Normal and Pathologic Concentrations of Uremic Toxins

Flore Duranton,* Gerald Cohen,[†] Rita De Smet,[‡] Mariano Rodriguez,[§] Joachim Jankowski,[‖] Raymond Vanholder,[‡] and Angel Argiles,* on behalf of the European Uremic Toxin Work Group

*RD-Néphrologie, Montpellier, France; [†]Medizinische Universität Wien, University Klinik für Innere Medizin III, Abteilung für Nephrologie und Dialyse, Wien, Austria; [‡]Nephrology Section, University Hospital Ghent, Gent, Belgium; [§]Unidad de Investigacion, University Hospital Reina Sofia, Cordoba, Spain; and [‖]Charité, Experimental Nephrology and Hypertension, Berlin, Germany

ABSTRACT

An updated review of the existing knowledge regarding uremic toxins facilitates the design of experimental studies. We performed a literature search and found 621 articles about uremic toxicity published after a 2003 review of this topic. Eighty-seven records provided serum or blood measurements of one or more solutes in patients with CKD. These records described 32 previously known uremic toxins and 56 newly reported solutes. The articles most frequently reported concentrations of β2-microglobulin, indoxyl sulfate, homocysteine, uric acid, and parathyroid hormone. We found most solutes (59%) in only one report. Compared with previous results, more recent articles reported higher uremic concentrations of many solutes, including carboxymethyllysine, cystatin C, and parathyroid hormone. However, five solutes had uremic concentrations less than 10% of the originally reported values. Furthermore, the uremic concentrations of four solutes did not exceed their respective normal concentrations, although they had been previously described as uremic retention solutes. In summary, this review extends the classification of uremic retention solutes and their normal and uremic concentrations, and it should aid the design of experiments to study the biologic effects of these solutes in CKD.

The uremic syndrome is characterized by the retention of various solutes that would normally be excreted by the kidneys. The substances that interact negatively with biologic functions are called uremic toxins. In the past years, research on uremic toxicity has been very dynamic and resulted in the identification of dozens of retention solutes, including several uremic toxins. In 2003, the European Uremic Toxin Work Group (http://www.uremic-toxins.org/) proposed a classification of 90 retention solutes providing data on normal and pathologic serum concentrations.[1] In 2007, results were further discussed and expanded with the addition of 14 solutes.[2,3] This collaborative work focused on the highest mean or median concentration of the solutes measured in a uremic population and the highest individual uremic concentration. These data were particularly relevant for researchers on uremic toxicity, and they became a successful tool for allowing use of standardized and biologically relevant concentrations in experimental settings. More recently, scientific and technological progress resulted in the identification of many new uremic retention solutes, particularly thanks to nontargeted approaches such as metabolomic and proteomic profiling.[4,5] To maintain experimental guidelines in keeping with current knowledge, it seemed necessary to propose an update of the encyclopedic review.[1] It was decided to study the publications from after the first review and compare results with previous findings. With this comparison,

Correspondence: Dr. Àngel Argilés, RD-Néphrologie, 104 rue de la Galéra, FR-34090 Montpellier, France. Email: argiles@rd-n.org

it became possible to identify new uremic retention solutes and provide an external validation of the original tool. We reviewed all original articles on uremic toxicity published between 2003 and April 2011 and extracted all serum concentrations of uremic retention solutes measured in uremic populations and healthy controls.

RESULTS

Overview of Uremic Toxin Research

Between 2003 and April 2011, 621 articles dealing with uremic toxins in renal patients matched a corresponding PubMed electronic search (www.pubmed.com). Eighty-seven articles (14%) provided data on uremic retention solute concentrations in CKD patients and were also included in the analysis (Supplemental Material 1). Most studies were performed in patients undergoing dialysis (chronic hemodialysis, 80.5% of studies; peritoneal dialysis, 2% of studies), and 14% of studies were performed in nondialyzed CKD patients. The remaining 3.5% of studies included both dialyzed and nondialyzed CKD patients. According to the classification of uremic retention solutes based on size and binding properties,[2] free water-soluble low molecular mass compounds (<0.5 kD) represented 46% of the 88 solutes; 28% of solutes were middle molecules (0.5–60 kD), and 24% of solutes were protein-bound solutes (Table 1). Most of the 88 solutes (59%) had only been quantified within one study; these solutes were mainly free water-soluble compounds (64%), whereas 23% of solutes were middle molecules, and the remaining 13% of solutes were protein-bound solutes. Among the 36 solutes for which several concentrations could be included, 45% were protein-bound solutes, 36% were middle molecules, and 19% were small water-soluble compounds. Uremic concentrations are presented in Tables 2–4.[6–86] Molar concentrations ranged from a few picomoles per liter for ILs to micromoles per liter for phenylacetic acid.[22,31] The highest mass concentration was detected for the acute phase macromolecule α1-acid glycoprotein.[54] Most frequently reported concentrations concerned β2-microglobulin, indoxyl sulfate, homocysteine, uric acid, and parathyroid hormone (PTH). There were large variations in binding of protein-bound solutes. The free fraction of acrolein represented less than 1% (range=0.72–0.84) of total acrolein. On average, the free fraction of p-cresylsulfate was 9.0% (4.2–13.8), the free fraction of indoxyl sulfate was 9.4% (4.5–12.9), and the free fraction of indole-3-acetic acid was 16.3% (14.3–18.3). The free fraction was greater for hippuric acid (60%; range=49–71). Our approach failed to include 58 uremic retention solutes that had been listed along with their uremic and normal concentrations in the previous classification.[1] Several isolation and detection techniques were applied to quantify toxins, including chromatography (ion exchange chromotography [IEC], gas chromatography [GC], and HPLC), spectrophotometry, fluorometry, chemilumisnescence, nephelometry, radioimmunometry, nuclear magnetic resonance, and mass spectrometry (MS). However, uremic and normal concentrations were generally, but not always, measured with similar techniques (85% of solutes) (Tables 2–4).

Comparison with Normal Concentrations

To evaluate the relative solute retention in uremia, we calculated the ratio of the mean of all reported uremic concentrations (M) to the normal concentration (N) measured in healthy controls (M/N). The ratio M/N ranged from 333 for phenylacetic acid to 0.3 for spermine (Tables 5–7). There were 21 solutes for which uremic concentrations were more than 10 times higher than normal (Table 5). There was a limited degree of retention in the case of 18 solutes for which the ratio M/N ranged between one and two (Table 6). For four compounds from the original uremic retention list, the ratio M/N was below one, which suggests that they might not be retention solutes (spermine, spermidine, guanidinoacetic acid, and malondialdehyde) (Table 7).

Comparison with Previously Reported Concentrations

Of the 88 retention solutes, 56 solutes had not been presented in the list of uremic toxins published in 2003 and 2007 (Table 1).[1,2] Because their uremic concentrations exceeded those concentrations of healthy controls, they were subsequently added to the list. However, there were 32 solutes that had already been included in the list of known

Table 1. Contingency table of uremic retention solutes depending on solute classification, solute status, and number of records retrieved for each solute

Solute Classification	Solute Status		Total (Count)
	Known Retention Solute (Count)	Newly Identified Retention Solute (Count)	
Free water-soluble low molecular weight molecules			
results based on one report (count)	8	25	33
results based on several reports (count)	3	4	7
total	11	29	40 (46%)
Protein-bound solutes			
results based on one report (count)	3	4	7
results based on several reports (count)	8	8	16
total	11	12	23 (25%)
Middle molecules			
results based on one report (count)	2	10	12
results based on several reports (count)	8	5	13
total	10	15	25 (28%)
Total (count)	32 (37%)	56 (63%)	88 (100%)

Solutes that had been presented in the previous reviews[1,2] were considered as known retention solutes.

Table 2. Mean and highest concentrations of uremic retention solutes found in uremic populations and normal concentrations found in the general population: Free water-soluble low molecular weight molecules

Molecule	Molecular Weight	Group	Uremic Concentrations			Normal Concentration N (SD)	Methods (U; N)	New Retention Solute[a]
			Number of Original Papers	Mean Uremic Concentration M (SD)	Highest Uremic Concentration H (SD or Range)			
2-Heptenal (μg/L)	112	RCC	1	—	54.7 (16.3)[6]	17.7 (5.33)[6]	GC/MS	✓
2-Hexenal (μg/L)	98	RCC	1	—	61.7 (20.5)[6]	22.1 (6.6)[6]	GC/MS	✓
2-Nonenal (μg/L)	140	RCC	1	—	101.8 (58.4)[6]	18.5 (5.2)[6]	GC/MS	✓
2-Octenal (μg/L)	126	RCC	1	—	32.5 (21.2)[6]	26.1 (16.4)[6]	GC/MS	✓
4-Decenal (μg/L)	154	RCC	1	—	100.1 (26.8)[6]	15.9 (5.3)[6]	GC/MS	✓
4-HO-decenal (μg/L)	170	RCC	1	—	36.6 (22.3)[6]	10.3 (7.1)[6]	GC/MS	✓
4-HO-hexenal (μg/L)	114	RCC	1	—	63.8 (25.3)[6]	25.1 (9.9)[6]	GC/MS	✓
4-HO-nonenal (μg/L)	156	RCC	1	—	117.3 (47.7)[6]	16.4 (9.0)[6]	GC/MS	✓
4-HO-octenal (μg/L)	142	RCC	1	—	27.8 (13.8)[6]	10.7 (3.6)[6]	GC/MS	✓
4-Pyridone-3-carboxamide-1-β-D-ribonucleoside (μg/L)	272	Nicotinamide	1	—	156.1 (169.2)[7]	3.54 (1.63)[7]	HPLC	✓
8-Hydroxy-2'-deoxyguanosine (μg/L)	283	Purine	1	—	0.82 (0.25)[8]	0.64 (0.23)[9]	HPLC; ELISA	✓
α-Keto-δ-guanidinovaleric acid (μg/L)	173	Guanidine	1	—	39.8 (31.1–60.6)[10]	8.23 (0.66)[11]	IEC	✓
Anthranilic acid (μg/L)	137	Guanidine	1	—	16.7 (6.5)[12]	4.23 (1.62)[12]	HPLC	✓
Arginic acid (μg/L)	175	Guanidine	1	—	57.8 (40.3–78.8)[10]	21.5 (3.5)[11]	IEC	✓
Asymmetric dimethylarginine (μg/L)[a]	202	Guanidine	5	385.0 (288.4)	878.7[b] (38.4)[14]	<60.6[15]	HPLC; ELISA	
Cysteine (μg/L)	121	Aminoacid	1	—	67.8 (3.6)[16]	43.6 (2.4)[16]	HPLC	✓
Decanal (μg/L)	156	RCC	1	—	23.4 (8.81)[6]	17.2 (5.0)[6]	GC/MS	✓
Dimethylamine (mg/L)	45	Amine	1	—	10.3 (1.6)[5]	2.18 (0.33)[5]	HPLC	✓
Ethylamine (μg/L)	45	Amine	1	—	69.0 (10.2)[17]	25.8 (5.8)[17]	HPLC	✓
Guanidine (μg/L)	59	Guanidine	1	—	96.2 (90.9–112.1)[10]	<11.8[18]	IEC	
Guanidinoacetic acid (μg/L)	117	Guanidine	1	—	220.0 (168.5–251.6)[10]	222.3 (79.6)[19]	IEC	
Guanidino succinic acid (mg/L)	175	Guanidine	1	—	1.43 (0.99–1.72)[10]	0.03 (0.01)[18]	IEC	
Heptanal (μg/L)	114	RCC	1	—	61.1 (44.1)[6]	51.1 (11.2)[6]	GC/MS	✓
Hexanal (μg/L)	100	RCC	1	—	51.7 (33.0)[6]	21.7 (10.8)[6]	GC/MS	✓
Hypoxanthine (mg/L)	136	Purine	1	—	2.57 (1.13)[20]	1.5 (0.5)[21]	HPLC	
Malondialdehyde (μg/L)	72	RCC	3	217.9 (148.4)	388.8 (21.6)[22]	257.7 (81.7)[23]	Spectrophotometry	
Methylguanidine (μg/L)	73	Guanidine	1	—	139.4 (72.3–218.3)[10]	<7.3[18]	IEC; HPLC	✓
Monomethylamine (μg/L)	31	Amine	2	332 (351)	580 (100)[5]	320 (40)[5]	HPLC	✓
Neopterin (μg/L)	253	Purine	1	—	83.3 (10.8)[24]	1.38 (0.47)[24]	ELISA	✓
Nicotinamide (μg/L)	122	Nicotinamide	1	—	35.4 (29.3)[25]	3.17 (1.22)[25]	HPLC	✓
N-methyl-2-pyridone-5-carboxamide (mg/L)	152	Nicotinamide	3	4.02 (3.28)	7.80 (3.59)[26]	1.37 (0.68)[26]	HPLC	✓
N-methyl-4-pyridone-3-carboxamide (mg/L)	152	Nicotinamide	3	498.6 (162.8)	636.9 (471.2)[22]	39.5 (13.7)[25]	HPLC	✓
Nonanal (μg/L)	142	RCC	1	—	68.9 (26.8)[6]	37.4 (23.3)[6]	GC/MS	✓
Noradrenalin (μg/L)	382	Catecholamine	1	—	2.02 (0.88)[27]	0.25 (0.07)[28]	RIA	✓

Table 2. Continued

Molecule	Molecular Weight	Group	Uremic Concentrations			Normal Concentration N (SD)	Methods (U; N)	New Retention Solute[a]
			Number of Original Papers	Mean Uremic Concentration M (SD)	Highest Uremic Concentration H (SD or Range)			
Oxalate (mg/L)	90		1	—	3.9 (0.6)[29]	0.3 (0.1)[30]	Spectrophotometry; IEC	✓
Phenylacetic acid (mg/L)	136	Guanidine	2	467.2 (10.6)	474.6 (44.9)[31]	<1.4[31]	NMR	
Symmetric dimethylarginine (μg/L)	202	Amine	1	—	646.4 (606.0)[32]	76.1 (21.0)[13]	HPLC; IEC	✓
Trimethylamine (μg/L)	59	Amine	1	—	82.0 (28.5)[33]	24.7 (7.3)[33]	GC/MS	✓
Trimethylamine-N-oxide (mg/L)	75	Amine	1	—	7.49 (2.39)[33]	2.84 (1.53)[33]	GC/MS	
Uric acid (mg/L)	168	Purine	12	64.4 (20.4)	83 (13)[34]	40.5 (13.9)[35]	Spectrophotometry	

GC/MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; IEC, ion exchange chromatography; NMR, nuclear magnetic resonance; RCC, reactive carbonyl compound; RIA, radioimmunoassay.
[a]Refers to solutes not present in refs. 1 and 2.
[b]Highest uremic concentrations found after suppression of outliers: asymmetric dimethylarginine=364±81 μg/L.[15]

uremic retention solutes. These solutes are presented in Figure 1 along with the ratio of the highest uremic concentrations found in the present analysis (H) to the concentrations found in the original review (h). This index ranged from 0.04 for TNF-α to 4.30 for carboxymethyllysine. Interestingly, the ratio H/h exceeded one for nine solutes, signifying that recent publications provided a more elevated highest concentration and justifying the need for updating the list of uremic concentrations. There were 12 solutes for which the highest concentration was less than one-half of the original highest report (ratio H/h below 0.5). For five of the solutes, it was even below 10% of the original report (H/h<0.1). For these compounds, we evaluated the representativeness of highest concentrations by graphical analyses. Data from both the present work and the previous review[1] were pooled, and possible outliers were sought using box plots. Results concerning solutes with four concentrations or more are displayed in Figure 2. There were, indeed, outlying maximal values for all four solutes that had all been published before 2003. After exclusion of suspected outliers, the corrected H/h ratios substantially increased, reaching scores above 10% (H/h: IL-6, 0.73; TNF-α, 0.47; methylguanidine, 0.16; guanidino succinic acid, 0.14).

Variability in Concentrations

To analyze variability in reported concentrations, the ratio H/L of the H to the lowest concentrations (L) found in uremic populations was used as an index of the range of the observed uremic concentrations. Substantial variability, as previously defined by a ratio exceeding 8.5,[2] was found for several protein-bound solutes (carboxymethyllysine, free indoxyl sulfate, and phenol) and middle molecules (PTH, TNF-α, leptin, osteocalcin, and IL-8) (Supplemental Material 2). Additional graphical analyses of dispersion for compounds including four values or more concluded with suspected outliers for asymmetric dimethyl arginine (ADMA), homocysteine, and PTH (Supplemental Material 3). Second highest uremic concentrations could be more representative of highest uremic concentrations (ADMA=364±81 μg/L[15]; homocysteine=7.8 mg/L [range=3.3–16.0][85]; PTH=1676±69 ng/L[86]). Asymmetries were present in several box plots, where mean values largely exceeded the medians. For these solutes, median concentrations could be more representative of uremic populations (median: ADMA=323 μg/L; carboxymethyllysine=1.8 mg/L; cystatin C=6.3 mg/L; PTH=329 ng/L; TNF-α=12.3 ng/L; homocysteine=4.3 mg/L). Opposite asymmetries were also present for several compounds, which suggested possible underestimated mean uremic concentrations (indole-3-acetic acid, free and total indoxyl sulfate, total p-cresylsulfate, and uric acid).

Sensitivity Analyses

Sensitivity analyses were performed to evaluate the influence of methodological choices on results. Total indoxyl sulfate concentrations were increased in patients undergoing dialysis (P=0.02). Receiving dialysis or not did not affect other compound

Table 3. Mean and highest concentrations of uremic retention solutes found in uremic populations and normal concentrations found in the general population: Protein-bound molecules

Molecule	Molecular Weight	Group	Number of Original Papers	Uremic Concentrations		Normal Concentration N (SD)	Methods (U; N)	New Retention Solute[a]
				Mean Uremic Concentration M (SD)	Highest Uremic Concentration H (SD or Range)			
3-Carboxy-4-methyl-5-propyl-2-furan-propanoic acid (mg/L)	240		3	6.1 (2.4)	8.8 (5.0)[36]	3.6 (0.2)[37]	HPLC; GC/MS	✓
Acrolein, total (mg/L)	56	RCC	2	9.8 (0.4)	10.1[38]	1.7 (0.5)[38]	ELISA	✓
Acrolein, free (μg/L)	56	RCC	2	76.2 (4.8)	79.5 (47.0)[38]	28 (10.1)[38]	HPLC	✓
Carboxymethyllysine (mg/L)	204	AGE	6	5.4 (7.4)	18.5 (5.0)[39]	0.35 (0.13)[40]	ELISA	✓
Dihydroxyphenylalanine (mg/L)	197	Catecholamine	1	—	11.4 (3.2)[41]	6.6 (0.73)[41]	Fluorometry	✓
Hippuric acid, total (mg/L)	179	Hippurate	5	71.3 (13.7)	87.2 (61.7)[42]	3.0 (2)[5]	HPLC	
Hippuric acid, free (mg/L)	179	Hippurate	3	41.3 (14.3)	51.1 (40.1)[42]	1.0[5,43]	HPLC; fluorometry	✓
Homocysteine (mg/L)	135	Aminoacid	12	4.90 (2.15)	9.37[b] (2.05)[44]	1.35 (0.14)[44]	HPLC	✓
Indican (mg/L)	295	Indole	1	—	27.3 (13.3)[17]	10.0 (0.4)[17]	HPLC	
Indole-3-acetic acid, total (mg/L)	175	Indole	4	2.03 (0.38)	2.4 (2.2)[36]	0.5 (0.3)[45]	HPLC	✓
Indole-3-acetic acid, free (mg/L)	175	Indole	2	0.37 (0.10)	0.44 (0.51)[36]	—	HPLC	
Indoxyl sulfate, total (mg/L)	212	Indole	18	23.1 (13.0)	44.5 (15.3)[46]	0.53 (0.29)[47]	HPLC	✓
Indoxyl sulfate, free (mg/L)	213	Indole	6	3.22 (1.21)	4.49 (2.67)[46]	ND[48]	HPLC	✓
Indoxyl-β-D-glucoronide (mg/L)	408	Indole	1	—	3.87 (3.88)[47]	1.26 (0.52)[47]	HPLC	
Kynurenic acid (μg/L)	189	Indole	1	—	151.0 (76.4)[5]	5.48 (1.32)[5]	HPLC	
p-Cresylsulfate, total (mg/L)	31	Phenol	6	20.9 (12.2)	41 (13.3)[5]	1.9 (1.3)[5]	HPLC	✓
p-Cresylsulfate, free (mg/L)	31	Phenol	2	1.75 (1.20)	2.6 (5.1)[49]	0.08 (0.09)[49]	HPLC	✓
Pentosidine (μg/L)	342	AGE	2	509.7 (98.6)	579.5 (299.3)[36]	51.6 (18.8)[50]	HPLC; ELISA	
Phenol (mg/L)	94	Phenol	2	2.79 (3.83)	5.5 (3.7)[51]	0.6 (0.2)[52]	HPLC; GC/MS	
Putrescine (μg/L)	88	Polyamine	2	9.11 (0.44)	9.42 (7.59)[53]	4.36 (2.75)[53]	HPLC	
Spermidine (μg/L)	145	Polyamine	1	—	9.99 (7.74)[53]	10.4 (5.1)[53]	HPLC	
Spermine (μg/L)	202	Polyamine	1	—	1.86 (1.53)[53]	6.20 (7.98)[53]	HPLC	
Thiocyanate (mg/L)	58		1	—	1.86 (0.17)[16]	0.29 (0.06)[16]	HPLC	✓

AGE, advanced glycation end-product; ELISA, Enzyme-linked immunosorbant assay; GC/MS, Gas chromatography–mass spectrometry; HPLC, High performance liquid chromatography; RCC, reactive carbonyl compound.

[a]Refers to solutes not present in refs. 1 and 2.

[b]Highest uremic concentrations found after suppression of outliers: homocysteine=7.8±1.3 mg/L.[85]

Table 4. Mean and highest concentrations of uremic retention solutes found in uremic populations and normal concentrations found in the general population: Middle molecules

Molecule	Molecular Weight	Group	Uremic Concentrations			Normal Concentration N (SD)	Methods (U; N)	New Retention Solute[a]
			Number of Original Papers	Mean Uremic Concentration M (SD)	Highest Uremic Concentration H (SD or Range)			
α1-Acid glycoprotein (g/L)	43,000	Protein	2	1.24 (0.09)	1.3 (0.1)[54]	0.63 (0.19)[13]	Nephelometry	✓
α1-Microglobulin (mg/L)	33,000	Protein	1	—	332 (45)[54]	<54.0[55]	Nephelometry; spectrophotometry	✓
β-Trace protein (mg/L)	26,000	Protein	1	—	12.3 (6.0–19.8)[56]	<0.74[57]	Nephelometry	✓
β2-Microglobulin (mg/L)	11,818	Protein	24	30.2 (7.8)	43.1 (18)[27]	1.9 (1.6)[45]	ELISA	
Adiponectin (mg/L)	30,000	Protein	1	—	16.6 (14.4–18.6)[58]	<11.1[59]	Fluorometry; ELISA	
Angiogenin (μg/L)	14,400	Protein	1	—	803 (74)[60]	<150.0[61]	ELISA	
Calcitonin (ng/L)	3450	Protein	1	—	21.8 (21.0)[54]	<20.0[62]	RIA	✓
Complement factor D (mg/L)	23,750	Protein	2	20.6 (13.0)	29.8 (8.6)[27]	1.9 (0.5)[63]	ELISA; RIA	
Cystatin C (mg/L)	13,300	Protein	4	10.4 (8.3)	22.9 (9.0)[64]	<1.6[65]	Nephelometry; RIA	
Fibroblast growth factor-23 (ng/L)	32,000	Protein	1	—	149.6 (102.8)[66]	26.3 (0.8)[66]	ELISA	✓
Glutathion, oxidized (mg/L)	613	Tripeptide	2	73.2 (57.6)	114.0 (73.1–160.2)[67]	20.8 (7.3)[35]	Spectrophotometry	✓
IGF-1 (μg/L)	7650	Protein	1	—	220 (93)[27]	145.0[68]	RIA; chemiluminescence	✓
IL-6 (ng/L)	24,500	Cytokine	7	5.91 (1.98)	8.6 (3.7)[22]	4.0[69]	ELISA	✓
IL-8 (ng/L)	8000	Cytokine	2	20.2 (25.1)	38 (0.42–659)[70]	1.64 (1.85)[71]	ELISA	✓
IL-10 (ng/L)	18,000	Cytokine	1	—	10.6 (6)[46]	7.1 (1.5)[72]	ELISA	
Leptin (μg/L)	16,000	Protein	6	37.6 (25.1)	70.1 (50.4)[73]	8.4 (6.7)[74]	RIA	✓
Myoglobin (μg/L)	17,000	Protein	1	—	163.6 (67.8)[54]	39.0 (2)[75]	RIA	✓
Osteocalcin (μg/L)	5800	Protein	2	189.7 (235.7)	356.4 (607.4)[54]	<18.0[76]	RIA	
PTH (ng/L)	9500	Protein	12	673 (660)	2195[b] (370)[77]	<60.0[78]	N/A; RIA	
Prolactin (μg/L)	22,000	Protein	2	25.8 (8.4)	31.7 (6.5)[54]	<19.3[79]	Chemiluminescence; spectrophotometry	✓
Resistin (μg/L)	12,500	Cytokine	1	—	47.3 (35.3–62.2)[80]	15.1 (0.7)[80]	ELISA	✓
Retinol binding protein (mg/L)	21,200	Protein	2	169.1 (53.5)	206.9 (20.5)[81]	<80.0[65]	ELISA; RIA	
Soluble intracellular adhesion molecule-1 (μg/L)	4270	Protein	1	—	80 (40–157)[70]	48 (5)[82]	ELISA	✓
TNF-α (ng/L)	26,000	Cytokine	4	21.6 (24.5)	57.8 (10.8)[83]	7.0[69]	ELISA	
Vascular endothelial growth factor (ng/L)	34,250	Protein	1	—	346 (887)[45]	60.0 (9)[45]	ELISA	✓

N/A, not available; PTH, parathyroid hormone; RIA, radioimmunoassay.

[a]Refers to solutes not present in refs. 1 and 2.

[b]Highest uremic concentration found after suppression of outliers: parathyroid hormone=1676±69 ng/L.[86]

Table 5. Comparison of the average uremic concentration (M) with normal concentrations (N): 21 solutes scoring above 10

Molecule	M/N
Phenylacetic acid	334
Neopterin	60.3
Guanidino succinic acid	47.7
4-Pyridone-3-carboxamide-1-β-D-ribonucleoside	44.2
Indoxyl sulfate, total	43.2
Hippuric acid, free	41.3
Kynurenic acid	27.6
Hippuric acid, total	23.8
p-Cresylsulfate, free	21.9
Methylguanidine	19.1
β-Trace protein	16.6
β2-Microglobulin	15.9
Carboxymethyllysine	15.4
Oxalate	13.0
N-Methyl-4-pyridone-3-carboxamide	12.5
IL-8	12.3
PTH	11.2
Nicotinamide	11.2
p-Cresylsulfate, total	11.0
Complement factor D	10.8
Osteocalcin	10.5

Table 6. Comparison of the average uremic concentration (M) with normal concentrations (N): 18 solutes scoring between one and two

Molecule	M/N
α1-Acid glycoprotein	1.96
Nonanal	1.84
Dihydroxyphenylalanine	1.72
Hypoxanthine	1.72
3-Carboxy-4-methyl-5-propyl-2-furan-propanoic acid	1.70
Soluble intracellular adhesion molecule-1	1.67
Uric acid	1.59
Cysteine	1.56
IGF-1	1.52
Adiponectin	1.50
IL-10	1.49
IL-6	1.48
Decanal	1.36
Prolactin	1.33
8-Hydroxy-2'-deoxyguanosine	1.28
2-Octenal	1.25
Heptanal	1.20
Calcitonin	1.09

Table 7. Comparison of the average uremic concentration (M) with normal concentrations (N): four solutes scoring below one

Molecules	M/N
Guanidinoacetic acid	0.99
Spermidine	0.96
Malondialdehyde	0.85
Spermine	0.30

DISCUSSION

With the appearance of extrarenal blood purification techniques, survival of patients suffering from ESRD greatly improved, thus confirming the major influence of uremic toxins and fluid control on outcome. Subsequently, many uremic solutes have been shown to be associated with mortality or morbidity in epidemiologic studies,[40,49,87–89] and direct adverse effects have been proven in experimental models.[31,45,90] Still, the uremic milieu is complex, and solutes may affect various biologic systems, possibly in a synergetic manner. This effect requires caution when extrapolating results from *in vitro* systems, and only clinical trials can allow for drawing conclusions on the clinical significance of biologic effects. Randomized clinical trials of retention solute removal on hard outcomes such as mortality are scarce, and results are not always supportive of improved survival.[91,92] Nonetheless, there is a substantial number of studies which quantified the circulating levels of various compounds in uremic patients to identify retention solutes or to evaluate solute removal. The present report is the continuation of the first encyclopedic work[1] published in 2003, which aimed to identify and classify uremic retention solutes. The goal of the present update was to reflect the practical definition of uremic retention solutes in recent clinical studies, extend the list of uremic retention solutes with the new compounds that have been reported in the literature since 2003, and compare the values reported in both analyses.

The review of the literature resulted in the inclusion of 88 published articles, with data on 32 known uremic toxins and 56 newly identified retention solutes, which were consequently added to the list of uremic toxins. A time limit was necessary to perform this analysis, but in such a dynamic field, new toxins are continuously identified. Recently, the strong vasoconstrictor uridine adenosine tetraphosphate (Up$_4$A) was described as a uremic toxin. CKD patients had, on average, a 5.2-fold higher Up$_4$A plasma concentration compared with healthy subjects, and this increased Up$_4$A concentration may influence blood pressure, proliferation rate of vascular smooth muscle cells, and calcification processes in CKD patients.[93,94] A molecular mass threshold was also applied, and molecules above 60 kD were excluded from the analysis, because these solutes cannot be filtered by the glomeruli. However, although their concentrations are not directly associated with glomerular function, several large acute phase proteins (α2-macroglobulin, fibrinogen,

concentrations (Supplemental Material 4). There was no sign of a general over- or underestimation of normal concentrations N compared with other available control concentrations ($P=0.10$), and using these control concentrations as normal concentrations did not substantially influence M/N ratios ($r^2=0.68$).
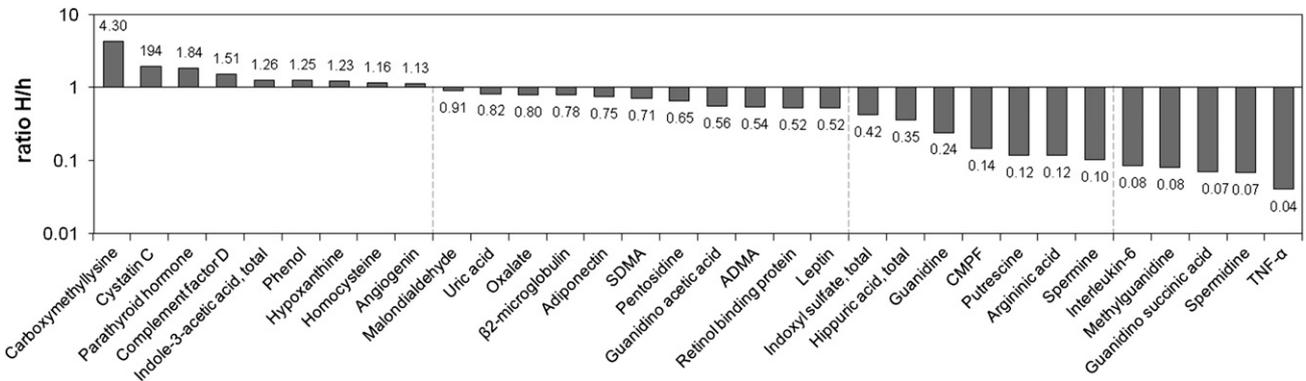
Figure 1. Relative change in highest uremic concentrations of known retention solutes. The H/h index is the ratio of the highest uremic concentration found in the present analysis to the highest concentration presented in the previous reviews.[1,2]
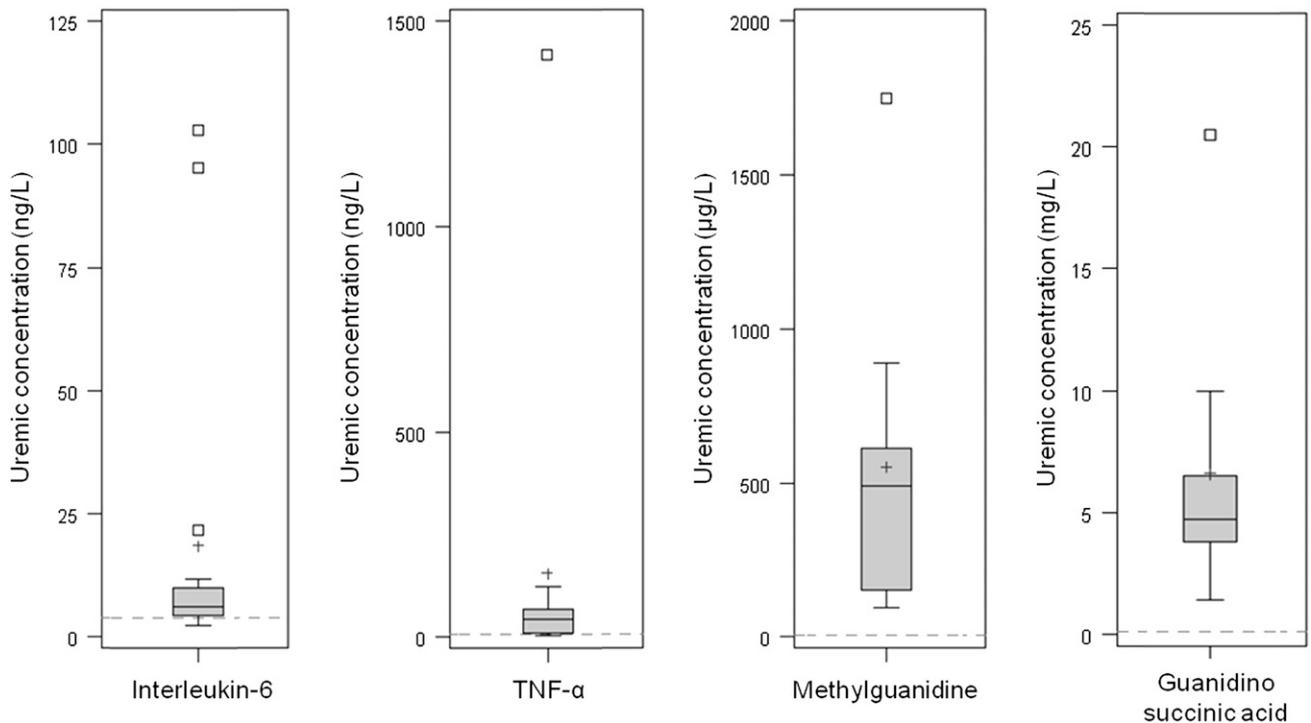
Figure 2. Distribution of the concentrations of IL-6, TNF-α, methylguanidine, and guanidino succinic acid found in uremic populations. Data from the present analysis and the previous review[1] were pooled and displayed. Dashed lines represent concentrations found in the general population. Values out of Tukey's inner fence were identified as suspected outliers.

myeloperoxidase, and IL-12)[56,95–97] and endothelium-related proteins (vascular cell adhesion molecule 1, vascular endothelial growth factor 1, and soluble vascular endothelial growth factor receptor)[45,70] were increased in CKD, and they could have a significant diagnostic and pathophysiologic value.[98,99] However, reviewing all solutes that were evaluated in a sample of publications on uremic patients, we identified molecules that were reduced in uremia (bilirubin, reduced glutathione, α1-antitrypsin, arginine, and homoarginine).[10,35,81,100] Because these solutes are associated with antioxidant, anti-inflammatory, and vasodilating

properties, their reduced concentration could be involved in additional adverse effects of uremia.

Performing this analysis, we faced several limitations related to data reporting. For instance, it is still frequent for -omics studies to report only strengths of associations without reporting concentrations.[4,64] We were also limited by the use relative units, particularly concerning enzymes and advanced glycation end products (AGEs).[24,39,101,102] Furthermore, we chose not to include results on κ-light chains, because the concentration found in uremic patients was less than 1% of the values previously

found in healthy or CKD populations (30–80 mg/L).[60,103] Finally, because it was shown in 2005 that the reported serum p-cresol concentrations were actually caused by an artifact linked to deproteinization of p-cresylsulfate and p-cresylglucuronide,[104,105] results on p-cresol were also excluded. Still, we found eight reports on p-cresol published after 2005. Warnings concerning the study of p-cresol have recently been discussed elsewhere.[106]

Our research identified 32 solutes that had been included in the previous report in 2003 and observed considerable differences, with a general tendency to lower concentrations. Several reasons could explain these discrepancies. A general improvement in dialysis techniques could result in a more efficient toxin removal with, eventually, better blood profiles of dialyzed patients. This finding was shown with hemodiafiltration over hemodialysis for middle molecules and small water-soluble molecules such as urea.[27,32,56,107] Methodological choices, in terms of quantification techniques, could also influence reported concentrations. A cross-sectional comparison of HPLC, MS, and ELISA determinations of ADMA showed large variability across and within techniques, and it found that MS was the most reliable measurement method.[108] A similar technical issue could explain the lower concentrations in guanidino compounds found in our analysis. Indeed, recent results were measured using IEC, whereas the previously reported values had been quantified by HPLC.

There were four compounds that did not clearly show uremic retention, although they had been presented as uremic solutes in previous classifications.[1,2] Results on guanidinoacetic acid confirmed that it is not a uremic toxin, because concentrations consistently decreased with CKD severity.[13,18,19,64] Concerning spermine and spermidine, normal concentrations were not presented in the original review.[1] Results from age-matched normal and uremic patients showed that both concentrations and particularly, spermine were reduced in uremia.[53] Furthermore, there was a clear increase in both acrolein, a toxic spermine oxidation product, and in the activity of the responsible oxidase.[53] Interestingly, concentrations in malondialdehyde actually increased with CKD severity, which supports its classification as a uremic retention solute.[22,84]

Whether normal and uremic concentrations of retention solutes were comparable is a difficult aspect of this study, especially when results are drawn from different sources. When available, we used internal controls to estimate normal concentrations. Furthermore, to minimize the impact of the methodological choices, efforts were made to select studies using the same techniques. When reference ranges were given by age strata, we used age-matched populations. Comfortingly, sensitivity analyses suggested that the choice of normal concentrations did not substantially modify results.

We believe that this analysis provided relevant information on uremic retention solutes, which should be taken into account when testing uremic retention solute toxicity. With the addition of 56 uremic retention solutes, this extended classification of uremic retention solutes proved that it was timely and useful. It should be used jointly with the previous publications[1,2] as a complementary tool, giving additional insight into concentration values and variability of retention solutes found in uremic populations.

CONCISE METHODS

Search and Selection Criteria

The PubMed database was searched for articles published from January 1, 2003 to April 1, 2011 dealing with uremic toxins in renal patients using the following keywords: (uremi* OR uraemi*) AND (toxin* OR toxic*) AND patients AND (renal failure chronic OR kidney disease OR CKD). The aim was to retrieve uremic and normal serum or plasma concentrations of uremic retention solutes and exclude common solutes such as creatinine and urea, inorganic compounds, and large molecules over 60 kD that are not filtered by the glomerulus. To be included in our study, plasma concentrations had to be measured in adult patients with CKD stages 3–5. All solutes that had been listed in the previous encyclopedic reviews were included. For other solutes, only those solutes in which uremic concentrations exceeded normal concentrations were included. For populations receiving renal replacement therapy by peritoneal or hemodialysis, only pretreatment concentrations were included. To avoid the effect of metabolic differences, reactive carbonyl compounds (RCCs), AGEs, and advanced lipoperoxidation end products were measured in nondiabetic patients. When necessary, blood concentrations were estimated using a blood protein concentration of 70 g/L and serum albumin concentration of 35 g/L.[1,109]

All concentrations are presented in grams per liter. Data that were originally reported in moles per liter were transformed into mass concentrations by multiplying with the molecular weight. Suspected unit errors were recorded and studied separately. All original concentrations were given as mean (SD) or median (range). For each solute, we calculated M and identified H and L reported concentrations. In contrast with our previous study, the maximal individual concentrations were not recorded or estimated. However, under the hypothesis of a normal distribution, it can be approximated using the formula $C_{individual\ max} = M + 2SD$.[1]

For each solute, we reported N measured in healthy controls. N values were preferably extracted from the same publication reporting the highest concentration H or if not possible, another included study. Otherwise, normal concentration was taken from the 2003 encyclopedic review or external sources. N levels were preferably recorded as means (SD). If reported as a range or confidence interval, the upper bound or maximal value was extracted and presented using a less than symbol. Finally, for solutes of which data were presented in the original review, the 2003 h value was also extracted.

Calculations and Analyses

The relative solute retention in uremia was studied using M/N. Solutes scoring more than 10 were arbitrarily defined as largely increased, whereas scorings below 2 were interpreted as showing limited evidence of uremic retention. For solutes that had been included in the 2003 review, highest reported values were compared using the H/h ratio. Between-study variability was assessed using H/L, which is an

index of the width of the concentration range. Low evidence of variability has been previously defined as a ratio below 3, whereas a ratio exceeding 8.5 is evidence of substantial variability.[5] Additionally, the coefficient of variability was calculated for results based on four data or more using the formula coefficient of variability= SD/M. Finally, graphical analyses of data dispersion using box plots were undertaken for solutes that had four values or more. Values outside Tukey's inner fence were considered to be suspected outliers.[2,110] Tukey's inner fence is defined as the range between 1.5 times the interquartile range below the first quartile to 1.5 times the interquartile range above the third quartile. Sensitivity analyses were performed to evaluate the influence of methodological choices. The effect of CKD stage (predialytic versus dialytic stages) on concentrations was assessed by exact Wilcoxon tests for two independent samples. The difference between N and other available control concentrations was assessed by a paired t test. The correlation between M/N ratios using N or other control concentrations was evaluated using Pearson's product moment correlation coefficient. Statistical tests were performed with a 5% type I error. Calculations and analyses were performed with Excel 2007 (Microsoft Corp., Redmond, WA) and SAS version 9.2 (SAS Institute, Cary, NC).

ACKNOWLEDGMENTS

DISCLOSURES

None.

REFERENCES

1. Vanholder R, De Smet R, Glorieux G, Argilés A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn PP, Deppisch R, Descamps-Latscha B, Henle T, Jörres A, Lemke HD, Massy ZA, Passlick-Deetjen J, Rodriguez M, Stegmayr B, Stenvinkel P, Tetta C, Wanner C, Zidek W; European Uremic Toxin Work Group (EUTox): Review on uremic toxins: Classification, concentration, and interindividual variability. *Kidney Int* 63: 1934–1943, 2003

2. Meert N, Schepers E, De Smet R, Argiles A, Cohen G, Deppisch R, Drüeke T, Massy Z, Spasovski G, Stegmayr B, Zidek W, Jankowski J, Vanholder R: Inconsistency of reported uremic toxin concentrations. *Artif Organs* 31: 600–611, 2007

3. Vanholder R, Meert N, Schepers E, Glorieux G, Argiles A, Brunet P, Cohen G, Drüeke T, Mischak H, Spasovski G, Massy Z, Jankowski J; European Uremic Toxin Work Group (EUTox): Review on uraemic solutes II—variability in reported concentrations: causes and consequences. *Nephrol Dial Transplant* 22: 3115–3121, 2007

4. Rhee EP, Souza A, Farrell L, Pollak MR, Lewis GD, Steele DJ, Thadhani R, Clish CB, Greka A, Gerszten RE: Metabolite profiling identifies markers of uremia. *J Am Soc Nephrol* 21: 1041–1051, 2010

5. Aronov PA, Luo FJ-G, Plummer NS, Quan Z, Holmes S, Hostetter TH, Meyer TW: Colonic contribution to uremic solutes. *J Am Soc Nephrol* 22: 1769–1776, 2011

6. Alhamdani M-SS, Al-Kassir A-HAM, Jaleel NA, Hmood AM, Ali HM: Elevated levels of alkanals, alkenals and 4-HO-alkenals in plasma of hemodialysis patients. *Am J Nephrol* 26: 299–303, 2006

7. Slominska EM, Carrey EA, Foks H, Orlewska C, Wieczerzak E, Sowinski P, Yacoub MH, Marinaki AM, Simmonds HA, Smolenski RT: A novel nucleotide found in human erythrocytes, 4-pyridone-3-carboxamide-1-beta-D-ribonucleoside triphosphate. *J Biol Chem* 281: 32057–32064, 2006

8. Goto S, Fujii H, Hamada Y, Yoshiya K, Fukagawa M: Association between indoxyl sulfate and skeletal resistance in hemodialysis patients. *Ther Apher Dial* 14: 417–423, 2010

9. Forlenza MJ, Miller GE: Increased serum levels of 8-hydroxy-2'-deoxyguanosine in clinical depression. *Psychosom Med* 68: 1–7, 2006

10. Taes YEC, Marescau B, De Vriese A, De Deyn PP, Schepers E, Vanholder R, Delanghe JR: Guanidino compounds after creatine supplementation in renal failure patients and their relation to inflammatory status. *Nephrol Dial Transplant* 23: 1330–1335, 2008

11. Derave W, Marescau B, Vanden Eede E, Eijnde BO, De Deyn PP, Hespel P: Plasma guanidino compounds are altered by oral creatine supplementation in healthy humans. *J Appl Physiol* 97: 852–857, 2004

12. Tankiewicz A, Pawlak D, Pawlak K, Szewc D, Myśliwiec M, Buczko W: Anthranilic acid-uraemic toxin damaged red cell's membrane. *Int Urol Nephrol* 37: 621–627, 2005

13. Pere P, Salonen M, Jokinen M, Rosenberg PH, Neuvonen PJ, Haasio J: Pharmacokinetics of ropivacaine in uremic and nonuremic patients after axillary brachial plexus block. *Anesth Analg* 96: 563–569, 2003

14. Kielstein JT, Böger RH, Bode-Böger SM, Martens-Lobenhoffer J, Lonnemann G, Frölich JC, Haller H, Fliser D: Low dialysance of asymmetric dimethylarginine (ADMA)—in vivo and in vitro evidence of significant protein binding. *Clin Nephrol* 62: 295–300, 2004

15. Zhang D-L, Liu J, Liu S, Zhang Y, Liu W-H: The differences of asymmetric dimethylarginine removal by different dialysis treatments. *Ren Fail* 32: 935–940, 2010

16. Hasuike Y, Nakanishi T, Moriguchi R, Otaki Y, Nanami M, Hama Y, Naka M, Miyagawa K, Izumi M, Takamitsu Y: Accumulation of cyanide and thiocyanate in haemodialysis patients. *Nephrol Dial Transplant* 19: 1474–1479, 2004

17. Raff AC, Lieu S, Melamed ML, Quan Z, Ponda M, Meyer TW, Hostetter TH: Relationship of impaired olfactory function in ESRD to malnutrition and retained uremic molecules. *Am J Kidney Dis* 52: 102–110, 2008

18. Tanaka A, Takahashi Y, Mizokuchi M, Shimada N, Koide H: Plasma, urinary, and erythrocyte concentrations of guanidino compounds in patients with chronic renal failure. *Ren Fail* 21: 499–514, 1999

19. De Deyn P, Marescau B, Lornoy W, Becaus I, Lowenthal A: Guanidino compounds in uraemic dialysed patients. *Clin Chim Acta* 157: 143–150, 1986

20. Shahbazian H, Zand Moghadam A, Ehsanpour A, Khazaali M: Changes in plasma concentrations of hypoxanthine and uric acid before and after hemodialysis. *Iran J Kidney Dis* 3: 151–155, 2009

21. Kock R, Delvoux B, Sigmund M, Greiling H: A comparative study of the concentrations of hypoxanthine, xanthine, uric acid and allantoin in the peripheral blood of normals and patients with acute myocardial infarction and other ischaemic diseases. *Eur J Clin Chem Clin Biochem* 32: 837–842, 1994

22. Rutkowski P, Słominska EM, Szołkiewicz M, Aleksandrowicz E, Smolenski RT, Wołyniec W, Renke M, Wisterowicz K, Swierczynski J, Rutkowski B: Relationship between uremic toxins and oxidative stress in patients with chronic renal failure. *Scand J Urol Nephrol* 41: 243–248, 2007

23. Bayés B, Pastor MC, Bonal J, Juncà J, Romero R: Homocysteine and lipid peroxidation in haemodialysis: Role of folinic acid and vitamin E. *Nephrol Dial Transplant* 16: 2172–2175, 2001

24. Fragedaki E, Nebel M, Schupp N, Sebekova K, Völkel W, Klassen A, Pischetsrieder M, Frischmann M, Niwa T, Vienken J, Heidland A, Stopper H: Genomic damage and circulating AGE levels in patients undergoing daily versus standard haemodialysis. *Nephrol Dial Transplant* 20: 1936–1943, 2005

25. Rutkowski B, Slominska E, Szolkiewicz M, Smolenski RT, Striley C, Rutkowski P, Swierczynski J: N-methyl-2-pyridone-5-carboxamide: a novel uremic toxin? *Kidney Int Suppl* 84: S19–S21, 2003

26. Carrey EA, Smolenski RT, Edbury SM, Laurence A, Marinaki AM, Duley JA, Zhu L, Goldsmith DJ, Simmonds HA: Origin and characteristics of an unusual pyridine nucleotide accumulating in erythrocytes: Positive correlation with degree of renal failure. *Clin Chim Acta* 335: 117–129, 2003

27. Beerenhout CH, Luik AJ, Jeuken-Mertens SGJ, Bekers O, Menheere P, Hover L, Klaassen L, van der Sande FM, Cheriex EC, Meert N, Leunissen KM, Kooman JP: Pre-dilution on-line haemofiltration vs low-flux haemodialysis: a randomized prospective study. *Nephrol Dial Transplant* 20: 1155–1163, 2005

28. Abd-Allah NM, Hassan FH, Esmat AY, Hammad SA: Age dependence of the levels of plasma norepinephrine, aldosterone, renin activity and urinary vanillylmandelic acid in normal and essential hypertensives. *Biol Res* 37: 95–106, 2004

29. Mydlík M, Derzsiová K: Oxalic Acid as a uremic toxin. *J Ren Nutr* 18: 33–39, 2008

30. Marangella M, Petrarulo M, Mandolfo S, Vitale C, Cosseddu D, Linari F: Plasma profiles and dialysis kinetics of oxalate in patients receiving hemodialysis. *Nephron* 60: 74–80, 1992

31. Jankowski J, van der Giet M, Jankowski V, Schmidt S, Hemeier M, Mahn B, Giebing G, Tolle M, Luftmann H, Schluter H, Zidek W, Tepel M: Increased plasma phenylacetic acid in patients with end-stage renal failure inhibits iNOS expression. *J Clin Invest* 112: 256–264, 2003

32. Kalousová M, Kielstein JT, Hodková M, Zima T, Dusilová-Sulková S, Martens-Lobenhoffer J, Bode-Boger SM: No benefit of hemodiafiltration over hemodialysis in lowering elevated levels of asymmetric dimethylarginine in ESRD patients. *Blood Purif* 24: 439–444, 2006

33. Bain MA, Faull R, Fornasini G, Milne RW, Evans AM: Accumulation of trimethylamine and trimethylamine-N-oxide in end-stage renal disease patients undergoing haemodialysis. *Nephrol Dial Transplant* 21: 1300–1304, 2006

34. Taki K, Tsuruta Y, Niwa T: Indoxyl sulfate and atherosclerotic risk factors in hemodialysis patients. *Am J Nephrol* 27: 30–35, 2007

35. Müller C, Eisenbrand G, Gradinger M, Rath T, Albert FW, Vienken J, Singh R, Farmer PB, Stockis JP, Janzowski C: Effects of hemodialysis, dialyser type and iron infusion on oxidative stress in uremic patients. *Free Radic Res* 38: 1093–1100, 2004

36. De Smet R, Dhondt A, Eloot S, Galli F, Waterloos MA, Vanholder R: Effect of the super-flux cellulose triacetate dialyser membrane on the removal of non-protein-bound and protein-bound uraemic solutes. *Nephrol Dial Transplant* 22: 2006–2012, 2007

37. Sassa T, Matsuno H, Niwa M, Kozawa O, Takeda N, Niwa T, Kumada T, Uematsu T: Measurement of furancarboxylic acid, a candidate for uremic toxin, in human serum, hair, and sweat, and analysis of pharmacological actions in vitro. *Arch Toxicol* 73: 649–654, 2000

38. Sakata K, Kashiwagi K, Sharmin S, Ueda S, Irie Y, Murotani N, Igarashi K: Increase in putrescine, amine oxidase, and acrolein in plasma of renal failure patients. *Biochem Biophys Res Commun* 305: 143–149, 2003

39. Ueda S, Yamagishi S, Takeuchi M, Kohno K, Shibata R, Matsumoto Y, Kaneyuki U, Fujimura T, Hayashida A, Okuda S: Oral adsorbent AST-120 decreases serum levels of AGEs in patients with chronic renal failure. *Mol Med* 12: 180–184, 2006

40. Wagner Z, Molnár M, Molnár GA, Tamaskó M, Laczy B, Wagner L, Csiky B, Heidland A, Nagy J, Wittmann I: Serum carboxymethyllysine predicts mortality in hemodialysis patients. *Am J Kidney Dis* 47: 294–300, 2006

41. Sutherland WHF, Gieseg SP, Walker RJ, de Jong SA, Firth CA, Scott N: Serum protein-bound 3,4-dihydroxyphenylalanine and related products of protein oxidation and chronic hemodialysis. *Ren Fail* 25: 997–1009, 2003

42. Dhondt AW, Vanholder RC, De Smet RV, Claus SA, Waterloos MA, Glorieux GL, Delanghe JR, Lameire NH: Studies on dialysate mixing in the Genius single-pass batch system for hemodialysis therapy. *Kidney Int* 63: 1540–1547, 2003

43. Farrell PC, Gotch FA, Peters JH, Berridge BJ Jr, Lam M: Binding of hippurate in normal plasma and in uremic plasma pre- and post-dialysis. *Nephron* 20: 40–46, 1978

44. Perna AF, Satta E, Acanfora F, Lombardi C, Ingrosso D, De Santo NG: Increased plasma protein homocysteinylation in hemodialysis patients. *Kidney Int* 69: 869–876, 2006

45. Jourde-Chiche N, Dou L, Sabatier F, Calaf R, Cerini C, Robert S, Camoin-Jau L, Charpiot P, Argiles A, Dignat-George F, Brunet P: Levels of circulating endothelial progenitor cells are related to uremic toxins and vascular injury in hemodialysis patients. *J Thromb Haemost* 7: 1576–1584, 2009

46. Lee C-T, Kuo C-C, Chen Y-M, Hsu CY, Lee WC, Tsai YC, Ng HY, Kuo LC, Chiou TT, Yang YK, Cheng BC, Chen JB: Factors associated with blood concentrations of indoxyl sulfate and p-cresol in patients undergoing peritoneal dialysis. *Perit Dial Int* 30: 456–463, 2010

47. Ujhelyi L, Balla G, Jeney V, Varga Z, Nagy E, Vercellotti GM, Agarwal A, Eaton JW, Balla J: Hemodialysis reduces inhibitory effect of plasma ultrafiltrate on LDL oxidation and subsequent endothelial reactions. *Kidney Int* 69: 144–151, 2006

48. Niwa T, Takeda N, Tatematsu A, Maeda K: Accumulation of indoxyl sulfate, an inhibitor of drug-binding, in uremic serum as demonstrated by internal-surface reversed-phase liquid chromatography. *Clin Chem* 34: 2264–2267, 1988

49. Liabeuf S, Barreto DV, Barreto FC, Meert N, Glorieux G, Schepers E, Temmar M, Choukroun G, Vanholder R, Massy ZA; European Uraemic Toxin Work Group (EUTox): Free p-cresylsulphate is a predictor of mortality in patients at different stages of chronic kidney disease. *Nephrol Dial Transplant* 25: 1183–1191, 2010

50. Taneda S, Monnier VM: ELISA of pentosidine, an advanced glycation end product, in biological specimens. *Clin Chem* 40: 1766–1773, 1994

51. Nakabayashi I, Nakamura M, Kawakami K, Ohta T, Kato I, Uchida K, Yoshida M: Effects of synbiotic treatment on serum level of p-cresol in haemodialysis patients: a preliminary study. *Nephrol Dial Transplant* 26: 1094–1098, 2011

52. Hida M, Aiba Y, Sawamura S, Suzuki N, Satoh T, Koga Y: Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oral administration of Lebenin, a lactic acid bacteria preparation, to uremic patients undergoing hemodialysis. *Nephron* 74: 349–355, 1996

53. Sakata K, Kashiwagi K, Sharmin S, Ueda S, Igarashi K: Acrolein produced from polyamines as one of the uraemic toxins. *Biochem Soc Trans* 31: 371–374, 2003

54. Testa A, Gentilhomme H, Le Carrer D, Orsonneau JL: In vivo removal of high- and low-molecular-weight compounds in hemodiafiltration with on-line regeneration of ultrafiltrate. *Nephron Clin Pract* 104: c55–c60, 2006

55. Ekström B, Berggård I: Human alpha1-microglobulin. Purification procedure, chemical and physiochemical properties. *J Biol Chem* 252: 8048–8057, 1977

56. Lindström V, Grubb A, Alquist Hegbrant M, Christensson A: Different elimination patterns of beta-trace protein, beta2-microglobulin and cystatin C in haemodialysis, haemodiafiltration and haemofiltration. *Scand J Clin Lab Invest* 68: 685–691, 2008

57. Pöge U, Gerhardt TM, Stoffel-Wagner B, Palmedo H, Klehr HU, Sauerbruch T, Woitas RP: beta-Trace protein is an alternative marker for glomerular filtration rate in renal transplantation patients. *Clin Chem* 51: 1531–1533, 2005

58. Kim S, Oh KH, Chin HJ, Na KY, Kim YS, Chae DW, Ahn C, Han JS, Kim S, Joo KW: Effective removal of leptin via hemodiafiltration with on-line endogenous reinfusion therapy. *Clin Nephrol* 72: 442–448, 2009

59. Guebre-Egziabher F, Bernhard J, Funahashi T, Hadj-Aissa A, Fouque D: Adiponectin in chronic kidney disease is related more to metabolic disturbances than to decline in renal function. *Nephrol Dial Transplant* 20: 129–134, 2005

60. Ouseph R, Hutchison CA, Ward RA: Differences in solute removal by two high-flux membranes of nominally similar synthetic polymers. *Nephrol Dial Transplant* 23: 1704–1712, 2008

61. Shapiro R, Strydom DJ, Olson KA, Vallee BL: Isolation of angiogenin from normal human plasma. *Biochemistry* 26: 5141–5146, 1987

62. d'Herbomez M, Caron P, Bauters C, Do Cao C, Schlienger JL, Sapin R, Baldet L, Carnaille B, Wémeau JL; French Group GTE (Groupe des Tumeurs Endocrines): Reference range of serum calcitonin levels in humans: Influence of calcitonin assays, sex, age, and cigarette smoking. *Eur J Endocrinol* 157: 749–755, 2007

63. Pascual M, Steiger G, Estreicher J, Macon K, Volanakis JE, Schifferli JA: Metabolism of complement factor D in renal failure. *Kidney Int* 34: 529–536, 1988

64. Toyohara T, Akiyama Y, Suzuki T, Takeuchi Y, Mishima E, Tanemoto M, Momose A, Toki N, Sato H, Nakayama M, Hozawa A, Tsuji I, Ito S, Soga T, Abe T: Metabolomic profiling of uremic solutes in CKD patients. *Hypertens Res* 33: 944–952, 2010

65. Kabanda A, Jadoul M, Pochet JM, Lauwerys R, van Ypersele de Strihou C, Bernard A: Determinants of the serum concentrations of low molecular weight proteins in patients on maintenance hemodialysis. *Kidney Int* 45: 1689–1696, 1994

66. Shigematsu T, Kazama JJ, Yamashita T, Fukumoto S, Hosoya T, Gejyo F, Fukagawa M: Possible involvement of circulating fibroblast growth factor 23 in the development of secondary hyperparathyroidism associated with renal insufficiency. *Am J Kidney Dis* 44: 250–256, 2004

67. Yu M, Kim YJ, Kang D-H: Indoxyl sulfate-induced endothelial dysfunction in patients with chronic kidney disease via an induction of oxidative stress. *Clin J Am Soc Nephrol* 6: 30–39, 2011

68. Friedrich N, Alte D, Völzke H, Spilcke-Liss E, Lüdemann J, Lerch MM, Kohlmann T, Nauck M, Wallaschofski H: Reference ranges of serum IGF-1 and IGFBP-3 levels in a general adult population: Results of the Study of Health in Pomerania (SHIP). *Growth Horm IGF Res* 18: 228–237, 2008

69. Rysz J, Banach M, Cialkowska-Rysz A, Stolarek R, Barylski M, Drozdz J, Okonski P: Blood serum levels of IL-2, IL-6, IL-8, TNF-alpha and IL-1beta in patients on maintenance hemodialysis. *Cell Mol Immunol* 3: 151–154, 2006

70. Stinghen AE, Gonçalves SM, Martines EG, Nakao LS, Riella MC, Aita CA, Pecoits-Filho R: Increased plasma and endothelial cell expression of chemokines and adhesion molecules in chronic kidney disease. *Nephron Clin Pract* 111: c117–c126, 2009

71. Berrahmoune H, Lamont JV, Herbeth B, FitzGerald PS, Visvikis-Siest S: Biological determinants of and reference values for plasma interleukin-8, monocyte chemoattractant protein-1, epidermal growth factor, and vascular endothelial growth factor: Results from the STANISLAS cohort. *Clin Chem* 52: 504–510, 2006

72. Sarris AH, Kliche KO, Pethambaram P, Preti A, Tucker S, Jackow C, Messina O, Pugh W, Hagemeister FB, McLaughlin P, Rodriguez MA, Romaguera J, Fritsche H, Witzig T, Duvic M, Andreeff M, Cabanillas F: Interleukin-10 levels are often elevated in serum of adults with Hodgkin's disease and are associated with inferior failure-free survival. *Ann Oncol* 10: 433–440, 1999

73. Ottonello L, Gnerre P, Bertolotto M, Mancini M, Dapino P, Russo R, Garibotto G, Barreca T, Dallegri F: Leptin as a uremic toxin interferes with neutrophil chemotaxis. *J Am Soc Nephrol* 15: 2366–2372, 2004

74. Dagogo-Jack S, Ovalle F, Landt M, Gearing B, Coyne DW: Hyperleptinemia in patients with end-stage renal disease undergoing continuous ambulatory peritoneal dialysis. *Perit Dial Int* 18: 34–40, 1998

75. Chen IW, David R, Maxon HR, Sperling M, Stein EA: Age-, sex-, and race-related differences in myoglobin concentrations in the serum of healthy persons. *Clin Chem* 26: 1864–1868, 1980

76. Kusec V, Lukić M, Stavljenić-Rukavina A: Osteocalcin reference range in a Croatian population sample. *Acta Med Croatica* 48: 59–62, 1994

77. Scholze A, Jankowski V, Henning L, Haass W, Wittstock A, Suvd-Erdene S, Zidek W, Tepel M, Jankowski J: Phenylacetic acid and arterial vascular properties in patients with chronic kidney disease stage 5 on hemodialysis therapy. *Nephron Clin Pract* 107: c1–c6, 2007

78. Tsukamoto Y, Hanaoka M, Matsuo T, Saruta T, Nomura M, Takahashi Y: Effect of 22-oxacalcitriol on bone histology of hemodialyzed patients with severe secondary hyperparathyroidism. *Am J Kidney Dis* 35: 458–464, 2000

79. Beltran L, Fahie-Wilson MN, McKenna TJ, Kavanagh L, Smith TP: Serum total prolactin and monomeric prolactin reference intervals determined by precipitation with polyethylene glycol: Evaluation and validation on common immunoassay platforms. *Clin Chem* 54: 1673–1681, 2008

80. Hung C-Y, Chen Y-L, Chen C-S, Yang C-S, Peng S-J: Association of leptin with hemodialysis-related muscle cramps: A cross-sectional study. *Blood Purif* 27: 159–164, 2009

81. Ficheux A, Gayrard N, Szwarc I, Andress D, Soullier S, Duny Y, Goubert G, Thomas M, Bismuth-Mondolfo J, Daurès JP, Brunet P, Servel MF, Argilés A: The use of SDS-PAGE scanning of spent dialysate to assess uraemic toxin removal by dialysis. *Nephrol Dial Transplant* 26: 2281–2289, 2011

82. Hackman A, Abe Y, Insull W Jr, Pownall H, Smith L, Dunn K, Gotto AM Jr, Ballantyne CM: Levels of soluble cell adhesion molecules in patients with dyslipidemia. *Circulation* 93: 1334–1338, 1996

83. Nolin TD, Appiah K, Kendrick SA, Le P, McMonagle E, Himmelfarb J: Hemodialysis acutely improves hepatic CYP3A4 metabolic activity. *J Am Soc Nephrol* 17: 2363–2367, 2006

84. Terrier-Lenglet A, Nollet A, Liabeuf S, Barreto DV, Brazier M, Lemke HD, Vanholder R, Choukroun G, Massy ZA; Groupe EUTox (European Uremic toxin): Plasma malondialdehyde may not predict mortality in patient with chronic kidney disease. *Nephrol Ther* 7: 219–224, 2011

85. Splendiani G, De Angelis S, Tullio T, Ferranini M, Dessì MR, Pastore A, Casciani S, Liberatoscioli L, Federici G, Cortese C: Selective adsorption of homocysteine using an HFR-ON LINE technique. *Artif Organs* 28: 592–595, 2004

86. Chen ZJ, Cao G, Tang WX, Lv XY, Huang SM, Qin W, Ping F, Ye T: A randomized controlled trial of high-permeability haemodialysis against conventional haemodialysis in the treatment of uraemic pruritus. *Clin Exp Dermatol* 34: 679–683, 2009

87. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM: Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol* 15: 2208–2218, 2004

88. Bammens B, Evenepoel P, Keuleers H, Verbeke K, Vanrenterghem Y: Free serum concentrations of the protein-bound retention solute p-cresol predict mortality in hemodialysis patients. *Kidney Int* 69: 1081–1087, 2006

89. Cheung AK, Rocco MV, Yan G, Leypoldt JK, Levin NW, Greene T, Agodoa L, Bailey J, Beck GJ, Clark W, Levey AS, Ornt DB, Schulman G, Schwab S, Teehan B, Eknoyan G: Serum beta-2 microglobulin levels predict mortality in dialysis patients: Results of the HEMO study. *J Am Soc Nephrol* 17: 546–555, 2006

90. Schepers E, Glorieux G, Dou L, Cerini C, Gayrard N, Louvet L, Maugard C, Preus P, Rodriguez-Ortiz M, Argiles A, Brunet P, Cohen G, Jankowski J, Jankowski V, Massy Z, Rodriguez M, Vanholder R; European Uremic Toxin Work Group (EUTox): Guanidino compounds as cause of cardiovascular damage in chronic kidney disease: an in vitro evaluation. *Blood Purif* 30: 277–287, 2010

91. Zoungas S, McGrath BP, Branley P, Kerr PG, Muske C, Wolfe R, Atkins RC, Nicholls K, Fraenkel M, Hutchison BG, Walker R, McNeil JJ: Cardiovascular morbidity and mortality in the Atherosclerosis and Folic Acid Supplementation Trial (ASFAST) in chronic renal failure: a multicenter, randomized, controlled trial. *J Am Coll Cardiol* 47: 1108–1116, 2006

92. Locatelli F, Martin-Malo A, Hannedouche T, Loureiro A, Papadimitriou M, Wizemann V, Jacobson SH, Czekalski S, Ronco C, Vanholder R; Membrane Permeability Outcome (MPO) Study Group: Effect of membrane permeability on survival of hemodialysis patients. *J Am Soc Nephrol* 20: 645–654, 2009

93. Jankowski V, Tölle M, Vanholder R, Schönfelder G, van der Giet M, Henning L, Schlüter H, Paul M, Zidek W, Jankowski J: Uridine adenosine tetraphosphate: A novel endothelium-derived vasoconstrictive factor. *Nat Med* 11: 223–227, 2005

94. Schuchardt M, Tölle M, Prüfer J, Prüfer N, Huang T, Jankowski V, Jankowski J, Zidek W, van der Giet M: Uridine adenosine tetraphosphate activation of the purinergic receptor P2Y enhances in vitro vascular calcification. *Kidney Int* 81: 256–265, 2012

95. Maduell F, Navarro V, Torregrosa E, Rius A, Dicenta F, Cruz MC, Ferrero JA: Change from three times a week on-line hemodiafiltration to short daily on-line hemodiafiltration. *Kidney Int* 64: 305–313, 2003

96. Capeillère-Blandin C, Gausson V, Nguyen AT, Descamps-Latscha B, Drüeke T, Witko-Sarsat V: Respective role of uraemic toxins and myeloperoxidase in the uraemic state. *Nephrol Dial Transplant* 21: 1555–1563, 2006

97. Lim WH, Kireta S, Leedham E, Russ GR, Coates PT: Uremia impairs monocyte and monocyte-derived dendritic cell function in hemodialysis patients. *Kidney Int* 72: 1138–1148, 2007

98. Schrijvers BF, Flyvbjerg A, De Vriese AS: The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. *Kidney Int* 65: 2003–2017, 2004

99. Suliman ME, Qureshi AR, Heimbürger O, Lindholm B, Stenvinkel P: Soluble adhesion molecules in end-stage renal disease: A predictor of outcome. *Nephrol Dial Transplant* 21: 1603–1610, 2006

100. Moon SJ, Kim DK, Chang JH, Kim CH, Kim HW, Park SY, Han SH, Lee JE, Yoo TH, Han DS, Kang SW: The impact of dialysis modality on skin hyperpigmentation in haemodialysis patients. *Nephrol Dial Transplant* 24: 2803–2809, 2009

101. Brandenburg VM, Schlieper G, Heussen N, Holzmann S, Busch B, Evenepoel P, Vanholder R, Meijers B, Meert N, Fassbender WJ, Floege J, Jahnen-Dechent W, Ketteler M: Serological cardiovascular and mortality risk predictors in dialysis patients receiving sevelamer: A prospective study. *Nephrol Dial Transplant* 25: 2672–2679, 2010

102. Campo A, Lanfranco G, Gramaglia L, Goia F, Cottino R, Giusto V: Could plasma cystatin C be useful as a marker of hemodialysis low molecular weight proteins removal? *Nephron Clin Pract* 98: c79–c82, 2004

103. Cohen G, Rudnicki M, Schmaldienst S, Hörl WH: Effect of dialysis on serum/plasma levels of free immunoglobulin light chains in end-stage renal disease patients. *Nephrol Dial Transplant* 17: 879–883, 2002

104. de Loor H, Bammens B, Evenepoel P, De Preter V, Verbeke K: Gas chromatographic-mass spectrometric analysis for measurement of p-cresol and its conjugated metabolites in uremic and normal serum. *Clin Chem* 51: 1535–1538, 2005

105. Martinez AW, Recht NS, Hostetter TH, Meyer TW: Removal of P-cresol sulfate by hemodialysis. *J Am Soc Nephrol* 16: 3430–3436, 2005

106. Vanholder R, Bammens B, de Loor H, Glorieux G, Meijers B, Schepers E, Massy Z, Evenepoel P: Warning: The unfortunate end of p-cresol as a uraemic toxin. *Nephrol Dial Transplant* 26: 1464–1467, 2011

107. Kerr PB, Argilés A, Flavier JL, Canaud B, Mion CM: Comparison of hemodialysis and hemodiafiltration: A long-term longitudinal study. *Kidney Int* 41: 1035–1040, 1992

108. Horowitz JD, Heresztyn T: An overview of plasma concentrations of asymmetric dimethylarginine (ADMA) in health and disease and in clinical studies: Methodological considerations. *J Chromatogr B Analyt Technol Biomed Life Sci* 851: 42–50, 2007

109. Meert N, Waterloos M-A, Van Landschoot M, Dhondt A, Ledebo I, Glorieux G, Goeman J, Van der Eycken J, Vanholder R: Prospective evaluation of the change of predialysis protein-bound uremic solute concentration with postdilution online hemodiafiltration. *Artif Organs* 34: 580–585, 2010

110. Tukey JW: *Exploratory Data Analysis*, Reading, MA, Addison-Wesley, 1977

CORRECTION

Duranton F, Cohen G, De Smet R, Rodriguez M, Jankowski J, Vanholder R, et al. Normal and pathologic concentrations of uremic toxins. *J Am Soc Nephrol* 23: 1–13, 2012.

The authors have observed errors in Tables 2 and 3 because of mismatches in "newly identified retention solutes" status or molecular weights that did not affect the paper otherwise. The corrected tables are printed below, and a corrected version of the manuscript has been posted online. We apologize for the inconvenience.

Table 2. Mean and highest concentrations of uremic retention solutes found in uremic populations and normal concentrations found in the general population: free water-soluble low molecular weight molecules

Molecule	Molecular Weight	Group	Number of Original Papers	Mean Uremic Concentration M (SD)	Highest Uremic Concentration H (SD or Range)	Normal Concentration N (SD)	Methods (U; N)	New Retention Solute[a]
2-Heptenal (μg/L)	112	RCC	1	–	54.7 (16.3)[6]	17.7 (5.33)[6]	GC/MS	✓
2-Hexenal (μg/L)	98	RCC	1	–	61.7 (20.5)[6]	22.1 (6.6)[6]	GC/MS	✓
2-Nonenal (μg/L)	140	RCC	1	–	101.8 (58.4)[6]	18.5 (5.2)[6]	GC/MS	✓
2-Octenal (μg/L)	126	RCC	1	–	32.5 (21.2)[6]	26.1 (16.4)[6]	GC/MS	✓
4-Decenal (μg/L)	154	RCC	1	–	100.1 (26.8)[6]	15.9 (5.3)[6]	GC/MS	✓
4-HO-decenal (μg/L)	170	RCC	1	–	36.6 (22.3)[6]	10.3 (7.1)[6]	GC/MS	✓
4-HO-hexenal (μg/L)	114	RCC	1	–	63.8 (25.3)[6]	25.1 (9.9)[6]	GC/MS	✓
4-HO-nonenal (μg/L)	156	RCC	1	–	117.3 (47.7)[6]	16.4 (9.0)[6]	GC/MS	✓
4-HO-octenal (μg/L)	142	RCC	1	–	27.8 (13.8)[6]	10.7 (3.6)[6]	GC/MS	✓
4-Pyridone-3-carboxamide-1-β-D-ribonucleoside (μg/L)	272	Nicotinamide	1	–	156.1 (169.2)[7]	3.54 (1.63)[7]	HPLC	✓
8-Hydroxy-2'-deoxyguanosine (μg/L)	283	Purine	1	–	0.82 (0.25)[8]	0.64 (0.23)[9]	HPLC; ELISA	✓
α-Keto-δ-guanidinovaleric acid (μg/L)	173	Guanidine	1	–	39.8 (31.1–60.6)[10]	8.23 (0.66)[11]	IEC	✓
Anthranilic acid (μg/L)	137		1	–	16.7 (6.5)[12]	4.23 (1.62)[12]	HPLC	✓
Argininic acid (μg/L)	175	Guanidine	1	–	57.8 (40.3–78.8)[10]	21.5 (3.5)[11]	IEC	
Asymmetric dimethylarginine (μg/L)	202	Guanidine	5	385.0 (288.4)	878.7 (38.4)[14,b]	<60.6[15]	HPLC; ELISA	
Cysteine (μg/L)	121	Aminoacid	1	–	67.8 (3.6)[16]	43.6 (2.4)[16]	HPLC	✓
Decanal (μg/L)	156	RCC	1	–	23.4 (8.81)[6]	17.2 (5.0)[6]	GC/MS	✓
Dimethylamine (mg/L)	45	Amine	1	–	10.3 (1.6)[5]	2.18 (0.33)[5]	HPLC	✓

Table 2. Continued

Molecule	Molecular Weight	Group	Uremic Concentrations			Normal Concentration N (SD)	Methods (U; N)	New Retention Solute[a]
			Number of Original Papers	Mean Uremic Concentration M (SD)	Highest Uremic Concentration H (SD or Range)			
Ethylamine (μg/L)	45	Amine	1	–	69.0 (10.2)[17]	25.8 (5.8)[17]	HPLC	✓
Guanidine (μg/L)	59	Guanidine	1	–	96.2 (90.9–112.1)[10]	<11,8[18]	IEC	
Guanidinoacetic acid (μg/L)	117	Guanidine	1	–	220.0 (168.5–251.6)[10]	222.3 (79.6)[19]	IEC	
Guanidino succinic acid (mg/L)	175	Guanidine	1	–	1.43 (0.99–1.72)[10]	0.03 (0.01)[18]	IEC	
Heptanal (μg/L)	114	RCC	1	–	61.1 (44.1)[6]	51.1 (11.2)[6]	GC/MS	✓
Hexanal (μg/L)	100	RCC	1	–	51.7 (33.0)[6]	21.7 (10.8)[6]	GC/MS	✓
Hypoxanthine (mg/L)	136	Purine	1	–	2.57 (1.13)[20]	1.5 (0.5)[21]	HPLC	
Malondialdehyde (μg/L)	72	RCC	3	217.9 (148.4)	388.8 (21.6)[22]	257.7 (81.7)[23]	Spectrophotometry	
Methylguanidine (μg/L)	73	Guanidine	1	–	139.4 (72.3–218.3)[10]	<7.3[18]	IEC; HPLC	
Monomethylamine (μg/L)	31	Amine	2	332 (351)	580 (100)[5]	320 (40)[5]	HPLC	✓
Neopterin (μg/L)	253	Purine	1	–	83.3 (10.8)[24]	1.38 (0.47)[24]	ELISA	✓
Nicotinamide (μg/L)	122	Nicotinamide	1	–	35.4 (29.3)[25]	3.17 (1.22)[25]	HPLC	✓
N-Methyl-2-pyridone-5-carboxamide (mg/L)	152	Nicotinamide	3	4.02 (3.28)	7.80 (3.59)[26]	1.37 (0.68)[26]	HPLC	✓
N-Methyl-4-pyridone-3-carboxamide (μg/L)	152	Nicotinamide	3	498.6 (162.8)	636.9 (471.2)[22]	39.5 (13.7)[25]	HPLC	✓
Nonanal (μg/L)	142	RCC	1	–	68.9 (26.8)[6]	37.4 (23.3)[6]	GC/MS	✓
Noradrenalin (μg/L)	169	Catecholamine	1	–	0.90 (0.49)[27]	0.25 (0.07)[28]	RIA	✓
Oxalate (mg/L)	90		1	–	3.9 (0.6)[29]	0.3 (0.1)[30]	Spectrophotometry; IEC	
Phenylacetic acid (mg/L)	136		2	467.2 (10.6)	474.6 (44.9)[31]	<1.4[31]	NMR	✓
Symmetric dimethylarginine (μg/L)	202	Guanidine	1	–	646.4 (606.0)[32]	76.1 (21.0)[13]	HPLC; IEC	
Trimethylamine (μg/L)	59	Amine	1	–	82.0 (28.5)[33]	24.7 (7.3)[33]	GC/MS	✓
Trimethylamine-N-oxide (mg/L)	75	Amine	1	–	7.49 (2.39)[33]	2.84 (1.53)[33]	GC/MS	✓
Uric acid (mg/L)	168	Purine	12	64.4 (20.4)	83 (13)[34]	40.5 (13.9)[35]	Spectrophotometry	

GC/MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; IEC, ion exchange chromatography; NMR, nuclear magnetic resonance; RCC, reactive carbonyl compound; RIA, radioimmunoassay.
[a]Refers to solutes not present in references 1 and 2.
[b]Highest uremic concentrations found after suppression of outliers: asymmetric dimethylarginine = 364±81 μg/L[15].

Table 3. Mean and highest concentrations of uremic retention solutes found in uremic populations and normal concentrations found in the general population: protein-bound molecules

Molecule	Molecular Weight	Group	Uremic Concentrations			Normal Concentration N (SD)	Methods (U; N)	New Retention Solute[a]
			Number of Original Papers	Mean Uremic Concentration M (SD)	Highest Uremic Concentration H (SD or Range)			
3-Carboxy-4-methyl-5-propyl-2-furan-propanoic acid (mg/L)	240		3	6.1 (2.4)	8.8 (5.0)[36]	3.6 (0.2) [37]	HPLC; GC/MS	
Acrolein, total (mg/L)	56	RCC	2	9.8 (0.4)	10.1[38]	1.7 (0.5)[38]	ELISA	✓
Acrolein, free (μg/L)	56	RCC	2	76.2 (4.8)	79.5 (47.0)[38]	28 (10.1)[38]	HPLC	✓
Carboxymethyllysine (mg/L)	204	AGE	6	5.4 (7.4)	18.5 (5.0)[39]	0.35 (0.13)[40]	ELISA	✓
Dihydroxyphenylalanine (mg/L)	197	Catecholamine	1	–	11.4 (3.2)[41]	6.6 (0.73)[41]	Fluorometry	✓
Hippuric acid, total (mg/L)	179	Hippurate	5	71.3 (13.7)	87.2 (61.7)[42]	3 (2)[5]	HPLC	
Hippuric acid, free (mg/L)	179	Hippurate	3	41.3 (14.3)	51.1 (40.1)[42]	1[5,43]	HPLC; Fluorometry	✓
Homocysteine (mg/L)	135	Aminoacid	12	4.90 (2.15)	9.37 (2.05)[44b]	1.35 (0.14)[44]	HPLC	
Indican (mg/L)	295	Indole	1	–	27.3 (13.3)[17]	10 (0.4)[17]	HPLC	✓
Indole-3-acetic acid, total (mg/L)	175	Indole	4	2.03 (0.38)	2.4 (2.2)[36]	0.5 (0.3)[45]	HPLC	
Indole-3-acetic acid, free (mg/L)	175	Indole	2	0.37 (0.10)	0.44 (0.51)[36]	–	HPLC	✓
Indoxyl sulfate, total (mg/L)	213	Indole	18	23.1 (13.0)	44.5 (15.3)[46]	0.53 (0.29)[47]	HPLC	
Indoxyl sulfate, free (mg/L)	213	Indole	6	3.22 (1.21)	4.49 (2.67)[46]	ND[48]	HPLC	✓
Indoxyl-β-D-glucuronide (mg/L)	309	Indole	1	–	2.93 (2.94)[47]	1.26 (0.52)[47]	HPLC	✓
Kynurenic acid (μg/L)	189	Indole	1	–	151.0 (76.4)[5]	5.48 (1.32)[5]	HPLC	
p-Cresylsulfate, total (mg/L)	188	Phenol	6	20.9 (12.2)	41 (13.3)[5]	1.9 (1.3)[5]	HPLC	✓
p-Cresylsulfate, free (mg/L)	188	Phenol	2	1.75 (1.20)	2.6 (5.1)[49]	0.08 (0.09)[49]	HPLC	✓
Pentosidine (μg/L)	378	AGE	2	509.7 (98.6)	579.5 (299.3)[36]	51.6 (18.8)[50]	HPLC; ELISA	
Phenol (mg/L)	94	Phenol	2	2.79 (3.83)	5.5 (3.7)[51]	0.6 (0.2)[52]	HPLC; GC/MS	
Putrescine (μg/L)	88	Polyamine	2	9.11 (0.44)	9.42 (7.59)[53]	4.36 (2.75)[53]	HPLC	
Spermidine (μg/L)	145	Polyamine	1	–	9.99 (7.74)[53]	10.4 (5.1)[53]	HPLC	
Spermine (μg/L)	202	Polyamine	1	–	1.86 (1.53)[53]	6.20 (7.98)[53]	HPLC	
Thiocyanate (mg/L)	58		1	–	1.86 (0.17)[16]	0.29 (0.06)[16]	HPLC	✓

GE, advanced glycation end-product; ELISA, enzyme-linked immunosorbant assay; GC/MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; RCC, reactive carbonyl compound.
[a]Refers to solutes not present in references 1 and 2.
[b]Highest uremic concentrations found after suppression of outliers: homocysteine= 7.8±1.3 mg/L[85].

Normal and pathologic concentrations of uremic toxins, a systematic review

Flore Duranton MSc, Gerald Cohen PhD, Rita De Smet MSc, Mariano Rodriguez MD PhD, Joachim Jankowski PhD, Raymond Vanholder MD PhD, Angel Argiles MD PhD

Supplementary material

Supplement 1. List of additional references included in the analysis.

Supplement 2. Table of the coefficient of variation and the ratio of the highest (H) to the lowest (L) concentrations found in uremic populations.

Data concerning solutes with four uremic concentrations or more are displayed.

Supplement 3. Distribution of retention solute concentrations found in uremic populations.

Data concerning solutes with four uremic concentrations or more are displayed. Values out of Tukey's inner fence were identified as suspected outliers. Results are grouped by pattern.

Supplement 4. Uremic toxin concentrations in predialytic patients and in patients undergoing hemodialysis or peritoneal dialysis.

Supplement 1. List of additional references included in the analysis.

Aveles, P. R., Criminácio, C. R., Gonçalves, S., Bignelli, A. T., Claro, L. M., Siqueira, S. S., Nakao, Lia S, et al. (2010). Association between biomarkers of carbonyl stress with increased systemic inflammatory response in different stages of chronic kidney disease and after renal transplantation. *Nephron. Clinical practice*, *116*(4), c294-9.

Barreto, D. V., Barreto, F. C., Liabeuf, S., Temmar, M., Lemke, H.-D., Tribouilloy, C., Choukroun, G., et al. (2010). Plasma interleukin-6 is independently associated with mortality in both hemodialysis and pre-dialysis patients with chronic kidney disease. *Kidney international*, *77*(6), 550-6.

Feliciani, A., Riva, M. A., Zerbi, S., Ruggiero, P., Plati, A. R., Cozzi, G., & Pedrini, L. A. (2007). New strategies in haemodiafiltration (HDF): prospective comparative analysis between on-line mixed HDF and mid-dilution HDF. *Nephrology, dialysis, transplantation*, *22*(6), 1672-9.

Guz, G., Bali, M., Poyraz, N. Y., Bagdatoglu, O., Yeğin, Z. A., Doğan, I., Atasever, T., et al. (2004). Gastric emptying in patients on renal replacement therapy. *Renal failure*, *26*(6), 619-24.

Ingrosso, Diego, Cimmino, A., Perna, A. F., Masella, L., De Santo, Natale G, De Bonis, M. L., Vacca, M., et al. (2003). Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. *Lancet*, 361(9370), 1693-9.

Kokot, F., Schinzel, R., Chudek, J., Heidland, A, Adamczak, M., Ficek, R., & Wiecek, A. (2003). Relationship between plasma level of parathyroid hormone and carboxymethyllysine in hemodialyzed patients--does it exist? *Clinical nephrology*, *59*(1), 31-4.

Krieter, D. H., Falkenhain, S., Chalabi, L., Collins, G., Lemke, H.-D., & Canaud, B. (2005). Clinical cross-over comparison of mid-dilution hemodiafiltration using a novel dialyzer concept and post-dilution hemodiafiltration. *Kidney international*, *67*(1), 349-56.

Krieter, D. H., Hackl, A., Rodriguez, A., Chenine, L., Moragues, H. L., Lemke, H.-D., Wanner, C., et al. (2010). Protein-bound uraemic toxin removal in haemodialysis and post-dilution haemodiafiltration. *Nephrology, dialysis, transplantation*, *25*(1), 212-8.

Krishnan, A. V., Phoon, R. K. S., Pussell, B. A., Charlesworth, J. A., Bostock, H., & Kiernan, M. C. (2005). Altered motor nerve excitability in end-stage kidney disease. *Brain*, *128*(Pt 9), 2164-74.

Lim, W. H., Kireta, S., Leedham, E., Russ, G. R., & Coates, P. T. (2007). Uremia impairs monocyte and monocyte-derived dendritic cell function in hemodialysis patients. *Kidney international*, *72*(9), 1138-48.

Lonnemann, G., Bahlmann, F. H., Freise, J., Hertel, B., & Dinarello, C. A. (2009). Low-flux hemodialysis suppresses interferon-gamma production: the possible role of beta2-microglobulin. *Clinical nephrology*, *72*(3), 170-6.

Meert, N., Beerenhout, C., Schepers, E., Glorieux, G., Kooman, J., & Vanholder, R. (2009). Evolution of protein-bound uraemic solutes during predilution haemofiltration. *Journal of nephrology*, *22*(3), 352-7.

Nasri, H., Baradaran, A., & Naderi, A. S. A. (2004). Close association between parathyroid hormone and left ventricular function and structure in end-stage renal failure patients under maintenance hemodialysis. *Acta medica Austriaca*, *31*(3), 67-72.

Okuno, S., Ishimura, E., Kohno, Kaori, Fujino-Katoh, Y., Maeno, Y., Yamakawa, T., Inaba, M., et al. (2009). Serum beta2-microglobulin level is a significant predictor of mortality in maintenance haemodialysis patients. *Nephrology, dialysis, transplantation*, *24*(2), 571-7.

Pedrini, L. A., De Cristofaro, V., Comelli, M., Casino, F. G., Prencipe, M., Baroni, A., Campolo, G., et al. (2011). Long-term effects of high-efficiency on-line haemodiafiltration on uraemic toxicity. A multicentre prospective randomized study. *Nephrology, dialysis, transplantation*, *26*(8), 2617-24.

Pedrini, L. A., Feliciani, A., Zerbi, S., Cozzi, G., & Ruggiero, P. (2009). Optimization of mid-dilution haemodiafiltration: technique and performance. *Nephrology, dialysis, transplantation*, *24*(9), 2816-24.

Pellicano, R., Polkinghorne, K. R., & Kerr, P. G. (2008). Reduction in beta2-microglobulin with super-flux versus high-flux dialysis membranes: results of a 6-week, randomized, double-blind, crossover trial. *American journal of kidney diseases*, *52*(1), 93-101.

Righetti, M., Filiberti, O., Ranghino, A., Ferrario, G., Milani, S., Serbelloni, P., Guida, G. E., et al. (2010). Internal hemodiafiltration versus low-flux bicarbonate dialysis: Results from a long-term prospective study. *The International journal of artificial organs*, *33*(11), 796-802.

De Smet, R., Van Kaer, J., Van Vlem, B., De Cubber, A., Brunet, P., Lameire, N., & Vanholder, R. (2003). Toxicity of free p-cresol: a prospective and cross-sectional analysis. *Clinical chemistry*, *49*(3), 470-8.

Tiranathanagul, K., Praditpornsilpa, K., Katavetin, P., Srisawat, N., Townamchai, N., Susantitaphong, P., Tungsanga, K., et al. (2009). On-line hemodiafiltration in Southeast Asia: a three-year prospective study of a single center. *Therapeutic apheresis and dialysis*, *13*(1), 56-62.

Tsubakihara, Y., Takabatake, Y., Oka, K., Shoji, T., Togawa, M., Okada, N., Takahito, I., et al. (2003). Effects of the oral adsorbent AST-120 on tryptophan metabolism in uremic patients. *American journal of kidney diseases*, *41*(3 Suppl 1), S38-41.

Tsujimoto, M., Higuchi, K., Shima, D., Yokota, H., Furukubo, T., Izumi, S., Yamakawa, T., et al. (2010). Inhibitory effects of uraemic toxins 3-indoxyl sulfate and p-cresol on losartan metabolism in vitro. *The Journal of pharmacy and pharmacology*, *62*(1), 133-8.

De Vriese, A. S., Langlois, M., Bernard, D., Geerolf, I., Stevens, L., Boelaert, J. R., Schurgers, M., et al. (2003). Effect of dialyser membrane pore size on plasma homocysteine levels in haemodialysis patients. *Nephrology, dialysis, transplantation*, *18*(12), 2596-600.

Zhang, X., Frischmann, M., Kientsch-Engel, R., Steinmann, K., Stopper, H., Niwa, T., & Pischetsrieder, M. (2005). Two immunochemical assays to measure advanced glycation end-products in serum from dialysis patients. *Clinical chemistry and laboratory medicine*, *43*(5), 503-11.

Wu, I.-W., Hsu, K.-H., Lee, C.-C., Sun, C.-Y., Hsu, H.-J., Tsai, C.-J., Tzen, C.-Y., et al. (2011). p-Cresyl sulphate and indoxyl sulphate predict progression of chronic kidney disease. *Nephrology, dialysis, transplantation*, 26(3), 938-47.

Supplement 2. Table of the coefficient of variation and the ratio of the highest (H) to the lowest (L) concentrations found in uremic populations.

Data concerning solutes with four uremic concentrations or more are displayed.

Molecule	Coefficient of variations (CV)	Ratio H/L	Number of publications
Asymmetric dimethylarginine	75%	5.37	5
β2-microglobulin	26%	3.65	24
Carboxymethyllysine	137%	92.5	6
Cystatin C	80%	3.74	4
Hippuric acid. total	19%	1.71	5
Homocysteine	44%	3.71	13
Indole-3-acetic acid, total	19%	1.6	4
Indoxyl sulfate. total	57%	21.14	18
Indoxyl sulfate. free	43%	4.49	6
Interleukin-6	34%	2.53	7
Leptin	67%	9.1	6
p-Cresylsulfate. total	58%	5.73	6
Parathyroid hormone	98%	9.84	12
Tumor Necrosis Factor-α	114%	14.49	4
Uric acid	32%	11.19	12

Supplement 3. Distribution of retention solute concentrations found in uremic populations.

Data concerning solutes with four uremic concentrations or more are displayed. Values out of Tukey's inner fence were identified as suspected outliers. Results are grouped by pattern.

a) **Group 1: Mean similar to Median**
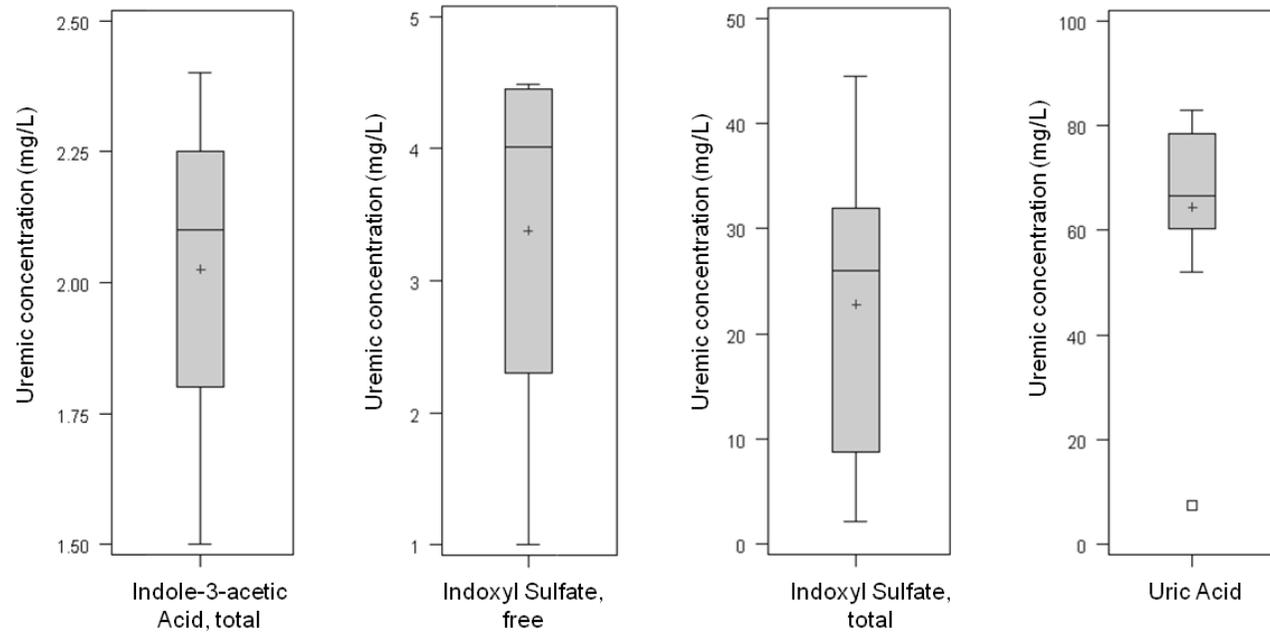
Supplement 3. Distribution of retention solute concentrations found in uremic populations.

b) Group 2: Mean > Median
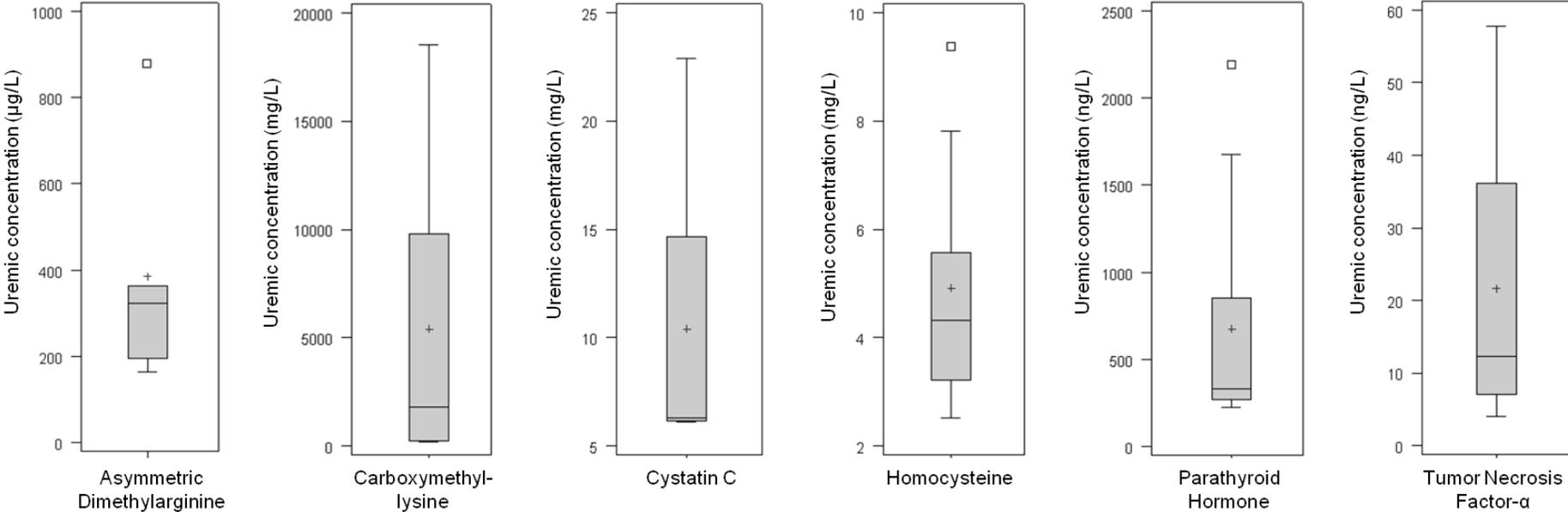
Supplement 3. Distribution of retention solute concentrations found in uremic populations.

    c) Group 3: Mean < Median
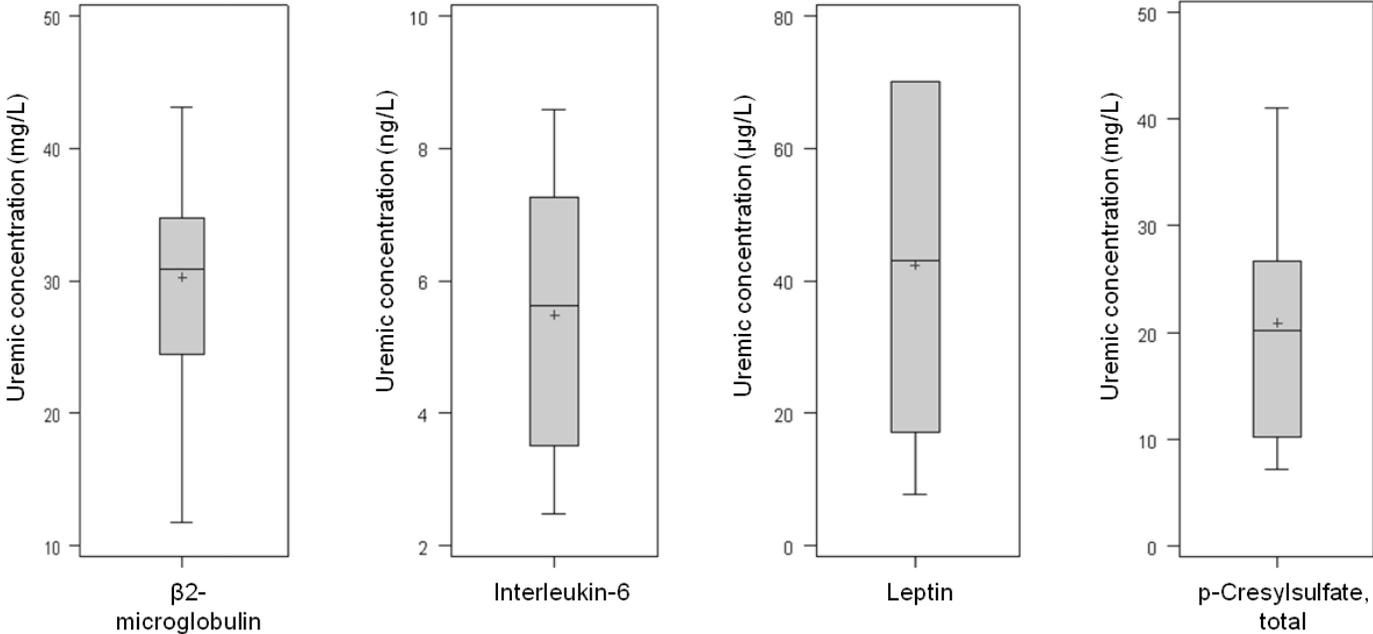
Supplement 4. Uremic toxin concentrations in predialytic patients and in patients undergoing hemodialysis or peritoneal dialysis.

Molecule	Mean uremic concentration (sd)		p-value*
	Inclusion of preHD patients	**HD or PD patients only**	
α1-Acid glycoprotein (g/L)	1.17	1.3	-
Carboxymethyllysine (mg/L)	10.1 (11.9)	3.01 (4.58)	0.53
Cystatin C (mg/L)	22.9	6.24 (0.14)	0.44
Fibrinogen (g/L)	4.06	4.86	-
Glutathion, oxidized (mg/L)	32.5	114	-
Indoxyl sulfate, total (mg/L)	6.7 (1.8)	27.8 (10.8)	0.007
Interleukin-6 (ng/L)	5.8 (2.5)	5.97 (1.89)	1.00
Interleukin-8 (ng/L)	38.0	2.48	-
Malondialdehyde (µg/L)	266.4 (173.1)	121.0	0.67
N-Methyl-2-pyridone-5-carboxamide (mg/L)	1.88	5.08 (3.85)	0.67
N-Methyl-4-pyridone-3-carboxamide (µg/L)	623.2	429.4 (155.8)	0.67
Parathyroid hormone (ng/L)	854.05	654.9	0.55
p-Cresylsulfate, total (mg/L)	12.1 (6.1)	17.4 (11.8)	0.70
p-Cresylsulphate, free (mg/L)	2.6	0.9	-
Tumor Necrosis Factor-α (ng/L)	3.99	27.5 (26.4)	0.50
Uric acid (mg/L)	73.4 (6.6)	58.0 (25.0)	0.27

Abbreviations: HD: hemodialysis; PD: peritoneal dialysis; preHD: predialytic stages of chronic kidney disease; sd: standard deviation.

* bilateral Wilcoxon exact test.