

# Contribution of studies on renal effects of heavy metals and selected organic compounds to our understanding of the progression of chronic nephropathies towards renal failure

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**Abstract.** Risk assessment for a number of workplace or environmental chemicals, especially heavy metals and industrial organic compounds, relies mostly on clinical and epidemiologic findings. The low incidence of chronic nephropathies raises methodological issues in carrying out and interpreting human data on the progression of early changes towards end-stage renal disease. To overcome such limitations of epidemiological studies, two main approaches have been explored: (i) human studies relying on biomarkers and (ii) experimental animal models. Animal experiments have been useful to characterize early changes, such as hyperfiltration, eventually leading to chronic renal failure. Animal studies provided insights into the mechanisms underlying microalbuminuria and microproteinuria. Such biomarkers of early changes, developed for use at the workplace, have then been used to monitor such chronic disorders and multifactorial diseases as diabetes and arterial hypertension. Another area where Occupational Medicine has provided evidence is the effectiveness of primary prevention over other possible approaches. Avoidance of exposure to heavy metals and volatile hydrocarbons and their derivatives, mainly in individuals with diagnosed renal disorders, remains the best approach towards a substantial reduction in the burden of renal diseases.

**Key words:** kidney, lead, organic solvents, microalbuminuria, microproteinuria

## Introduction

Owing to its diverse functions and small mass in relation to the resting cardiac output that it handles, the kidney is a target both for chemicals that are pharmacologically active and for toxic chemicals. The nephron and its related cells perform a diversity of physiological functions. It is the major organ of excretion and homeostasis for water-soluble molecules; because it is a metabolically active organ, it can concentrate certain substances actively. In addition, its cells have the potential to biotransform chemicals and metabolically activate a variety of compounds. Specific physiological characteristics are localized to specific cell types. This makes them susceptible to, and the target for, toxic chemicals. The effect of any chemical on

a cell may be pharmacological, in which case the effect is dose-dependent and occurs only as long as the concentration of the effector is high enough to be active. Alternatively, the chemical may cause even severe damage to the cell; usually renal cells respond to injury by repair and the kidney as a whole responds to cellular lesion by renal and extra-renal adaptation to compensate for loss of that cell function. Although there is a substantial capacity within the kidney for repair, there are also several circumstances where damage may be irreversible. This depends on exposure levels, on exposure time, which may vary over a long period of time or is limited to a single event, and it may be due to a single substance or to multiple chemicals. Some chemicals cause an acute injury and others produce chronic renal changes that may lead to end-stage renal failure (1).

## The epidemiological approach to the nephrotoxicity

Epidemiology of nephrotoxicity by individual chemicals or mixed exposures has been inadequately studied. The contribution of chemicals to the overall incidence of nephropathy and of chronic renal failure is, with few exceptions, undefined. In the case of some occupationally exposed groups and analgesic-associated renal disease, there has been extensive research that has shown variations in incidence between groups and countries. It was finally estimated that up to 5% of end-stage renal disease may be due to toxic nephropathies and about 50% of end-stage renal disease is of unknown etiology (1).

A major problem in assigning a cause to end-stage renal disease is the long latency and subsequent slow development of chronic renal failure, which makes retrospective identification of the causative agent difficult. More importantly, the etiology may be obscured by lack of reliable information on the likely causative agents, the levels and duration of exposure, and other possible contributing and exacerbating factors (2).

The health significance of nephrotoxicity is also difficult to assess because of the diverse array of chemicals that target different parts of the kidney, the spectrum of disease consequences, and several interacting factors. Many industrial and environmental chemicals have been shown in experimental studies to be nephrotoxic, but the extent of their contribution to the overall incidence of chronic renal failure is not known. However, nearly 50% of these patients were considered possible (but not diagnosed) cases of toxic nephropathy (3, 4). Of those patients identified as having chemical-related renal disease, analgesic nephropathy is the most important recognized outcome, the prevalence varying greatly between countries, whereas some patients had other specific drug or chemical-related nephropathies (3).

The major occupational exposure is to workplace solvents, but other organic compounds, including pesticides, and toxic metals are of great concern. The well documented occurrence of nephropathies in subjects occupationally exposed to lead or cadmium, the excess of mortality for renal diseases in cohorts of workers with previous exposure to these two heavy metals (5),

and evidence that subclinical renal effects caused by cadmium are early signs of an accelerated and irreversible decline of renal function (6) point to the importance of occupational exposure to nephrotoxic agents as causal agents or modifying factors contributing to the burden of end-stage renal disease. Significantly increased risks of chronic renal failure (CRF) were found for exposure to lead [odds ratio 2.11 (95% CI 1.23-4.36)], copper [2.54 (1.16-5.53)], chromium [2.77 (1.21-6.33)], tin [3.72 (1.22-11.3)], mercury [5.13 (1.02-25.7)], welding fumes [2.06 (1.05-4.04)], and oxygenated hydrocarbons [5.45 (1.84-16.2)] (7). However, the low incidence of chronic nephropathies raises methodological issues in carrying and interpreting epidemiological studies aimed to the identification of aetiological agents acting as pathogenetic factors, as well as of those risk factors which, interacting with occupational exposure, can modulate the progression of early changes towards overt renal dysfunction leading to end-stage renal disease (8). For instance, the frequencies of various occupational exposures were high among patients with diabetic nephropathy (7).

To overcome such limitations of epidemiological studies, two main approaches have been explored: (i) human studies relying on biomarkers of early renal effect and (ii) experimental models of nephrotoxicity. Biomarkers are expected to increase the sensitivity of traditional approaches based on crude measures of exposure (e.g., job titles) and of outcome (e.g., death certificates); for use in preventive medicine, biomarkers should not be regarded as diagnostic tests but rather as indicators that early, reversible changes have occurred that could later lead to clinical disease (2, 8). Experimental models have been aimed not only at evaluating morphological alterations or pathology, but also at investigating the biochemical and functional correlates of such changes (1). The originality of the approach used in occupational toxicology has been the integration between findings gathered from epidemiological investigations on groups at risk, the validation of sensitive biomarkers in the same groups, and the application of the same effect biomarkers used to monitor workers occupationally exposed to nephrotoxic chemicals in selected animal models (1, 2, 8, 9).

## Biomarkers of effect

A biomarker of effect has been defined as “a measurable biochemical, physiological or other alteration within an organism that, depending on magnitude, can be recognised as an established or potential health impairment or disease” (10). Such biomarkers are expected to reflect early modifications preceding progressive structural or functional damage at the molecular, cellular and tissue level. Therefore, they should identify early and reversible biochemical events that may also be predictive of later response (11). Unfortunately, the mechanism of action of many chemicals is still unknown. Changes occurring in target tissues or cells may not be mirrored by biochemical changes occurring in peripheral, accessible media. Finally, whereas early damage may be repaired and subsequent dysfunction compensated for, it may also trigger a “cascade of events” eventually leading to clinical disease (2, 11).

Three main strategies have been followed in developing biomarkers of effect: (i) epidemiological; (ii) clinical; (iii) experimental. Most biomarkers of nephrotoxicity have been identified on the basis of pathophysiological reasoning, starting from clinical conditions, and extrapolating backward changes supposed to precede illness. Such an assumption, together with different methodological contexts of application, may lead to misinterpretation of the health significance of observed changes, which greatly depends on the prevalence of the condition being examined (12).

## Biomarkers of renal changes

Work on biomarkers of nephrotoxicity dates back to the mid-twentieth century, when Friberg's pioneering studies on cadmium nephrotoxicity lead to the set-up of a qualitative test identifying low molecular weight proteinuria (13). It took 15 years to develop semi-quantitative methods to assess cadmium-induced low-molecular weight proteinuria (14-16) and 15 years more to characterize cadmium-induced proteinuria on the basis of the urinary excretion of single low and high molecular weight serum proteins and enzymes (17). Immunochemical methods available by

the early 1980s lead to the identification of kidney-derived antigens as early markers indicating that tubular cell damage and not simply dysfunction was associated with chronic exposure to cadmium (18, 19). Among several biomarkers available, a core battery of urinary markers has been recommended, including albumin, one low-molecular weight protein, such as  $\beta_2$ -microglobulin or retinol-binding protein (RBP) and one marker of cytolysis, such as the activity of the lysosomal enzyme NAG (*N*-acetyl- $\beta$ -D-glucosaminidase) (20). Small deviations falling within the 95<sup>th</sup>-99<sup>th</sup> percentile of reference values cannot be interpreted at the individual level, since alternative explanations are possible (21). When such markers are examined on a group basis, in the context of epidemiological studies, potential confounding factors (e.g. meat meal, physical workload) should be also considered.

## Toxic nephropathies from selected chemicals at workplace

Recognized occupational renal diseases include those arising from exposure to heavy metals, organic chemicals (aliphatic and aromatic solvents and halogenated hydrocarbons) and silica. Cause and effect are relatively easy to demonstrate when renal damage is acute, whereas establishing the contribution of an occupational xenobiotic to kidney disease is considerably more difficult if the toxicity is delayed. The identification of toxic compounds responsible for the progression of chronic nephropathies was thought crucial in terms of prevention, since the affected workers can be removed from the exposure, allowing to slow, if not to stop, the degenerative cascade leading to chronic renal failure.

### *Heavy Metals*

More than 45 naturally occurring elements are classified as heavy metals; among these, seven are generally recognized as nephrotoxic elements: lead, cadmium, mercury, uranium, chromium, copper, and arsenic, though chronic renal failure has been described for only lead, mercury, cadmium, uranium, and arsenic. Therapeutic use of cisplatin, gold, lithium, and

bismuth may also induce kidney damage. Other potentially nephrotoxic elements include barium, cobalt, manganese, nickel, silver, thallium, thorium, tin, and vanadium. Nephrotoxic properties of such elements arise mainly from the tubular re-absorption of metal-protein complexes, which increase the epithelial burden of elements interacting with organic macromolecules, thus starting a cascade of events leading to cell membrane damage and oxidative stress. The selective vulnerability of different nephronic subunits, though difficult to assess when the renal functional reserve is severely impaired, can result in an increased  $\beta_2$ -microglobulin excretion following  $\text{Na}_2\text{CrO}_4$  administration and chronic exposure to cadmium, thus revealing a damage preferentially occurring at the initial segment of proximal convoluted tubule (S1), whereas elements damaging intermediate and distal segments (S2-S3) include inorganic mercury and lead (22).

#### *a) Chromium and chromium compounds*

Epidemiological investigations and animal studies have shown that measuring the urinary excretion of low molecular weight proteins may be useful to monitor renal dysfunction (23). Cross-sectional investigations gave evidence of mild tubular dysfunction in chrome-platers and welders occupationally exposed to water-soluble chromium(VI) (18). It was clearly established that chromium(VI) compounds arises from its direct cytotoxicity towards epithelial tubular cells. Experimental studies have shown an epithelial impairment leading to a progressive dose-dependent tubular epithelial necrosis of this nephronic subunit (23). Franchini and Mutti (24) assessed dose-effect/response relationships between the urinary excretion of chromium and that of retinol-binding protein or the renal antigen BB-50. Most of the abnormal values were observed in subjects with urinary excretion of chromium greater than  $15 \mu\text{g/g}$  creatinine; however, above this threshold the degree of tubular impairment was not related to urinary excretion of chromium. It was concluded that the tubular damage following chromium exposure is presumably transient and mostly due to acute exposure (24).

#### *b) Mercury and mercury compounds*

The toxicity of mercury depends on both its chemical form and the route of absorption. In rats, rel-

atively high doses of  $\text{HgCl}_2$  ( $>10 \text{ mg/kg}$  b.w. s.c.) induce severe haemodynamic changes and backleak, which contributes to the reduction in creatinine clearance induced by outer cortical ischemia with relevant impairment of glomerular function, whereas non critical doses ( $0.6\text{--}1.8 \text{ mg/kg}$  s.c.) can determine dramatic increases in RBP excretion, reabsorbed mainly at distal level of proximal tubule, as well as a slight increase in albumin excretion (microalbuminuria) (22). Such microalbuminuria found in animal models using subcritical doses represents a biomarker of tubular impairment of reabsorption of filtered plasmaproteins, since the complete integrity of the whole tubular segment is required to efficiently perform this physiological function (22).

Occupational exposure to elemental mercury for a decade with urinary concentrations exceeding  $50 \mu\text{g/dl}$  is associated with increased human intestinal alkaline phosphatase (HIAP) excretion but little increase in urinary tissue non-specific alkaline phosphatase NAG, RBP,  $\beta_2$ -microglobulin, or microalbuminuria (25, 26). More recently, in subjects occupationally exposed to lower Hg concentrations, leading to Hg-U levels between  $2.3$  to  $35.0 \mu\text{g/g}$  creatinine, no significant prevalence of abnormal values for the above biomarkers have been observed (27).

Sporadic case reports of nephrotic syndrome following exposure to elemental or organic mercury have appeared since the middle of the past century but, in occupational setting, the causal relationship of mercury exposure to proteinuria and the nephrotic syndrome has been less compelling, because the dose-response is unpredictable and the etiology of nephrotic syndrome unrelated to mercury is rarely known. Observations in rats may provide a framework for understanding mercury-induced glomerular disease in humans. Multiple subcutaneous injections of  $\text{HgCl}_2$  in rats, in doses too small to produce acute tubular necrosis, induced membranous nephropathy, a renal disease characterized by glomerular deposition of immune complexes and heavy proteinuria (28). The response to mercury in the rat is under genetic control and dose-dependent (29, 30). As little as  $0.005 \text{ mg/100 g}$  b.w. elicit immunologically mediated glomerular disease in selected strains. Metallic mercury vapor ( $1 \text{ mg/m}^3$ ) is as effective as  $\text{HgCl}_2$  for inducing autoimmune disease

in susceptible rats. Immunoglobulin localization in the glomeruli is associated with heavy proteinuria, circulating immune complexes, and polyclonal B-cell activation owing to antiself Ia autoreactive T cells (30).

### c) Lead

As for other metallic elements, it is difficult to estimate a threshold and target selectivity for lead. Field studies have shown both glomerular and tubular effects, frequently co-existing in same workers (31). Renal biopsies in chronic lead nephropathy show non-specific tubular atrophy and interstitial fibrosis with minimal inflammatory response as well as mitochondrial swelling, loss of cristae, and increased lysosomal dense bodies within proximal tubule cells (32). Arteriolar changes indistinguishable from nephrosclerosis are found, often in the absence of clinical hypertension. Intranuclear inclusion bodies are often absent when the renal disease is long-standing or following the administration of chelating agents. Morphologic alterations are minimal in glomeruli until the reduction in GFR is advanced. The appearance of arteriolar nephrosclerosis before hypertension develops and the relatively short duration of hypertension before renal failure supervenes suggest that the early renal injury from lead may be in the microvascular endothelium (33) and not only in the tubulointerstitial area.

A cross-sectional study on 81 male lead-exposed workers and 45 age-matched controls (median blood lead concentrations 2.03 and 0.34  $\mu\text{mol/l}$  respectively) analyzing urinary biomarkers of renal integrity preferentially or exclusively located along the different nephron segments showed that not only tubular but also glomerular involvement could be shown in early phases of lead nephropathy, as revealed by increases in the median values of 6-keto-prostaglandin 1  $\alpha$  and decreases in fibronectin (34).

Experimental studies showed that Pb acetate at high doses (0.5%) in drinking water for 12 months can lead, even in the early stages of intoxication, kidney cortex hypertrophy, increases of glomerular filtration rate (GFR) and a parallel increase in tubular antigens excretion, whereas late stages are characterized mainly by tubulointerstitial changes leading to kidney remodelling and progressive glomerulo-angiosclerosis (35).

The renal haemodynamic response was estimated by determining the capacity of the kidney to increase the glomerular filtration rate (in terms of creatinine clearance) after an acute consumption of cooked red meat in male Pb workers and matched controls (36). Both control and Pb exposed workers showed a significant increment in creatinine clearance (on average by 15%) after oral protein load which was positively correlated with Pb-B, suggesting that exposure to Pb may be associated with a slight hyperfiltration state, which has been found to attenuate the age related decline in baseline creatinine clearance by a factor of two. However, the progression of lead nephropathy exhibits some similarities and differences in animals and man (37). The first stage is the period of acute effects and is limited to functional and morphological changes in proximal tubular cells. Such changes are substantially identical in workers and in rat.

As lead nephropathy progresses, pathological and clinical changes are more difficult to compare between experimental models and man. Progression in man is usually over several years and clinical manifestations are quite non specific, including increased BUN and reduced GFR. Glomerular injury probably occurs secondary to tubular atrophy, interstitial nephropathy and nephron loss. At late stages, hyperplasia, cytomegaly and dysplastic cellular changes in proximal tubular lining cells are common to man and experimental animals. However, these changes are associated with renal adenocarcinoma in a high percentage of lead exposed rats, but very few cases have been reported in man. The reasons for the difference in organ specificity between rodents and men are not known.

Exposure to low doses of lead, such as those characterizing work environments in Western Countries, can determine subclinical alterations revealed by biochemical changes of unlikely prognostic significance. Lead-exposed workers showed an increase in TXB<sub>2</sub> and a decrease in PGE<sub>2</sub> and 6-keto-PGF<sub>1 $\alpha$</sub>  in the urine (38), a finding that had been interpreted as an interference of Pb on prostaglandin metabolism; although the pathophysiological significance of the urinary eicosanoids is unclear, measurement of urinary PGE<sub>2</sub>, PGF<sub>2 $\alpha$</sub> , and 6-keto-PGF<sub>1 $\alpha$</sub>  may provide insight into the mechanisms of hypertension and injury to the glomerulus or renal medulla. Chia et al. (39) found

significant changes in urinary excretion of the thermo-stable isoform of N-acetyl-D- $\beta$ -glucosaminidase (NAG-B) among a cohort of workers occupationally exposed to lead.

#### d) Cadmium

The kidney is the critical organ for chronic cadmium (Cd) exposure. Whatever the source and absorption route, the highest Cd concentration is found in the renal cortex and in proximal tubular cells, mainly of S1 and S2 segments). The earliest sign of tubular lesion is a plasma-derived low molecular weight (< 40 kDa) tubular proteinuria (42), including  $\beta_2$ -microglobulin and RBP. In severe cases of Cd nephrotoxicity, tubular damage may lead to renal glucosuria, aminoaciduria, hyperphosphaturia ("Fanconi's syndrome"), hypercalciuria, polyuria due to decreased concentration capacity, and a reduced ability to handle an acid load (43). Advanced stages of intoxication are associated with functional changes in other segments of the nephron and are associated with glomerular damage, increased prevalence of kidney stones, lowered plasma concentrations of calcitriol (44). This injury may progress to a chronic interstitial nephritis.

Several studies have tried to identify specific biomarkers predicting nephrotoxic effects of cadmium in human. Because tubular proteinuria was the first and most extensively investigated sign of Cd-induced nephropathy, the determination of low molecular weight proteins in urine remains the most useful biomarker for detecting early renal effect from Cd exposures (41, 43, 45). In healthy subjects, tubular reabsorption of LMW proteins is almost complete; since in healthy subjects  $\beta_2$ -microglobulin in plasma is usually about 2 mg/L, daily excretion is less than 0.3 mg: when tubular reabsorption capacity drops of about 1% this leads to a 10-fold increased excretion of the  $\beta_2$ -microglobulin (42).

Other biomarkers of renal dysfunction have been proposed since the early 80s: among these, RBP apolipoprotein,  $\alpha_1$ -microglobulin and Human Clara Cell Protein (CC16 or protein 1). Lysosomal enzymes, such as NAG and human alkaline phosphatase have been also used to detect early kidney dysfunctions (41, 42, 45). In particular, urinary excretion of NAG – particularly of the NAG-B iso-enzyme, seems

to be very sensitive, showing any threshold without association between urine U-Cd and urinary excretion of the enzyme (45).

The lack of reversibility of Cd proteinuria was demonstrated by Roels et al. (46). In presence of severe microproteinuria ( $\beta_2$ -microglobulin >1500  $\mu$ g/g creatinine) and historical Cd-U values exceeding 20  $\mu$ g/g creatinine, Cd-induced tubular dysfunction was progressive in spite of reduction or cessation of Cd exposure. A strong association between cumulative cadmium exposure and the later increase in serum creatinine supported the notion that cadmium-induced renal disease progresses slowly after a latent period of several decades. Workers who were exposed to cadmium in a nonferrous smelter in Belgium for up to 5 years and who had tubular proteinuria were examined annually for 5 years after exposure had ceased (47). Cd levels in the kidney ranged from 133 to 355  $\mu$ g/g. The reduction in GFR was accompanied by an increase in mean serum  $\beta_2$ -microglobulin from 0.189 to 0.300 mg/dL and an increase in mean urinary  $\beta_2$ -microglobulin excretion from 1.770 to 2.500  $\mu$ g/L. The loss of GFR over a 5-year period was estimated to be 30 times the predicted loss of kidney function.

#### Organic chemicals as risk factors for the progression of chronic nephropathy

Although at least 40 clinical and case-control studies have examined the relationship between glomerulonephritis and exposure to organic solvents, the possible pathogenetic role of solvent exposure in the development of chronic glomerulonephritis is a controversial issue (48). A number of these studies concluded that patients with chronic glomerulonephritis have been exposed to organic solvents (aliphatic and aromatic) more frequently than patients with other diseases (49-52). Toxicological studies of the effects of gasoline distillates performed over the past two decades under the auspices of the American petroleum industry have identified an effect of gasoline constituents on the renal tubule of male rats. Referred to as "light hydrocarbon nephropathy", tubular injury is induced by exposing Fischer 344 male rats to petroleum hydrocarbon vapors from a

few hours up to a few years. Mice, guinea pigs, dogs, primates, and female rats do not develop the lesion. It is not known if similar morphologic tubular damage occurs in humans exposed to gasoline vapors. The hydrocarbons studied in animal models include n-nonane, C8, C10-C11 isoparaffinic solvent, jet fuels, methyl-isobutyl ketone, varnish, unleaded gasoline, naphthas, and a variety of complex organic solvents and distillates. These volatile hydrocarbons are cytotoxic to proximal tubules, where they and their metabolic products are selectively accumulated. The most prominent lesion is hyaline droplet formation within epithelial cells of proximal tubules. Sustained renal failure with permanently reduced GFR has not been reported in light hydrocarbon nephropathy in humans or experimental animals.

Experimental exposure to hydrocarbons has sporadically produced glomerular lesions, but this has generally occurred as a consequence of tubulo-interstitial damage. Although the role of tubulo-interstitial injury is now recognized as a key factor in the progression of renal diseases, the relevance of these models to the human beings is questionable, owing to the overt differences in biotransformation and in the delivery of solvent metabolites to the kidney. Some metabolites of compounds belonging to different classes of organic solvents are able to bind the rat specific protein  $\alpha_2$ -microglobulin and accumulate in proximal tubules, where the complex tends to precipitate in the form of insoluble crystals, eventually leading to cell degeneration and death. In a rat model of perchloroethylene (PCE)-induced nephropathy, the tubular accumulation of  $\alpha_2$ -microglobulin precipitating in the form of insoluble crystals in male rats exposed to PCE for 4 weeks gave rise to selective damage to S2 tract of proximal tubules and its amount was correlated with albuminuria, a widely accepted biomarker of glomerular dysfunction (53).  $\alpha_2$ -Microglobulin is also present at very low concentrations in female rats, which doesn't develop overt renal damage but only minor changes, and it is lacking in human beings. Smaller but significant increases in albuminuria, associated with low molecular weight proteinuria (RBP and  $\beta_2$ -microglobulin) were found in female rats. Thus, in the above model, exposure to PCE seems to determine glomerular proteinuria of tubular origin (53).

Many investigators have attempted to identify solvent-induced glomerulonephritis by assessing the urine of exposed workers for low-molecular-weight proteins and enzymes, markers for tubular, rather than glomerular disease (54, 55). Cross-sectional studies carried out in groups of workers occupationally exposed to solvent mixtures and perchloroethylene in dry-cleaning shops has shown mild functional changes suggesting diffuse abnormalities along the nephron. Possible generalized membrane alterations can be responsible of the observed increase in high molecular weight proteinuria, fibronectin and brush-border antigens (56). Although such tubular proteinuria is common, the massive albuminuria of solvent nephropathy is distinctly rare in association with perchloroethylene exposure.

Case-control studies suggest a possible role of exposure to volatile hydrocarbons not only in the development of chronic glomerulonephritis, but also in their progression towards end-stage renal disease (57, 58). In spite of the difficulty to implement experimental models of multifactorial diseases, for which the interaction between risk factors seems more relevant than a sum of single effect produced by each one, we recently evaluated the role of styrene, a widely used hydrocarbon, in the progression of a well known nephropathy (59). Adriamycin-induced nephrosis was chosen as a model because it is characterized by progressive worsening of proteinuria, followed by focal glomerulosclerosis and tubulo-interstitial fibrosis (60). Co-exposure to ADR and styrene resulted in a proteinuria much greater than that caused by ADR alone. The interactive effect of styrene and ADR was statistically significant for albuminuria and urinary fibronectin. A similar response was observed for GFR at the end of the experiment, styrene-exposed animals showing hyperfiltration as compared to their respective control group. At the end of the experiment, histopathological scoring for interstitial infiltration and fibrosis was also significantly higher in styrene-treated animals as compared to their respective control groups. In ADR-treated rats, L.M.W proteinuria was only slightly affected, suggesting minimal tubular dysfunction associated with extensive tubular atrophy. However, styrene-exposed animals showed L.M.W proteinuria higher than their respective controls.

Moreover, the urinary excretion rate of albumin and fibronectin correlates with the histopathological semi-quantitative scoring for interstitial infiltration and fibrosis. Indirect evidence of mechanisms increasing the production of reactive oxygen species was obtained from the parenchymal concentration of 8-hydroxy-2-deoxyguanosine DNA adduct and glutathione depletion, two associated phenomena.

This animal model confirmed the role of hydrocarbon exposure as a factor accelerating the progression of renal disease indicated by epidemiological investigations in patients suffering from chronic renal disease (57, 58), thus suggesting the need to avoid solvent exposure in patients suffering from renal diseases.

## Conclusion

Although clinical, epidemiological and experimental studies can be affected by methodological issues producing sometimes inconsistent results, they should be considered as complementary approaches. Experimental studies are essential to recognize potentially nephrotoxic compounds, to derive thresholds and safe doses, to study the mechanisms responsible of the progression towards severe kidney impairment and late-stage disease.

The target selectivity of some metals or organic chemicals, which can only be supposed on the basis of field studies in occupationally exposed workers, has been confirmed in selected nephrotoxicity studies, combining acute, subacute and chronic designs. The above studies have clarified, in part, the mechanisms underlying the different pattern of biomarkers used to assess early changes in renal integrity and function, along with their meaning. However, a full validation of biomarkers should rely on follow-up studies indicating the health significance of observed changes. Microalbuminuria and low-molecular weight proteinuria fulfil this condition in subjects suffering from diabetes mellitus and from chronic cadmium poisoning, respectively. In such situations, both markers are predictors of an accelerated deterioration of renal function. Outside of these two situations, no information is available to interpret the early changes resulting from chemical exposure and there is a need to conduct longitudinal

studies on populations with well-characterized exposure or risk. Therefore, when persistent microproteinuria is observed in the context of a documented chronic exposure to a suspected or established nephrotoxin, it is prudent to consider that it might have a similar meaning as in incipient diabetic or cadmium nephropathy (46, 47, 61). Such a view is corroborated by animal experiments, which support epidemiological studies suggesting that hydrocarbon exposure can accelerate the progression of renal disease towards chronic renal failure (59). This mechanism would be involved in several renal diseases, in which proteinuria is the main factor accelerating its course (62, 63). In subjects with incipient renal disease, avoidance of exposure to heavy metals and volatile hydrocarbons and their derivatives is essential to prevent end-stage renal disease.

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