Normal and Pathologic Concentrations of Uremic Toxins

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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.

J Am Soc Nephrol 23: 1258–1270, 2012. doi: 10.1681/ASN.2011121175

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,

Received December 14, 2011. Accepted April 13, 2012.

Published online ahead of print. Publication date available at www.jasn.org.

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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).

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.

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 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		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)[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 (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)	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		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 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	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]	3.0 (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 concentrations 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

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).

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_4A) was described as a uremic toxin. CKD patients had, on average, a 5.2-fold higher Up_4A plasma concentration compared with healthy subjects, and this increased Up_4A 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,

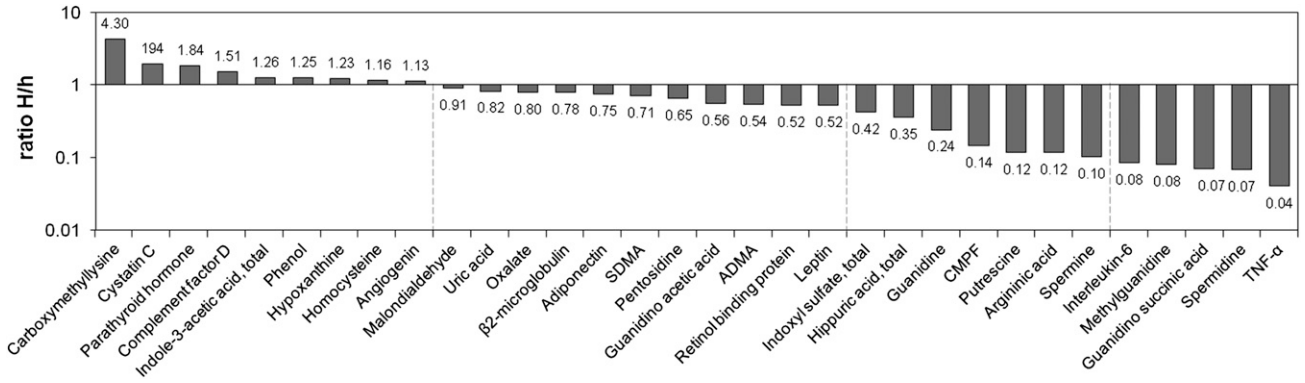

Figure 1. Relative change in highest uremic concentrations of known retention solutes. The H/h index is the ratio of the highest uremic concentration found in the present analysis to the highest concentration presented in the previous reviews.[1,2]

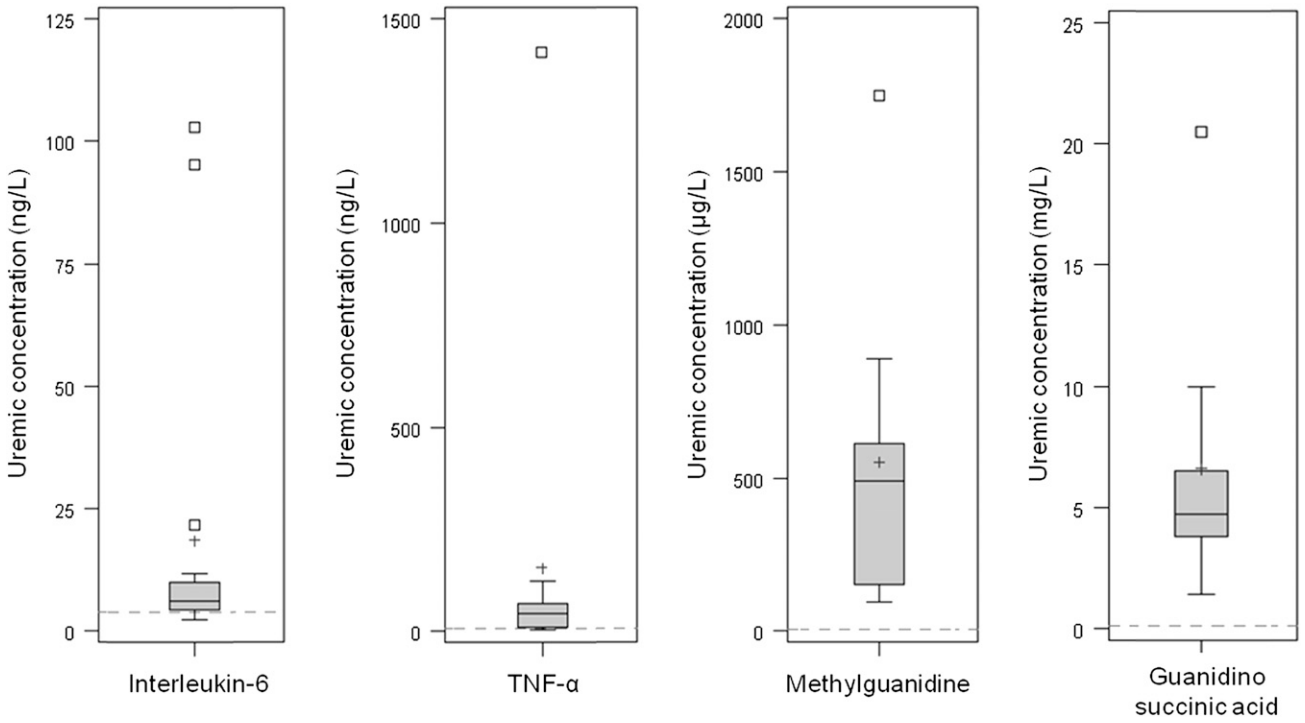

Figure 2. Distribution of the concentrations of IL-6, TNF-α, methylguanidine, and guanidino succinic acid found in uremic populations. Data from the present analysis and the previous review[1] were pooled and displayed. Dashed lines represent concentrations found in the general population. Values out 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-antitrypsine, 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

The European Uremic Toxin Work Group (EUTox) is a working group from the European Society for Artificial Organs (ESAO) endorsed by the European Renal Association–European Dialysis and Transplant Association (ERA-EDTA) that aims to analyze and discuss matters related to the identification, characterization, analytic determination, and evaluation of biological activity of uremic retention solutes. EUTox is constituted of 24 academic research groups and 7 corporate members (Abbott, Bayer Schering Pharma, BBraun Avitum, Fresenius Medical Care, Gambro, Membrana, and Vifor Pharma). Academic members are O. Abou Deif, A. Argiles, T. Drueke, U. Baurmeister, J. Beige, P. Brunet, G. Cohen, D. Fliser, G. Glorieux, S. Herget-Rosenthal, V. Jankowski, J. Jankowski, Z. Massy, H. Mischak, A. Ortiz, A. Perna, J.M. Rodriguez Portillo, G. Spasovski, B. Stegmayr, P. Stenvinkel, P. Thornalley, R. Vanholder, C. Wanner, and A. Wiecek. Member affiliations and other information about EUTox can be obtained at the website http://uremic-toxins.org. We also would like to thank Prof. J.P. Daurès for guidance and support.

DISCLOSURES

None.

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