.[5] In 2012 The World Health Organisation (WHO)[6] reported that preventable environmental risks accounted for 12.6 million deaths worldwide. In 2015 the estimated global anthropogenic mercury (Hg) release was approximately 1800 tonnes.[7] Hg and methylmercury are major pollutants of the environment and exposure can be via food, mining or due to contamination of water supplies.[8] According to the WHO, at least 140 million people in 50 countries have been drinking water containing arsenic (As) at levels above the WHO provisional guidelines.[9] The primary sources of As toxicity in the general population is contaminated water, soil and food products.[10] Commercial production of Cd rose throughout the 20th century and now fluctuates around 23 000 metric tonnes annually. The manufacture of Cd/Nickel batteries accounts for around 55% of this and it is expanding because of the greater demand for rechargeable batteries. Environmental levels of Pb have increased because of the use of leaded fuel, Pb-based paint, mining, plumbing and other industrial activities.[11] Pb is one of the most widely occurring divalent metals that induce nephrotoxicity, and this has been demonstrated even at low doses.[12] In addition to respiratory diseases silica causes kidney damage. As well as being part of e-waste, the rapid emergence of silica nanoparticles for drug delivery threatens to markedly increase the risk of silica-induced nephrotoxicity.[1] Exposure to pollutants present in waste is a common cause of kidney disease which is most prominent in, but not restricted to, developing countries. The kidney is particularly susceptible to environmental pollutants and following exposure, levels of circulating pollutants in blood rise. The kidney filtres approximately 180 L of blood per day, producing approximately 1.8 L of urine. The result is a high renal exposure to pollutants. A recent review[13] highlighted the epidemiological evidence for the association between both acute and chronic kidney disease, with environmental pollutants, including air pollution, heavy metal pollution and other environmental risk factors. The measurement of urinary biomarkers plays an increasingly important role in the monitoring of at-risk populations from exposure to heavy metals and metalloids.

All authors contributed equally to this work.

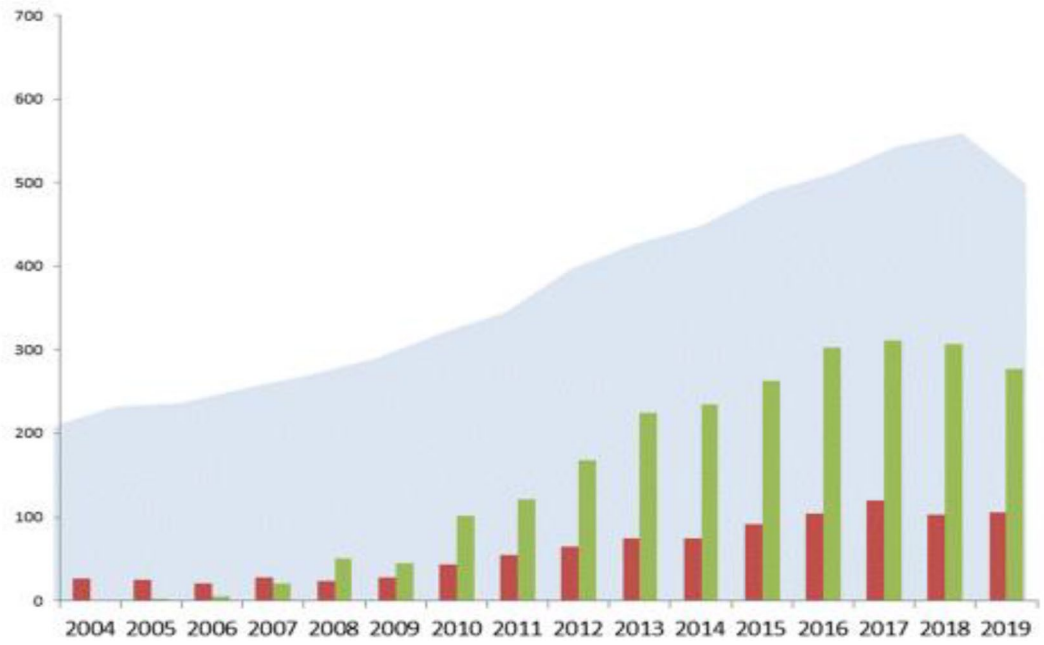

Figure 1. Biomarker Publications 2004 to 2019. The number of publications retrieved by year 2004 to 2019 in a search using the terms 'biomarker' and 'nephrotoxicity', brown columns, 'biomarker' and 'Acute Kidney Injury, AKI', green columns. The blue shaded area is the number of publications in 100 seconds retrieved by year 2004 to 2019 in a search using the term 'biomarker'.

In this review, we discuss the advent and principles of new biomarkers of nephrotoxicity with an in-depth focus on the most prominent entering use. To assess the potential utility of the biomarkers in environmental studies, we offer an overview of the methodologies used in their measurement. We then consider the mechanism of nephrotoxic damage and the evidence for biomarkers to detect the damage caused, by cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As) and silica (SiO_2).

Biomarkers

The use of biomarkers in the detection of acute kidney injury has increased substantially in the last 10 years. The number of publications retrieved from Pubmed using the search term 'biomarker' has increased 1.7-fold between 2009 and 2019, the number retrieved for 'biomarker' and 'nephrotoxicity' has increased 3.9-fold and for 'biomarker' and 'Acute Kidney Injury' the increase over the same period was 6.9-fold, more than four-fold greater than biomarkers in general (Figure 1).

Human kidneys have the potential to suffer significant impairment of function without obvious manifestation of symptoms. Biomarkers are therefore required for early detection of exposure to environmental nephrotoxins[13] present in soil, dust, water, food and air.[14] This information can be used to help to limit the adverse renal effects. Biomarkers should be objective measures of biological and pathogenic processes. Common and almost universally accepted renal biomarkers such as serum creatinine (sCr), blood urea nitrogen (BUN) and urinary albumin Alb/creatinine ratio (ACR) have proved to be insensitive and inadequately specific.[15] Many alternative tentative urinary biomarkers have been reported in the literature[16-19] of these 2 biomarkers, β_2-microglobulin (β_2-MG) and *N*-acetyl-β-D-glucosaminidase (NAG) have gained widespread use in the environmental field. Kidney injury molecule-1 (KIM-1) and neutrophil gelatinase associated lipocalin (NGAL) which are up-regulated in the renal proximal tubules are widely used to monitor the progression of acute kidney disease in clinical research. Both these biomarkers are now gaining ground in the environmental research field. Further information can be obtained from Wasung et al,[18] who reviewed a range of potential biomarkers of renal function used in monitoring the progression of acute kidney injury (AKI) and chronic kidney disease (CKD). An in-depth review considered the value of urine and serum biomarkers for the detection of AKI.[19]

β_2-MG is a low molecular mass protein filtered by the glomeruli and normally reabsorbed in the renal tubules. In common with other low molecular weight proteins, β_2-MG is virtually completely reabsorbed by megalin-mediated endocytosis in the proximal tubules and subsequently catabolised within the tubular cell. Under pathological conditions, β_2-MG appears in the urine indicating kidney injury.[18] Although still frequently used, β_2-MG is being replaced by other markers because of its instability in urine at pH < 7. However as partial degradation occurs before voiding has made it less reliable.[20] Evidence suggests that even when urine is stored at −80°C degradation reduced measured β_2-MG activity after only 6 hours.[21] Data from a clinical study[22] indicated that urinary β_2-MG is a good biomarker for proximal tubular injury. When the urine samples are collected from patients the pH requires adjustment to pH 6 to 8 with sodium hydroxide before overnight shipping to the laboratory for assay. This additional step could prove to be a disadvantage in studies where large numbers of the population are screened. Further, it was demonstrated[23] that β_2-MG is rapidly degraded in the bladder at low pH. An alternative

procedure to avoid the deterioration of β_2-MG in acidic urine involved instructing patients to take 400 mg of oral sodium bicarbonate the evening before the measurement of urinary β_2-MG was carried out after ingestion of tap water to enforce diuresis.[24] However, the need for these preliminary steps would make it difficult to apply to large populations in the environmental situation. In experiments[25] designed to define the extent, variability, and the mechanism of its instability in urine at pH 6 it was found that this was eliminated by heating to 80°C, a temperature that eliminates any enzymes present. This result suggests that the instability may be a consequence of enzyme activity. In an earlier study,[26] it was concluded that urinary retinal binding protein (RBP) was a better marker than β_2-MG because of its greater stability in urine and independence of pH. In a study comparing NAG and β_2-MG urinary activities in 1 month to 3-year-old babies, it was found[27] that the 3-month-old group had lower levels than the 12 months and 3-year-old groups. Interestingly the NAG values were almost constant throughout the 3 years. Since urine pH is easily affected by diet and children consume milk as the main part of their diet the decrease in β_2-MG activity may be explained as an effect of the proteins in milk. An association between urine pH and age in young infants was reported.[28] Data from a later study[29] suggested that β_2-MG may be a better marker of glomerular rather than tubular injury.

NAG is a lysosomal enzyme widely distributed in human tissue. It is released into serum from cells by exocytosis or from the breakdown of cells. The molecular weight of NAG is between 130 and 140 kDa preventing its filtration through the glomerulus although it is routinely cleared by the liver. The release of lysosomal hydrolases including NAG into the urine from the renal proximal tubules indicates tubular damage. The damage may be a result of either exposure to nephrotoxic agents including heavy metals such as Cd,[30,31] or other renal tubular abnormalities. NAG is the most active enzyme among proximal tubular lysosomal glycosidases, it is stable in urine and as a result, NAG is one of the most widely used biomarkers of renal tubular injury.[32-34] It is also used routinely as a reference test in assessing renal tubular injury in both humans and animal models.[35,36]

KIM-1 also named Hepatitis A virus cellular receptor 1 (HAVCR1) or T-cell immunoglobulin mucin receptor 1 (TIM1), is a multifunctional transmembrane protein. KIM-1 acts as an attachment receptor for a variety of virions and functions as a co-stimulatory molecule on T cells. In the kidney, KIM-1 is expressed in the s3 segment of the proximal tubule following injury. The extracellular domain is shed by a MAP kinase regulated process and its presence in urine correlates with its expression in the tissues.[37]

NGAL, also named Lipocalin-2 (LCN2), is stored in granules in neutrophils but also synthesised de novo by macrophages during inflammation. As a result of its size, 25 kDa together with its resistance to protease degradation, NGAL passes freely through the glomerulus and is retrieved via endocytosis in the proximal tubule. In man, NGAL expression can be stimulated in the Loop of Henle and the Collecting Duct.[38]

There are other biomarkers under investigation in clinical settings of kidney injury but they are used less widely for heavy metal toxicity screening (see Supplemental 1). In general, these tests measure proteins normally endocytosed by the proximal tubule, enzymes lost from damaged renal cells, markers of inflammation and more recently, micro-RNA. Biomarkers of failure of tubule reabsorption,[33] indicate a functional deficit and include low molecular mass proteins include alpha 1 microglobulin, α_1-MG; retinol binding protein, RBP; metallothionein, MT; cystatin C, Cys-C; Clara Cell Protein, CC16. Renal tubular enzymes released because of tubular injury include alkaline phosphatase, ALP; alpha-glutathione transferase, α-GST; glutathione-S-transferase Pi, π-GST; γ-glutamyl transpeptidase, GGT and lactic acid dehydrogenase, LDH. Acute kidney injury is also associated with the excretion of microRNAs for example, miR-21, miR-200c, miR-423.[5,16,18,39,40] The number of novel urinary biomarkers tested to detect nephron segment specific injuries caused by heavy metals is increasing dynamically. The tissue specificity and early release of circulating miRNAs following tissue injury make them promising biomarkers of tissue injury.[41] The recent epidemiological and toxicological advances in using miRNAs as biomarkers of chemical exposure in kidney damage have been reviewed.[42]

The use of multiple biomarkers has the potential to produce qualitative as well as quantitative information about nephrotoxic agents. Renal biomarkers are often the result of damage to a specific region of the kidney tubule carrying out a variety of different functions. This is illustrated in Figure 2, using NAG, KIM-1 and NGAL, as examples. Many small proteins are freely filtered by the glomerulus and reabsorbed in the proximal tubule for example NGAL which is taken up by megalin. Hence, the majority of urinary NGAL may be a result of a failure of megalin mediated endocytosis and reflects a functional problem predominantly in the s1 segment of the tubule. There remains some controversy over renal NGAL expression in different species. Megalin mediated endocytosis is one of the defining functions of the proximal tubule and its reduction or loss is seen as a key indicator of loss of function.

KIM-1 is expressed in the s3 segment of the proximal tubule, which is the most distal to the glomerulus, its expression is controlled at the transcriptional level by STAT3 (signal transducer and activator of transcription 3) transcription factor.[37] The expression of KIM-1 is, therefore, mediated by toxins or stimuli activating this pathway. The presence of KIM-1 in urine depends on the cleavage of the cell surface molecule by matrix metalloproteinase enzymes expressed in the tubule. The presence of NAG in urine is a result of necrosis, necroptosis and ferroptosis and it is expressed in all 3 segments of the proximal tubule, s1 > s2 > s3 (Figure 2).

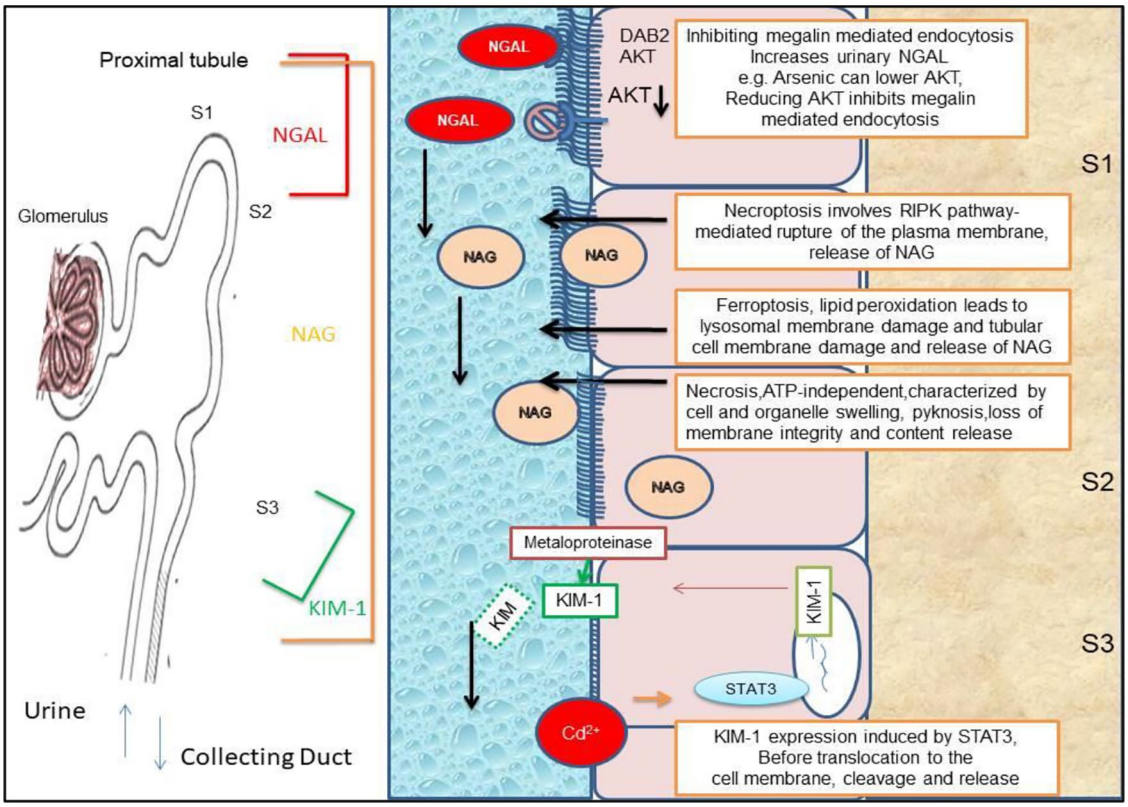

Figure 2. The molecular mechanisms involved in the release of NAG, NGAL and KIM-1 from the tubular epithelial cells in the s1, s2 and s3 segments of the proximal renal tubule (Right hand side), schematic representation of the renal tubule indicating the segmental origin of NAG, NGAL and KIM-1 (Left hand side).

Table 1. Characteristic of chromogenic tags available in NAG substrates.

CHROMOGENIC TAG	MOLAR EXTINCTION COEFFICIENT (ε) (L/MOL/CM)	ABSORBANCE WAVELENGTH (NM)
4-Nitrophenyl (ρNP)	12800	405
2-Chloro-4-nitrophenyl (CNP)	3580	410-420
Sodio-3,3′-dichlorophenolsulfonphthaleinyl (CPR)	20640	575
2-Methoxy-4-(2′-nitrovinyl)-phenyl- (MNP)	27000	505
5-[4-(2-Acetamido-2-deoxy-β-D-glucopyranosyloxy)-3-methoxyphenylmethylene]-2-thioxothiazolidin-4-one-3-ethanoate (VRA)	37000	505
m-Cresolsulphonphthaleinyl- (MCP)	40670	580

Urinary Biomarker Assay Methods

The sensitivity of any biomarker used in the detection of damage is dependent on the methods used to measure the biomarker. The measurement of urinary NAG activity has been quantified either spectrofluorimetrically or spectrophotometrically.[43] The assay must be sufficiently sensitive to allow the dilution of the inhibitory effect of urea. Initially, the most widely used substrates were 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide incorporating a fluorescent aglycone and the 4-nitrophenyl-*N*-acetyl-β-D-glucosaminide (PNP-GlcNAc) incorporating a chromogenic aglycone. More recently several novel colourimetric substrates (Table 1) with higher extinction coefficients and greater sensitivity have been developed.[33,44] Both manual and automated assays have been used.[44,45] Recently, kits utilising the 96 well plate absorbance readers have been widely used for testing across species. NAG activities and other urine enzyme activities need to be factored by urine creatinine to allow for the variation in urine flow. NAG activities are expressed as (μmol hydrolysed substrate/min × L urine) and or NAG indices (μmol hydrolysed substrate/min × mmol creatinine) to allow for urine flow.[43] A variety of other units have been used which makes comparisons between different laboratories difficult.[43] A rate procedure has recently been described to determine reference ranges for NAG in healthy Chinese adults.[46]

NGAL measurements are performed by ELISA or by a chemiluminescent microparticle immunoassay (CMIA). The 2-step CMIA for use on the ARCHITECT analyser developed by Abbots Laboratories was found to be superior to 4 other commercially available assays for urinary NGAL.[47] A comparison of this procedure with an ELISA procedure for NGAL[48] showed that the level of NGAL in urine varied between the 2 methods and comparison should be carried out with care.

KIM-1 is generally measured by ELISA or a microparticle Luminex xMAP Technology assay, although alternative assays have been developed for measurements in mouse urine. The sensitivity and specificity of ELISA assays are based on the characteristics of the antibody used. Most commercially available KIM-1 assays report comparable sensitivity. However, a meta-analysis published in 2014 reported distinct differences between ELISA and xMAP assays.[49]

β_2-MG has a low molecular weight (11.8 kDa) and, as a result, it is rapidly filtered by the glomerulus and normally 99% is reabsorbed by the tubules. Bernard et al[26] developed a highly sensitive procedure for the determination of β_2-MG in urine and serum which is based on the direct agglutination of β_2-MG by latex particles containing an antibody. The agglutination is quantified by counting the unagglutinated particles or by using turbidimetry. Data obtained using this procedure compared well with radioimmunoassay procedures (0.97 and 0.93 respectively). A fully automated nephelometric immunoassay to quantify β_2-MG in human serum which is also based on the light scattering signal produced by the agglutination of latex microparticles has been developed.[50] An immunoturbidimetric kit for the assay of β_2-MG in urine has also been validated.[51]

Nephrotoxic Effects of Environmental Pollutants

Environmental pollutants can be defined as elements or compounds introduced into the natural environment potentially causing unfavourable changes. Which do not necessarily cause adverse health effects but may create the environment more likely to result in adverse health effects.

Heavy Metals

Cadmium

Cd is a naturally occurring element and its adverse effect have been monitored in populations exposed to Cd in cigarettes, food and industrial sources. Monitoring the urinary level of this element is recommended as an essential biomarker in environmental studies.[52,53] These authors[54,55] have discussed the effect of Cd exposure on human health. While diet is the main source of Cd exposure in the general population, smoking also plays an important role and it is now established that smoking is an independent risk factor for renal disease.[55]

Cd is the seventh most toxic metal (US Agency for Toxic Substances and Disease Registry, ATSDR CAS number 7440-43-9) and remains a major health risk. Exposure to Cd typically results in renal tubulopathy, and a marked reduction in glomerular filtration rate (GFR), which may result in chronic renal failure (CRF).[34] Satarug et al[55] reviewed the nephrotoxic effect of Cd and Pb in relation to mortality and concluded that the currently accepted tolerable intake of Cd as well as the urinary Cd threshold limit does not provide adequate health protection. A summary of data obtained from epidemiological studies in the US, Spain, Korea, Germany and China indicated supported this conclusion. In view of the known interaction between Cd and Hg environmental exposure to these metals should be kept to a minimum.

The normal route of uptake of Cd is shown in Figure 3. There are several different molecular pathomechanisms behind Cd-induced renal injury which result in tubular dysfunction. These include reabsorption and lysosomal degradation of Cd^{2+}-metallothionein complexes in the proximal and distal tubules, release of free $Cd2^{+}$, perturbation of cellular Ca^{2+} homoeostasis, and interference with mitochondrial function.[56] The normal route of uptake of Cd is shown in Figure 3.

The severity of Cd-related renal dysfunction is dependent on the level of exposure and the availability of metallothionein. Also important is the presence of other pre-existing adverse health conditions such as diabetes mellitus, as well as age.[3,4] The concomitant exposure to other contaminants also has an effect.[57] Several studies determined the benchmark dose (BMD) and BMDL (BMD lower confidence limit) values for urinary Cd (UCd). These values have been determined in both Cd-polluted and non-polluted regions in China[58] Sweden[59] and Thailand.[60]

After cellular injury, proteins synthesised in the tubular cells may be released and detected in urine. In the case of Cd nephropathy, NAG and KIM-1 have been assayed most frequently.[55]

It is noteworthy that employees of certain industries, as well as some populations, are especially prone to Cd-elicited tubular nephropathies (Table 1) which is indicated by elevated urinary NAG and other tubular biomarkers. Even low-level environmental Cd and Hg exposure may influence renal tubular function disadvantageously in children.[61] The availability of datasets on UCd concentration together with urinary NAG and β_2-MG levels were used in the meta-analyses of BMD with a 95% lower confidence limit for UCd.[62] Liu et al[62] collected 92 datasets from 30 publications. calculated that 1.76 and 1.67 µg/g creatinine were the UCd BMD and BMDL (95%), respectively, at 5% extra risk of benchmark response (BMR). In another meta-analysis based on data from 13 studies,[63] 3.21 and 2.24 µg/g creatinine UCd, BMD5 and BMDL5 values were derived, respectively. UCd BMD5 and BMDL5 values calculated on the available β_2-MG concentrations (3.56 and 3.13 µg/g creatinine, respectively) were comparable to the NAG activity.[63] In a recent study[64] of males, females and children who had been living in a Cd polluted area for many years

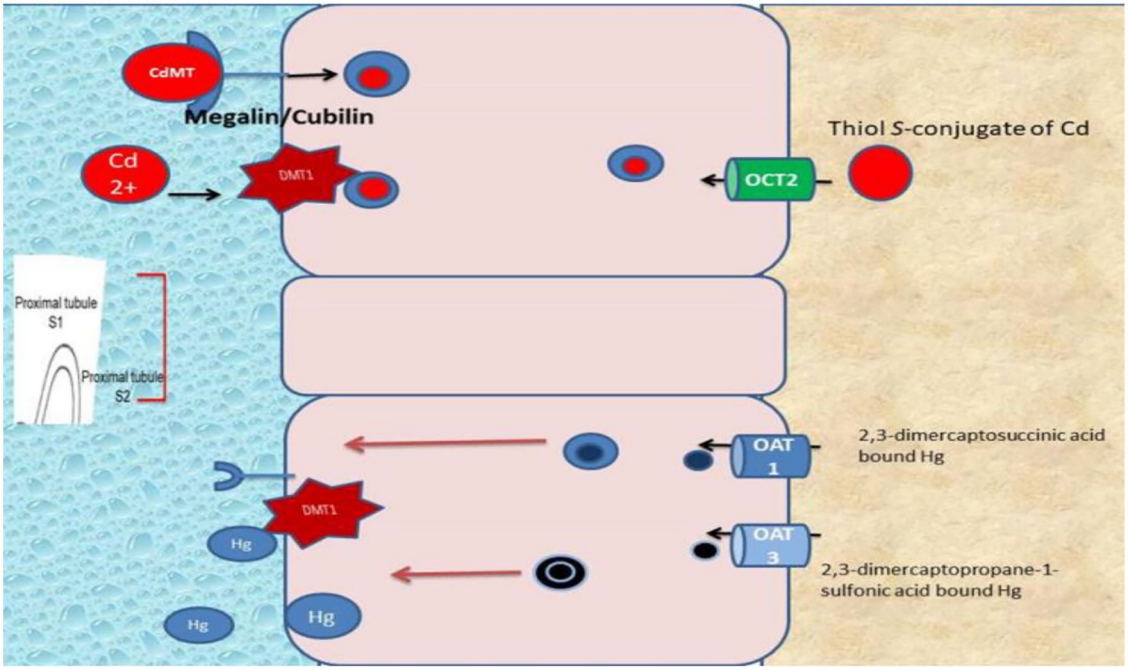

Figure 3. Mechanisms of Cd and Hg uptake by proximal tubule epithelial cells in s1/s2. Cd-metallothionein complexes are also taken up on the apical membrane, but it is endocytosed by megalin. Cd^{2+} ions have a strong affinity for sulphur groups and form complexes with select sulphydryl (thiol)-containing biomolecules. Cd^{2+}-thiol complexes are taken up by the basal organic cation transporter OCT2. Divalent Metal Transporter 1 (DMT1) allows transmembrane movement of a number of metals including Cd and Hg. Insert identifies section of proximal tubule illustrated in main figure.

found that 2.2 μg/g Cd would be a better threshold for clinical diagnosis. Females were more sensitive to Cd accumulation than males and in the long-term β_2-MG was a better biomarker of tubular damage than NAG. In the recent benchmark dose estimation study[65] it was found urinary β_2-MG and tubular albuminuria were also good biomarkers to assess the nephrotoxic effects of long-term environmental Cd exposures in Chinese women.

Urinary biomarkers, particularly Alb, β_2-MG and NAG, were suitable indicators with which to map improving renal function after reduction of dietary Cd-intake in Cd-contaminated areas.[66] As a result of the long half-life of Cd in the body[67] the deleterious effects of Cd exposure on renal function as indicated by elevated NAG and β_2-MG levels are difficult to assess even if low-Cd foods including rice are used. Moriguchi et al[68] established that NAG was a more sensitive biomarker for monitoring Cd exposure in the general population than either alpha1-macroglobulin (α_1-MG) or β_2-MG.

Chronic Cd exposure leading to Itai-Itai disease can also result in osteomalacia and multiple bone fractures in addition to kidney tubular damage. Uchida et al[69] found a correlation between urinary levels of vitamin D-binding protein β_2-MG, 2 megalin ligands and NAG. Bone mineral loss also correlated with Cd levels and changes in urinary markers in a Cd-exposed female population in Thailand.[70] Bone mineral density was also shown to be affected in Chinese women following Cd exposure which also correlated with renal symptoms.[71] However, low-level Cd exposure may affect kidney tubules without the involvement of glomeruli and impairment of the bones.[72] It is now apparent from the data accumulated from many studies that early monitoring of at-risk populations would be very beneficial.

KIM-1 was shown to be a useful urinary biomarker with which to detect renal tubular injury in Cd-exposed rats.[73] It was also demonstrated to be an earlier biomarker of renal tubular dysfunction than NAG and β_2-MG in a Cd-exposed population in Thailand.[74] These authors demonstrated that KIM-1 correlated with urinary and blood Cd levels as well as with NAG. A positive dose-dependence was recorded between urinary KIM-1 and Cd in both men and women.[75] Urinary α_1-MG was a sensitive marker of low-level Cd exposure while KIM-1, retinol binding protein (RBP) and possibly Alb were positively associated with urinary Cd only when overnight urine specimens were analysed. No correlation was found with β_2-MG.[75] The s1 and s2 segments of the early proximal tubule have been identified as the site of damage from Cd exposure, as well as Hg and Pb. However, the emerging role of ZIP8 in mediating the transmembrane movement of a broad range of divalent cations introduces a new dimension. ZIP8, like KIM-1, is expressed in the s3 segment of the proximal tubule.[76]

Multiple linear regression analyses showed that some type 2 diabetic-related biomarkers are confounders of associations between RBP and Hg or Cd biomarkers.[77] Additional data indicated a mediating effect of adiponectin on the relationship between urinary Cd and RBP.[77] Analysis of a large cohort from a Cd contaminated district in Thailand found that there was a clear dose-response relationship between urinary KIM-1 and Cd, and that the threshold values of KIM-1 in both genders

Table 2. Sources and targets of environmental Cd exposure.

ACTIVITY	REFERENCE
Workers exposed to Cd pigment dust	Kawada et al[83]
Welders	Verschoor et al[84]
Copper-Cd alloy workers	Mason et al[85]
Nickel-Cd battery manufacturers	Chia et al[86]
Ore refinery workers	van Sittert et al[87]
Cd smelters	Lei et al[88]
Cd plating workers	Kalahasthi et al[89]
Solderers	Mortada et al[78]
Residents in Cd contaminated areas	Phuc et al[90] and Cui et al[79]

were less than those of urinary NAG and β_2-MG.[74] Conflicting results were obtained by Li[64] who recommended β_2-MG as a better marker for exposure to Cd than NAG in a cohort of 1595 residents living near a contaminated Cd site. Ironworkers using soldering are liable to Cd overload as indicated by higher levels of Cd in blood and urine compared with controls.[78] This exposure may lead to kidney damage as indicated by the increase in the level of NAG and β_2-MG in urine.

More recently[79] it was reported that there were early renal effects of Cd exposure in children and adults living in a tungsten-molybdenum mining area of China. The investigated population studied had significantly higher accumulation of Cd and this was reflected in increasing levels of urinary NAG and β_2-MG in children and adults. The principal source of Cd was dietary intake in particular rice.

A recent Korean cross-sectional study considered the effect of exposure to Cd[80] on urinary NAG, β_2-MG and malondialdehyde (MDA) in adults living in a Cd-polluted area near an abandoned copper refinery. In both the high and low exposure groups urinary Cd levels were positively associated with urinary NAG levels but not with the erum copper to zinc ratio (CZR). After statistical adjustment of the data serum the CZR ratio was strongly associated with urinary β_2-MG levels in the low exposure group, and MDA was significantly associated with Cd regardless of Cd exposure. In both high and low Cd exposure groups, the copper-zinc imbalance is a risk factor for renal tubular damage as it induces oxidative stress independent of Cd exposure. Chen et al[81] assessed the effects of Cd exposure on serum 25-hydroxyvitamin D {25(OH)D} levels and renal tubular dysfunction in a population environmentally exposed to Cd. β_2-MG and RBP were used as indicators of renal dysfunction. Cd exposure did not affect serum 25(OH)D levels and that high 25(OH)D levels were associated with a decreased risk of renal tubular dysfunction. The same group studied the association between Cd exposure and the urinary biomarkers – microalbuminuria, NAG, NAGB isoenzyme, β_2-MG and RBP. They reported that the cumulative intake of Cd was lower than the critical standard previously reported suggesting an adverse effect on human health. Because it is difficult to find a population that was exposed exclusively to single metal, Lim et al[82] studied the effect of low exposure to Pb and Cd in a large cross-sectional study of the Korean adult population. The concentrations of both Pb and urinary Cd were positively associated with increased excretion of NAG and β_2-MG. They found an interactive effect of Pb and Cd exposure on urinary NAG and β_2-MG. This study highlighted the potential importance to health of the interactive effect of low-level exposure to multiple heavy metals. Workers in industry where Cd is used are also at risk of adverse effects which have been demonstrated in populations with high industrial and or environmental exposure (Table 2).

Table 3. Sources and targets of environmental lead exposure.

ACTIVITY	REFERENCE
Secondary lead refinery	Endo et al[94]
Battery plants	Pergande et al[95]
Lead stabiliser factories	Pergrande et al[95] and Lim et al[96]
Auto garage mechanics	Kumar and Krishnaswamy[97]
Workers auto repair shops	Oktem et al[98]
Police exposed to automobile exhaust	Mortada et al[99]
Residents living nearby a lead battery factory	Lin et al[100]

Lead

Renal dysfunctions including tubulopathy have been detected in employees in industries utilising Pb as well as in individuals who have been exposed domestically to Pb paint (Table 3). Pb as well as As are taken up in the s1/s2 segments of the proximal tubule by non-receptor mediated endocytosis (Figure 4). Urinary NAG activity is a sensitive and reliable marker for the detection of kidney tubular injury induced by heavy metals including Pb.[91] At the cellular level Pb^{2+} disturbs Ca^{2+} homoeostasis in the proximal tubules, which in turn interferes with normal mitochondrial function and elicits apoptotic cell death.[56] One explanation of the sensitivity of humans to Pb is the demonstration that õ-aminolevulinic acid dehydratase polymorphism influences the nephrotoxic effect of industrial workers exposed to Pb.[92] Metallothionein 1A polymorphism may also influence both urinary uric acid and NAG excretion in lead-exposed workers.[93]

The nephrotoxic effects of Cd co-contaminants including thallium and antimony should also be considered in Pb-elicited nephrotoxicity in Pb workers.[101,102] Pb also increases the nephrotoxic effects of low-level Cd in metal workers,[103]

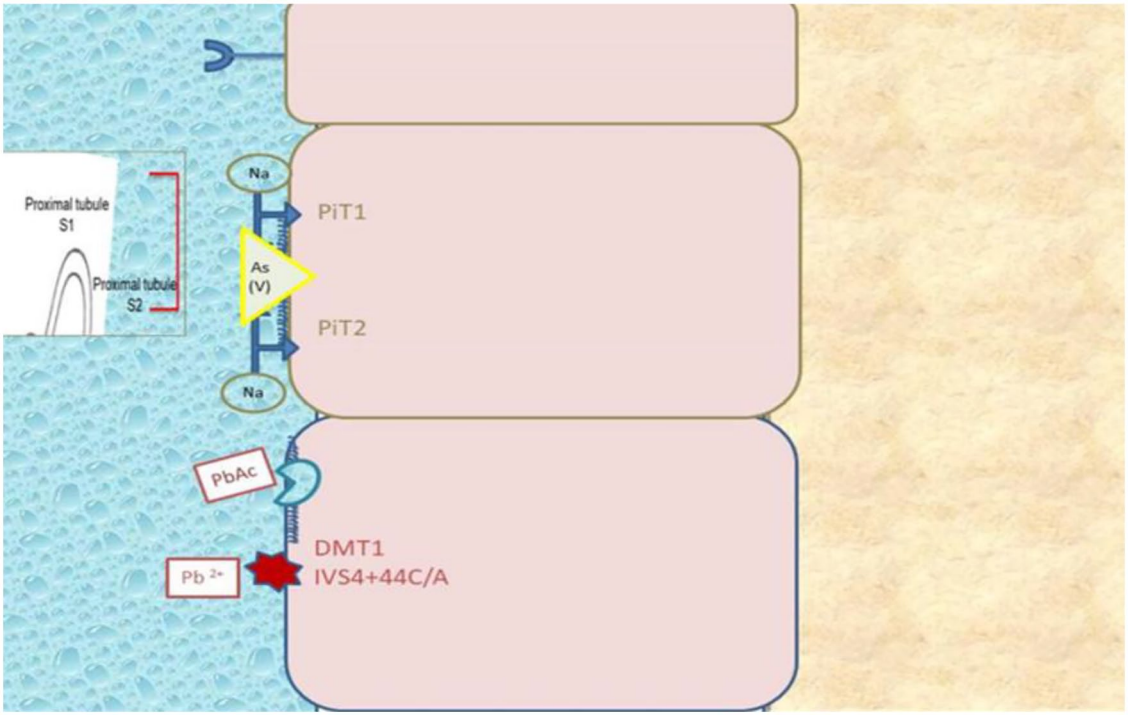

Figure 4. Mechanisms of Pb and As uptake by proximal tubule epithelial cells in s1/s2. Pb acetate is taken up by nonreceptor-mediated endocytosis. At least 4 DMT1 isoforms are expressed, IVS4+44C/A may play a particular role in the uptake of Pb in the kidney. The phosphate transporters PiT1 and PiT2 have a similar affinity for the Arsenate form of As as they do for Pi. Arsenate competes with Pi and reduces Pi transport in both PiT1 and PiT2, inducing inward currents similar to Pi. The insert identifies a section of the proximal tubules illustrated in the main figure.

however, even low exposure to these heavy metals affects renal function.[82] Exposure to Cd and Pb mixtures may also lower blood haemoglobin levels in humans.[104] Heavy metal mixtures containing Cd and Pb also cause significant renal dysfunction in residents living in contaminated areas.[105] The nephrotoxic effect of Pb exposure and cigarette smoking are synergistic in industrial workers.[106] These factors should be considered when planning large-scale occupational epidemiological screening studies. In a study of storage battery plant workers,[107] reported that the BMD and BMDL values for blood Pb, based on urinary excretion of total protein, β_2-MG and NAG activity were as low as 299.4 and 253.4 µg/L for NAG, underlining the sensitivity of NAG assays. A significant correlation was found between body Pb burden less than 200 µg and 24-hour urine NAG excretion. Other occupational studies gave comparable or even lower values suggesting that renal tubular damage might have preceded Pb-induced osteoporosis.[108] Future studies using biomarkers to detect early renal tubular injury caused by occupational Pb exposure should include KIM-1 in addition to NAG.[109]

Mercury

Hg toxicity continues to be a global health concern.[7,110] A significant level of inorganic mercury has been reported in the general population due to its presence in fish, a vapour in dental amalgams, and ethylmercury in vaccines as well as occupationally in gold mining (Table 4). Although traditional methods of measuring exposure using blood and hair levels are useful, intra-population levels vary so that the assay of biomarkers of effect are required. Hg compounds occur as either elementary organic or inorganic Hg compounds.[58] Global Hg emissions have grown over the 5 years between 2010 and 2015 at a rate of 1.8% per year from 2188 in 2010 to 2390 metric tonnes in 2015.[111] Proximal tubular damage can be extensive following Hg exposure which is linked to the depletion of the thiol pool of the cells and the consequent resulting oxidative stress.[56] Increased urinary NAG activity occurs in workers employed in industries where exposure to Hg is low but where exposure lasts for an extended period (Table 4).

Table 4. Sources and targets of environmental mercury exposure.

ACTIVITY	REFERENCE
Chlor-alkali plants	Jarosińska et al[112]
Fluorescent lamp production	El-Safty et al[113]
Mercury toxicity	Rosenman et al[114]
Thermometer manufacturing	Ehrenberg et al[115]
Natural gas production	Boogaard et al[116]
Mercury mining	Kobal et al[117]
Gold mining	Drake et al[118]

The renal tubular changes resulting from exposure to Hg cause physiological and biochemical changes resulting in the

release of NAG and other biomarkers. However, these changes can be reversed.[119] Increased urinary NAG activity was observed in chlor-alkali plant workers. When selenium concentration was low, changes in urinary NAG activity were detected which were associated with the lower selenium concentrations found in whole blood and serum at an early stage.[112] Lower serum glutathione peroxidase activity has also been recorded.[113] The titre of autoantibodies against myeloperoxidase was also higher in workers with high Hg exposure. The nephrotoxic effects of Hg exposure and smoking can be synergistic.[113] It should be borne in mind that most biomarkers of nephrotoxicity including NAG are general indicators of kidney injury[120] and, their correlation with urinary Hg levels is a useful indicator of the extent of tissue damage.

The safety of amalgam fillings is a recurring area of concern. Although they are largely considered safe in the USA, EU and the UK there is a trend away from their use in many countries. The evidence relating to amalgam filling association with nephrotoxicity and urinary NAG levels is ambiguous despite reported associations of elevated urinary NAG levels with amalgam fillings in several studies.[121,122] No differences in renal function were found in patients before and after the removal of their amalgam fillings.[123] Exposure to Hg vapour did not affect the health of the employees in the dental profession either.[124] No effect of amalgam fillings was indicated by urinary NAG activity in children.[125] Urinary creatinine levels vary in children due to changes in muscle mass and this should be considered when calculating results. No association was found in a population of Chinese children between urinary NAG and dental amalgam based on historic records of dental treatment. A relationship between NAG activity and urinary Hg level was however found by Mortada et al[121] and Ye et al[126] Previously, a correlation was found between blood and urine Hg concentrations, the number of fillings and urinary NAG activity and Alb excretion.[121] This data suggests that amalgam was not a suitable filling material because of a potential consequence of nephrotoxicity.[121] This view was supported by the finding that amalgam fillings probably affect kidney tubular function in children and that urinary NAG values were the most sensitive indicator.[122] Oxidative stress may be responsible for tubular damage because urinary NAG and malondialdehyde levels were positively associated.[122]

In a study of Japanese women urinary NAG and α_1-MG levels correlated with dietary intake of fish contaminated with Hg and with the Hg levels found in hair, toenails, and urine.[127] Dietary factors, selenium intake and co-exposures to other nephrotoxic agents all influenced urinary Hg and NAG levels in the general population, which had not been exposed occupationally to Hg.[112]

Arsenic

As is a metalloid and affects millions of people worldwide.[9,10] It is one of the most abundant contaminants found in water and soil. A link has been established between its presence and type 2 diabetes and cancer. Epidemiological and experimental studies evaluating As nephrotoxicity have used a combination of biomarkers of nephrotoxicity which included GFR, proteinuria, NAG, β_2-MG, α_1-MG as well as RBP.[128] More recently KIM-1, NGAL and interleukin-18 have also been used to evaluate As nephrotoxicity. The appearance of As in the environment for example in drinking water is typically geological and may cause kidney injury leading to CKD.[56] Decrease in the antioxidant capacity of the cells as well as disturbances in mitochondrial function, energy, amino acid and choline metabolism, result in injury to the brush border membrane (Figure 4). In addition to this, apoptotic cell death also occurs in the renal proximal tubules.[56]

Chronic As exposure increased urinary NAG levels in populations living in areas where As pollution is endemic.[129] At a relatively low level As exposure may elicit detectable renal tubular damage.[129] Cd exposure may enhance As nephrotoxicity when humans are co-exposed to Cd and As contaminants.[130] Cd and As exposure together caused more pronounced renal injury in people living in contaminated areas than in a population exposed to only one of these toxicants.[131] Studies of a Korean population co-exposed to Cd, Pb and As from a local abandoned copper smelter, demonstrated that urinary Cd was a risk factor for tubular dysfunction as indicated by elevated urinary NAG levels.[132]

Long-term exposure to even low concentrations of Cd and/or As may result in tubular damage resulting from oxidative stress indicated by the positive correlation between urinary NAG levels and the oxidative indices urinary malondialdehyde and 8-hydroxy-2′-deoxyguanosine levels.[133] Kidney patients exposed to Cd and As present in drinking water and/or in locally produced tobacco, showed higher urinary NAG levels than non-exposed patients.[134]

As toxicity is complex and involves the generation of free radicals and the induction of oxidative damage to cells. One way of reducing the toxic effect of As is the use of chelating agents, which form inert chelator-metal complexes which can be excreted.[135] A cross-sectional study of Mexican children exposed to tap and well water containing As and Cr at levels above the WHO recommended values identified a dose-dependent increase in KIM-1 excretion.[5] In a Mexican study of early kidney injury biomarkers including KIM-1 were associated with urinary fluoride but not As levels.[136] Environmental hazards from natural sources are widespread, particularly in northern Mexico. A cross-sectional study of children in northern Mexico using renal biomarkers was carried out by Cárdenas-González et al.[5] The local tap water had levels of As and Cr which were above the values recommended by the WHO. However, these authors failed to find any increase in functional biomarkers monitored or in miR-21 microRNA and NGAL but did find an increase in KIM-1 (As, Cr) and in miR-200c and miR-423 microRNAs (Cr).

A Sri Lankan study investigated the effect of heavy metals on CKD of unknown aetiology (CKDu). KIM-1 levels correlated with urinary As, Pb and Hg levels but not with Cd level[137]

while urinary fibrinogen did correlate with urinary As levels. In a systematic review of the association between As, Cd, Pb and chromium in drinking water and CKD, a positive correlation between Cd exposure and urinary NAG and KIM-1 has been reported.[138]

Silica

Silica (SiO_2) is a metalloid oxide of silicon but because silicon dioxide does not contain oxide ions, it has no basic properties. It is, in fact, weakly acidic, reacting with strong bases. A recent study[139] demonstrated that mesoporous SiO_2 particles (MSNs) have the potential to induce dose-dependent kidney injury in rats. The functional impairment was mediated via MSNs-induced oxidative stress, inflammation, fibrosis and tissue injury. Elevated NAG activity has been recorded in workers exposed to silica in the absence of silicosis.[140] An investigation into the possibility of subclinical nephrotoxicity in Egyptian pottery workers by measuring several parameters in the urine of 29 non-smoking and 35 smoking males was undertaken.[141] All of the parameters measured were elevated including KIM-1 suggesting that there were subclinical glomerular and tubular effects related to the length and intensity of exposure. In an earlier study, the urine of ceramic workers exposed to SiO_2 was compared to matched controls.[142] The renal biomarkers Alb, α_1-MG, NAG as well as Cu and Zn were measured as well as controls. The data demonstrated that exposure to silica resulted in renal changes which correlated with the level of exposure. A recent study by Ramadan et al[143] demonstrated that urinary liver-type fatty acid-binding protein (L-FABP) may also be used to screen for renal injuries in silica dust exposed handicraft pottery workers.

In Taiwan, KIM-1 and NGAL were significantly elevated in welding workers post-exposure to metal fumes, as were urinary Al, Cr, Mn, Fe, Co and Ni levels. The level of NGAL was more significantly associated with Al ($r = .737$, $P < .001$), Cr ($r = .705$, $P < .001$), Fe ($r = .709$, $P < .001$) and Ni ($r = .657$, $P < .001$) than KIM-1. This suggests that NGAL may be a urinary biomarker for PM2.5 exposure in welding workers.[144] In the light of these results the future application of NGAL in screening the nephrotoxic for the effect of high-metal content, fumes and dust has promise.

Conclusions

The exposure of at-risk populations to heavy metals is still a major problem. Recent changes in technology involving metals and the sensitivity of the kidney to them have added to the urgency to utilise established as well as to develop additional sensitive biomarkers. Each year the world produces in the region of 50 million tonnes of electronic waste (e-waste). The e-waste contains potentially harmful materials such as Cd, Hg and Pb. It is therefore becoming more important to monitor the health of people who are exposed to e-waste. Cd is one of the candidates responsible for the devastating increase in CKD in Sri Lanka, again emphasising the need for inexpensive and sensitive biomarkers for screening the affected populations. Estimated global Hg emissions increased 20% between 2010 and 2015. The WHO estimates that more than 200 million people worldwide are chronically exposed to unsafe levels of As in drinking water. Since exposure to environmental toxins can be monitored, progression to kidney disease is preventable. Biomarkers can therefore play an important role in this field. The first 2 decades of the 21st century have seen an accumulation of evidence for NAG and/or β_2-MG as the biomarkers of choice in many situations. Although β_2-MG is still widely used its stability raises problems for its use in large population studies and these need to be considered. β_2-MG may, in fact, be a better marker for glomerular rather than tubular pathology. The introduction of functional biomarkers such as NGAL and markers of distinct areas of the tubule such as KIM-1 offers greater qualitative measures of pollutant-induced nephrotoxicity. NAG is still the predominant biomarker used in the environmental field but the availability of KIM-1 and NGAL has added the potential to be able to monitor both the severity and progression of any nephrotoxic effect.

Literature Search Procedure

In the preparation of this review the literature was searched using PubMed, Biological Abstracts and various WHO publications for the terms nephrotoxicity, biomarkers, urinary enzymes, NAG, KIM-1, NGAL, β_2-MG, heavy metals, Cd, Hg, Pb, As and silica together with the phrase various contaminants.

Ethical Approval and Informed Consent

Where appropriate ethical approval and informed consent were obtained by the authors of each review or publication quoted in this article.

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Supplemental Material

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