Kidney International, Vol. 63 (2003), pp. 1934–1943

Review on uremic toxins: Classification, concentration, and interindividual variability

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Review on uremic toxins: Classification, concentration, and interindividual variability.

Background. The choice of the correct concentration of potential uremic toxins for in vitro, ex vivo, and in vivo experiments remains a major area of concern; errors at this level might result in incorrect decisions regarding therpeutic correction of uremia and related clinical complications.

Methods. An encyclopedic list of uremic retention solutes was composed, containing their mean normal concentration (C_N), their highest mean/median uremic concentration (C_U), their highest concentration ever reported in uremia (C_{MAX}), and their molecular weight. A literature search of 857 publications on uremic toxicity resulted in the selection of data reported in 55 publications on 90 compounds, published between 1968 and 2002.

Results. For all compounds, C_U and/or C_{MAX} exceeded C_N. Molecular weight was lower than 500 D for 68 compounds; of the remaining 22 middle molecules, 12 exceeded 12,000 D. C_U ranged from 32.0 ng/L (methionine-enkephalin) up to 2.3 g/L (urea). C_U in the ng/L range was found especially for the middle molecules (10/22; 45.5%), compared with 2/68 (2.9%) for a molecular weight <500 D ($P < 0.002$). Twenty-five solutes (27.8%) were protein bound. Most of them had a molecular weight <500 D except for leptin and retinol-binding protein. The ratio C_U/C_N, an index of the concentration range over which toxicity is exerted, exceeded 15 in the case of 20 compounds. The highest values were registered for several guanidines, protein-bound compounds, and middle molecules, to a large extent compounds with known toxicity. A ratio of C_{MAX}/C_U <4, pointing to a Gaussian distribution, was found for the majority of the compounds (74/90; 82%). For some compounds, however, this ratio largely exceeded 4 [e.g., for leptin (6.81) or indole-3-acetic acid (10.37)], pointing to other influencing factors than renal function, such as gender, genetic predisposition, proteolytic breakdown, posttranslation modification, general condition, or nutritional status.

Conclusion. Concentrations of retention solutes in uremia vary over a broad range, from nanograms per liter to grams per liter. Low concentrations are found especially for the middle molecules. A substantial number of molecules are protein bound and/or middle molecules, and many of these exert toxicity and are characterized by a high range of toxic over normal concentration (C_U/C_N ratio). Hence, uremic retention is a complex problem that concerns many more solutes than the current markers of urea and creatinine alone. This list provides a basis for systematic analytic approaches to map the relative importance of the enlisted families of toxins.

Key words: uremic toxins, uremic toxicity, renal failure, concentrations, retention.

Received for publication July 3, 2002
and in revised form October 24, 2002
Accepted for publication December 13, 2002

The uremic syndrome is attributed to the progressive retention of a large number of compounds, which under normal conditions are excreted by the healthy kidneys

[1–3]. These compounds are called uremic retention solutes, or uremic toxins, when they interact negatively with biologic functions.

A substantial number of publications have been devoted to the concentration changes of individual uremic retention solutes in several conditions of end-stage renal disease (ESRD). To our knowledge, no attempts have been made, however, to develop an in depth and systematic overview of all these data with the intention to open the field for new analytic approaches.

Various aspects of the uremic syndrome remain partly or entirely unexplored, although an exact knowledge of its nature, as well as of the relative importance of the various responsible toxins, is urgently needed to allow the development of better defined and more specific removal strategies than the empiric ones based on diffusion, convection, or molecular size exclusion, as applied in the current dialysis membranes today. The clinical importance to address this issue in depth has recently been stressed by the demonstration of an improved outcome when clearance of uremic toxins with a molecular weight range of approximately 1000 to 5000 D was enhanced [4].

In many studies, concentrations are applied that exceed those encountered in uremia, so that conclusions might have relatively little clinical relevance. To overcome these pitfalls, in the present publication, three lists are presented containing in total 90 retention solutes together with their normal and uremic concentrations, offering a "dictionary" of uremic retention solutes, in a standardized and homogeneous manner. The work has been constructed as an objective report of the available information, rather than as a critical analysis of how this information has been obtained. The report is followed by a number of reflections regarding the characteristics and the retention pattern of the depicted molecules. The aim is to offer a platform for more systematic future studies in the area of uremic toxicity.

METHODS

A database of 857 publications, published between 1966 and 2002, covering the field of uremic toxins and/or uremic toxicity, was considered. The references were collected based on a Medline literature search (Reference Manager 9) with as reference words (search items) "uremic toxins/uremic toxicity" and/or specific names of known retention solutes as recently reviewed [1, 3, 5]. This list was further completed based on the personal expertise of the authors. Among these publications, 149 were devoted to solute concentration. Only plasma/serum concentrations were taken into consideration.

A search was made to register a representative mean or median normal concentration, a representative mean or median uremic concentration, and the highest reported single uremic concentration. Subsequently, 55 publications were used for the present analysis.

Compounds were included only if their mean/median uremic concentration and/or maximum uremic concentration were in excess of the reported normal concentration.

For the sake of uniformity, all concentration (C) values were normalized to a volume of 1 liter (L), and were reported in grams per liter (g/L), or, if less concentrated, in milligrams per liter (mg/L), micrograms per liter (μg/L), or nanograms per liter (ng/L). Data that were reported in mole per liter (mol/L) or equivalent units were transformed to g/L or equivalent concentration values by the formula:

$$C\ (g/L) = [C\ (mol/L) \times \text{molecular weight}] \quad \text{(Eq. 1)}$$

Units of concentration were chosen in a way that the uremic concentration always exceeded 1.0. All data were reported up to one digit below unity, unless if normal values or their standard deviations (SD) were smaller than 0.1. The molecular weights utilized might be slightly different from other reported values, depending on the methods and procedures applied to identify the molecular size [i.e., sodium dodecyl sulfate (SDS)-gel electrophoresis, gel permeation chromatography, mass spectroscopy, or others].

Uremic concentrations were all expressed as mean ± SD, or if means were lacking, as median. If literature data were expressed in mean ± SEM, SEM was transformed to SD according to the formula:

$$SD = SEM \times \sqrt{(n-1)} \quad \text{(Eq. 2)}$$

Our primary research was aimed at finding the highest reported mean/median uremic concentrations (C_U). These values were noted, regardless of whether the patients reported were in the predialytic stage, or dialysed by hemodialysis or peritoneal dialysis. If possible, the corresponding normal values (C_N) were extracted from the same publications. If not available in these specific publications, normal values were collected from other sources. If normal values were indicated in the publications as being below a given detection limit, this limit was introduced as the highest normal value. If normal values were given as a range, also the highest value was taken as the reference. In both cases, the normal value is accompanied by the symbol "<" in the tables. In any other case, means ± SD are given and the means were taken as the reference value. Per C_N, C_U, or C_{MAX} value, only one literature reference was finally to be used for data collection, so that per molecule maximum three references can be found. It, however, occurred only once that we had to refer to three publications (homocysteine), whereas the characteristic concentrations of most molecules were only covered by one reference ($N = 67$). For 22 molecules, two references were used.

In addition, if available, the single highest uremic concentration ever reported was illustrated as well (C_{MAX}).

This information was not necessarily obtained from the same publication as the mean/median uremic value. If no range or individual values could be found, a hypothetical maximal value was calculated as:

$$C_{MAX} = \text{Mean} + 2\ SD \qquad \text{(Eq. 3)}$$

Finally, the number of patients on which the C_N and C_U values had been obtained, the molecular weight, the reference(s) from which the data had been collected, and (if available) the larger group to which the molecules belonged (e.g., guanidines, phenols, peptides) were reported.

Molecules were subdivided into three major classes: (*1*) small solutes (<500 D) with no known protein binding; (*2*) solutes with known or likely protein binding; and (*3*) middle molecules (≥500 D). For all the protein-bound solutes, only the total concentrations were illustrated.

The lower cutoff value for the so-called middle molecules (500 D) was based on the original literature data reporting on this class of uremic solutes [6]. In this literature, middle molecule molecular weight range is usually reported as 500 to 2000 D. Since most of the identified larger uremic solutes are characterized by a molecular weight in excess of 2000 D, the upper limit for middle molecules was not restricted to 2000 D, but all solutes with a molecular weight in excess of 2000 D were also enlisted as middle molecules.

Molecules that are not filterable through the glomerular basement membrane because of their molecular size (molecular weight > ~60,000 D) were not included [e.g., lipoprotein(a)]. Likewise, anorganic compounds were excluded, although it is acknowledged that they can exert toxicity (e.g., H_2O, K^+, phosphate, trace elements). The reader is referred for these compounds to specific publications [7–9].

Furthermore, the ratio between mean uremic and normal concentration was calculated (C_U/C_N) to obtain an index of the relative increase during uremia. In addition, also the ratio of maximal over mean uremic concentration (C_{MAX}/C_U) was calculated. The latter index describes whether data distribution was Gaussian or not.

For some of the advanced glycation end products (AGEs), published concentrations are expressed in the literature as picogram per milligram (pg/mg) protein or microgram per milligram (μg/mg) protein [10, 11]. Since in these publications, however, no serum total protein was mentioned, it was impossible to extrapolate the absolute AGE concentrations. In these cases, absolute content was calculated by multiplying with an average serum protein concentration (i.e., 70,000 mg/L). The reported AGE values all were obtained in uremic patient groups without diabetes mellitus.

For statistical comparison, an Instat® statistical package was used. Sets of values reported per group of molecules (small water-soluble, protein-bound, middle molecules) were compared statistically to each other by the Mann-Whitney U test. Dichotomous variables were compared by chi-square test. Statistical significance was accepted for $P < 0.05$.

RESULTS

The concentrations of in total 90 uremic solutes are listed in Tables 1 to 3. In Table 1, results regarding 45 low molecular weight solutes (molecular weight <500 D) without known protein binding (small free water-soluble compounds) are summarized [12–33]. Similarly, Table 2 contains data on 25 compounds with known protein binding or belonging to groups of solutes that are known to be protein bound [10, 11, 20, 34–53]. Most of these protein-bound solutes are also characterized by a molecular weight <500 D, although two of these compounds (leptin and retinol-binding protein) have a molecular weight conforming with that of the middle molecules. For these protein-bound compounds, the tables only contain total (free + bound) concentrations. Table 3 then contains information on the middle molecules ($N = 22$) [22, 45, 53–66].

Hence, 68 solutes are characterized by a molecular weight below 500 D. Among the 22 middle molecules, 12 (54.5%) have a molecular weight >12,000 D.

For several molecules, we found a discordance between the highest [13, 34, 63] and the lowest range of reported uremic concentrations [15, 36, 67]. The most striking examples [asymmetric dimethylarginine (ADMA); interleukin-6 (IL-6); 3-deoxyglucosone (3-DG)] are illustrated in Table 4, where, in each instance, a discrepancy by a factor of 8 or more was observed. In Tables 1 to 3 only the highest reported value is illustrated. It is of note that for ADMA, values intermediate between the lower range as observed by Marescau et al [15] and the higher range observed by Kang et al [13] have been reported by Vallance et al [14] (approximately 878.5 μg/L) and Zoccali et al [68] (777.7 μg/L).

The uremic concentration of the 90 reported molecules was spread over a broad range, from 2.3 g/L [urea, full urea concentration; for blood urea nitrogen (BUN) multiply by 28/60] to 32.2 ng/L (methionine-enkephalin) (factor $>70 \times 10^4$). The concentration range for the small water-soluble compounds ($N = 45$) extended from 2.3 g/L (urea) to 62.7 ng/L (β-lipotropin) (Table 1). The range for the protein-bound solutes ($N = 25$) extended from 247.0 mg/L (hippuric acid) to 175.8 ng/L (melatonin) (Table 2), and for the middle molecules ($N = 22$) from 192.0 mg/L (retinal-binding protein) to 32.2 ng/L (methionine-enkephalin) (Table 3) The median concentration value was 773.8 μg/L overall (methylguanidine) and was 1.2 mg/L for the small water-soluble compounds, as well as for the protein-bound compounds, but only 0.95 μg/L for the middle molecules. Considering these medians, in general, the concentration of the middle molecules was

Table 1. Free water-soluble low-molecular-weight solutes ($N = 45$)

Solute	C_N	C_U	C_{MAX}	MW	Ref	Group
1-methyladenosine $\mu g/L$	17.1 ± 5.1/10	104.0 ± 56.2/17	216.4	281	[12]	Ribonucleosides
1-methylguanosine $\mu g/L$	13.7 ± 16.9/10	41.6 ± 23.8/17	89.2	297	[12]	Ribonucleosides
1-methylinosine $\mu g/L$	13.5 ± 3.9/10	620.4 ± 203.4/14	1027.2	282	[12]	Ribonucleosides
ADMA mg/L	0.2 ± 0.06/6	1.6 ± 1.2/10	7.3[a]	202	[13, 14]	Guanidines
α-keto-δ-guanidinovaleric acid $\mu g/L$	<30.2/66	—	140.4[a]	151	[15]	Guanidines
α-*N*-acetylarginine $\mu g/L$	18.1 ± 24.8/16	328.3 ± 142.6/13	4580.0[a]	216	[16, 17]	Guanidines
Arab(in)itol mg/L	<0.6/33	15.0 ± 9.0/12	33.0	152	[18, 19]	Polyols
Argininic acid $\mu g/L$	<77.0/66	80.5 ± 56.0/11	197.8[a]	175	[15, 16]	Guanidines
Benzylalcohol mg/L	—	27.0 ± 50.7/17	187.9[a]	108	[20]	
β-guanidinopropionic acid $\mu g/L$	<3.3/24	28.8 ± 18.3/29	65.4	131	[21]	Guanidines
β-lipotropin ng/L	<55.3/10	62.7/22	108.8[a]	461	[22]	Peptides
Creatine mg/L	9.7 ± 3.3/24	134.0 ± 30.3/29	235.8[a]	131	[21]	Guanidines
Creatinine mg/L	<12.0/23	136.0 ± 46.0/19746	240.0[a]	113	[23, 24]	Guanidines
Cytidine $\mu g/L$	<468.0	683.3 ± 287.8/7	1263.6[a]	234	[25]	Purines
Dimethylglycine $\mu g/L$	<381.1/33	576.8/18	1040.3[a]	103	[26]	
Erythritol mg/L	<0.7/33	9.8 ± 14.0/12	37.0[a]	122	[18, 19]	Polyols
γ-guanidinobutyric acid $\mu g/L$	<3.6/24	33.3 ± 16.0/30	1750.0[a]	145	[27, 17]	Guanidines
Guanidine $\mu g/L$	<11.8/16	172.9 ± 83.8/13	800.0[a]	59	[16, 17]	Guanidines
Guanidinoacetic acid $\mu g/L$	222.3 ± 79.6/24	383.8 ± 143.9/29	693.8[a]	117	[21]	Guanidines
Guanidonosuccinic acid mg/L	0.03 ± 0.01/16	6.5 ± 3.4/13	47.0[a]	175	[16, 17]	Guanidines
Hypoxanthine mg/L	1.5 ± 0.5/145	2.0 ± 1.6/65	5.3	136	[28, 29]	Purines
Malondialdehyde $\mu g/L$	257.7 ± 81.7/30	428.8 ± 170.4/16	769.6	71	[30]	
Mannitol mg/L	<1.3/33	26.0 ± 25.0/12	76.0	182	[18, 19]	Polyols
Methylguanidine $\mu g/L$	<7.3/24	773.8 ± 508.8/5	1820.0[a]	73	[21, 17]	Guanidines
Myoinositol mg/L	<10.0/8	94.0 ± 69.0/12	232.0	180	[18]	Polyols
N^2,N^2-dimethylguanosine $\mu g/L$	9.0 ± 4.7/10	236.4 ± 89.7/14	415.8	311	[12]	Ribonucleosides
N^4-acetylcytidine $\mu g/L$	57.0 ± 17.1/10	159.6 ± 30.8/14	221.2	285	[12]	Ribonucleosides
N^6-methyladenosine $\mu g/L$	18.5 ± 8.4/10	70.3 ± 53.3/17	176.9	281	[12]	Ribonucleosides
N^6-threonylcarbamoyladenosine $\mu g/L$	35.5 ± 27.2/10	378.0 ± 151.2/17	680.4	378	[12]	Ribonucleosides
Orotic acid mg/L	0.5 ± 1.4/30	6.7 ± 16.0/22	38.7	174	[31]	Pyrimidines
Orotidine mg/L	1.2 ± 1.6/30	20.2 ± 13.5/22	47.2	288	[31]	Pyrimidines
Oxalate mg/L	0.3 ± 0.1/8	4.9 ± 1.4/8	7.6	90	[32]	
Phenylacetylglutamine mg/L	<4.7	53.3 ± 44.7/6	120.6[a]	264	[33]	
Pseudouridine mg/L	0.5 ± 5.8/30	13.1 ± 21.4/7	86.6[a]	244	[25, 31]	Ribonucleosides
SDMA $\mu g/L$	76.1 ± 21.0/66	640.3 ± 212.1/38	1232.2[a]	202	[15]	Guanidines
Sorbitol mg/L	<0.4/33	3.1 ± 2.1/12	7.3	182	[18, 19]	Polyols
Taurocyamine $\mu g/L$	<52.2/24	—	121.8[a]	174	[17]	Guanidines
Threitol $\mu g/L$	<319.6/33	990.0 ± 920.0/12	5697.4[a]	122	[18, 19]	Polyols
Thymine mg/L	—	2.8 ± 4.2/22	11.2	126	[31]	Pyrimidines
Uracil $\mu g/L$	<224.0	252.0 ± 154.6/7	448.0[a]	112	[25]	Purines
Urea g/L	<0.4/23	2.3 ± 1.1/16	4.6[a]	60	[24]	
Uric acid mg/L	<67.2	83.4 ± 44.5/7	146.7[a]	168	[25]	Purines
Uridine mg/L	1.5 ± 1.3/30	9.8 ± 11.4/22	32.6	244	[31]	Pyrimidines
Xanthine mg/L	0.5 ± 1.4/180	1.5 ± 0.8/65	3.0	152	[28, 29]	Purines
Xanthosine $\mu g/L$	23.9 ± 12.8/10	96.6 ± 62.9/11	222.4	284	[12]	Ribonucleosides

Abbreviations are: C_N, normal concentration; C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; MW, molecular weight; ref, reference; ADMA, asymmetrical dimethylarginine; SDMA, symmetrical dimethylarginine. The underlined numbers behind the slash point to the number of data on which the means or medians have been obtained. No underlined number indicates that no data about the number of samples were available. Normal values are reported as means ± SD, or in the case of a single value as a maximum (accompanied by <); uremic values are reported as means ± SD or, in the case of a single value, as a median.

[a]C_{MAX} values are original data (all other values were calculated as mean + 2 SD based on C_U)

lower compared to the water-soluble and protein-bound solutes and this was confirmed by comparative statistical analysis ($P = 0.01$ vs. small water-soluble compounds; $P = 0.02$ vs. protein-bound compounds). For 10 out of 22 of the middle molecules (45.5%) uremic concentration was in ng/L range, compared with 1 out of 45 small water-soluble compounds (2.2%; $P < 0.001$) and 1 out of 25 protein-bound compounds (2.5%; $P < 0.01$).

The index C_U/C_N offers an indication about the differences between uremic and normal concentration values, over which toxicity can be exerted. The 20 solutes scoring higher than 15 and the 19 solutes scoring lower than 2.5 are listed in Table 5. The cutoff values for these lists were set arbitrarily. Among the eight compounds with the highest C_U/C_N index, there were two guanidines, three protein-bound solutes, and two middle molecules. Among the 20 lowest scoring compounds, there were seven middle molecules. When considering the index per group of molecules, only the difference between protein-bound molecules and middle molecules was at the borderline of significance ($P = 0.06$).

The index C_{MAX}/C_U indicates which solutes show a non-Gaussian distribution, in a way that the highest ratios are obtained for the solutes with the most abnormal

Table 2. Protein-bound solutes ($N = 25$)

Solute	C_N	C_U	C_{MAX}	MW	Ref	Group
2-methoxyresorcinol *μg/L*	—	19.6 ± 81.2/17	322.0[a]	140	[20]	Phenols
3-deoxyglucosone *mg/L*	0.3 ± 0.1/30	1.7 ± 1.0/27	3.5	162	[34]	AGE
CMPF *mg/L*	7.7 ± 3.3/7	61.0 ± 16.5/15	94.0[a]	240	[35]	
Fructoselysine *mg/L*	—	58.1 ± 10.8/10	79.7	308	[10]	AGE
Glyoxal *μg/L*	67.0 ± 20.0	221.0 ± 28.0/20	277.0	58	[36]	AGE
Hippuric acid *mg/L*	<5.0	247.0 ± 112.0/7	471.0	179	[37]	Hippurates
Homocysteine *mg/L*	<1.7/24	8.1 ± 1.6/7	26.4[a]	135	[38–40]	
Hydroquinone *μg/L*	—	50.6 ± 84.7/17	286.0[a]	110	[20]	Phenols
Indole-3-acetic acid *μg/L*	17.5 ± 17.5/7	875.0 ± 560.0/42	9076.9[a]	175	[41,42]	Indoles
Indoxyl sulfate *mg/L*	0.6 ± 5.4/40	53.0 ± 91.5/20	236.0	251	[35]	Indoles
Kinurenine *μg/L*	<391/7	686.4 ± 178.9/21	952.6	208	[43]	Indoles
Kynurenic acid *mg/L*	<1.0	—	9.5[a]	189	[44]	Indoles
Leptin *μg/L*	8.4 ± 6.7/56	72.0 ± 60.6/8	490.0[a]	16000	[45, 46]	Peptides
Melatonin *ng/L*	26.5 ± 7.1/35	175.8 ± 130.2/13	436.2	126	[47]	Indoles
Methylglyoxal *μg/L*	47.0 ± 12.0/15	110.0 ± 18.0/20	146.0	72	[36]	AGE
N^{ε}–(carboxymethyl)lysine *mg/L*	1.1 ± 0.3/24	4.3 ± 1.3/44	6.9	204	[11]	AGE
p-cresol *mg/L*	0.6 ± 1.0/12	20.1 ± 10.3/20	40.7	108	[48]	Phenols
Pentosidine *μg/L*	51.6 ± 18.8/19	896.0 ± 448.0/24	2964.0[a]	342	[49]	AGE
Phenol *mg/L*	0.6 ± 0.2/12	2.7 ± 3.9/10	10.5	94	[48]	Phenols
P-OHhippuric axid *mg/L*	—	18.3 ± 6.6/13	31.5	195	[50]	Hippurates
Putrescine *μg/L*	21.1 ± 7.9/10	77.4 ± 27.3/25	132.0	88	[51]	Polyamines
Quinolinic acid *mg/L*	0.1 ± 0.05/10	1.5 ± 0.9/54	3.3	167	[52]	Indoles
Retinol-binding protein *mg/L*	<80	192.0 ± 78.0/112	369.2[a]	21200	[53]	Peptides
Spermidine *μg/L*	—	97.2 ± 45.0/25	187.2	145	[51]	Polyamines
Spermine *μg/L*	—	18.2 ± 16.2/25	66.7[a]	202	[51]	Polyamines

Abbreviations are: C_N, normal concentration; C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; MW, molecular weight; ref, reference; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid; AGE, advanced glycation end products. The underlined numbers behind the slash point to the number of data on which the means or medians have been obtained. No underlined number indicates that no data about the number of samples were available. Normal values are reported as means ± SD, or in the case of a single value as a maximum (accompanied by <); uremic values are reported as means ± SD.

[a] C_{MAX} values are original data (all other values were calculated as mean + 2 SD based on C_U).

Table 3. Middle molecules ($N = 22$)

Solute	C_N	C_U	C_{MAX}	MW	Ref	Group
Adrenomedullin *ng/L*	13.2 ± 4.6/17	41.8 ± 19.7/29	81.2	5729	[54]	Peptides
Atrial natriuretic peptide *ng/L*	28.0 ± 12.2/23	202.0 ± 117.3/27	436.6	3080	[55]	Peptides
β_2-microglobulin *mg/L*	<2.0	55.0 ± 7.9/10	100.0[a]	11818	[53, 56]	Peptides
β-endorphin *ng/L*	<173.3/10	301.5/22	492.0[a]	3465	[22]	Peptides
Cholecystokinin *ng/L*	<20.0	45.9 ± 32.3/38	131.5[a]	3866	[57]	Peptides
Clara cell protein (CC16) *mg/L*	<0.1	3.3 ± 2.0/112	12.5[a]	15800	[53]	Peptides
Complement factor D *mg/L*	1.9 ± 0.5/5	19.8 ± 4.1/5	26.0[a]	23750	[58]	
Cystatin C *mg/L*	<1.6	11.8 ± 3.0/112	20.0[a]	13300	[53]	Peptides
Degranulation inhibiting protein I[c] *μg/L*	321.7 ± 59.7/23	713.7 ± 390.0/125	1631.4[a]	14100	[59][b]	Peptides
Delta-sleep inducing peptide *μg/L*	—	1.5 ± 0.9/7	3.3	848	[60]	Peptides
Endothelin *ng/L*	20.8 ± 3.8/23	63.0 ± 33.2/12	129.4	4283	[55]	Peptides
Hyaluronic acid *μg/L*	<124.0/86	215.0 ± 257.0/184	1843.0[a]	25000	[61]	Peptides
Interleukin-1β *ng/L*	<160.0/15	428.0 ± 134.0/29	1700.0	32000	[62]	Cytokines
Interleukin-6 *ng/L*	13.3 ± 3.1/28	92.3 ± 117.9/230	328.1	24500	[63]	Cytokines
κ-Ig light chain *mg/L*	34.0 ± 15.0/15	70.0 ± 60.9/104	287.0[a]	25000	[64]	Peptides
λ-Ig light chain *mg/L*	31.0 ± 11.2/15	87.0 ± 60.9/104	328.0[a]	25000	[64]	Peptides
Leptin *μg/L*	8.4 ± 6.7/56	72.0 ± 60.6/8	490.0[a]	16000	[45, 46]	Peptides
Methionine-enkephalin *ng/L*	<18.3/10	32.2/22	75.5[a]	555	[22]	Peptides
Neuropeptide Y *ng/L*	<80.0	64.9 ± 25.5/19	115.9	4272	[57]	Peptides
Parathyroid hormone *μg/L*	<0.06	1.2 ± 0.6/10	2.4	9225	[65]	Peptides
Retinol-binding protein *mg/L*	<80	192.0 ± 78.0/112	369.2[a]	21200	[53]	Peptides
Tumor necrosis factor-α *ng/L*	13.3 ± 3.0/28	114.0 ± 147.0/230	408.0	26000	[63, 66]	Cytokines

Abbreviations are: C_N, normal concentration; C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; MW, molecular weight; ref, reference. The underlined numbers behind the slash point to the number of data on which the means or medians have been obtained. No underlined number indicates that no data about the number of samples were available. No number indicates that no n value was given. Normal values are reported as mean ± SD, or in the case of a single value as a maximum (accompanied by <); uremic values are reported as mean ± SD or, in the case of a single value, as a median.

[a] C_{MAX} values are original data (all other values were calculated as mean + 2 SD based on C_U)

[b] S Schmaldienst, Vienna: personal communication

[c] Degranulation inhibiting protein I corresponds to angiogenin

Table 4. Highest versus lowest reported concentrations

	Low range C_U	Ref	High range C_U	Ref	Low range C_{MAX}	Ref	High range C_{MAX}	Ref
ADMA *μg/L*	169.5 ± 31.2	[15]	1616.0 ± 1212.0	[13]	282.8	[15]	7272.0	[13]
IL-6 *ng/L*	11.7 ± 2.8	[67]	92.3 ± 117.9	[63]	80.0	[67]	328.1	[63]
3-DG *μg/L*	59.0 ± 13.0	[36]	1715.0 ± 1009.6	[34]	85.0	[36]	3500	[34]

Abbreviations are: C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; ADMA, asymmetrical dimethylarginine; IL-6, interleukin-6; 3-D6, 3-deoxyglucose.

Table 5. C_U/C_N ratio: Highest and lowest scoring molecules

20 solutes scoring ≥15		19 solutes scoring <2.5	
Guanidinosuccinic acid	216.67	Retinol-binding protein	2.40[a]
Methylguanidine	106.00[a]	Methylglyoxal	2.34
Indoxyl sulfate	88.33	Cholecystokinin	2.30[a]
Indole-3-acetic acid	50.00	3-deoxyglucosone	2.27
Hippuric acid	49.40[a]	Degranulation inhibiting protein I	2.21
1-methylinosine	45.96	κ-Ig light chain	2.20
p-cresol	33.50	Methionine-enkephalin	1.76[a]
Clara cell protein (CC16)	33.00[a]	Kinurenine	1.76[a]
β_2-microglobulin	27.50[a]	ADMA	1.75
N^2,N^2-dimethylguanosine	26.27	ß-endorphin	1.74[a]
Pseudouridine	26.20	Hyaluronic acid	1.73[a]
Arab(in)itol	25.00[a]	Guanidinoacetic acid	1.73
Parathyroid hormone	20.00[a]	Dimethylglycine	1.51[a]
Mannitol	20.00[a]	Cytidine	1.46[a]
Interleukin-6	19.50	Hypoxanthine	1.33
α-*N*-acetylarginine	18.14	Uric acid	1.24[a]
Pentosidine	17.36	β-Lipotropin	1.13[a]
Orotidine	16.83	Argininic acid	1.05[a]
Oxalate	16.33	Neuropeptide Y	0.81[a]
Quinolinic acid	15.00		

Abbreviations are: C_U, mean/median uremic concentration; C_N, normal concentration; ADMA, asymmetrical dimethylarginine. The cut-off values of 15 and 2.5 were set arbitrarily. Solutes with a high score display a high differential concentration, uremic vs. normal, over which biologic activity (toxicity) can be exerted. Conversely, this range is limited for solutes displaying a low value.

[a]C_U/C_N obtained with C_N as a maximal (not as a mean) value

Table 6. C_{MAX}/C_U ratio: Highest scoring molecules

γ-Guanidinobutyric acid	52.55	Pseudouridine	6.61
2-Methoxyresorcinol	16.43	Oxalate	6.55
α-*N*-acetylarginine	13.95	Orotic acid	5.78
Indole-3-acetic acid	10.37	Threitol	5.75
Hyaluronic acid	8.57	Hydroquinone	5.65
Guanidinosuccinic acid	7.23	Guanidine	4.63
Benzylalcohol	6.96	Indoxyl sulfate	4.45
Interleukin-6	6.84	Erythreitol	4.11
Leptin	6.81	Thymine	4.00

Abbreviations are: C_{MAX}, maximal uremic concentration; C_U, mean/median uremic concentration.

distribution. In Table 6, the compounds with a C_{MAX}/C_U above or equal to 4.0 are displayed. It contains 17 compounds, four of which are guanidines, but also some middle molecules, such as leptin (6.81), IL-6 (6.84), and hyaluronic acid (8.57) are included. We found no statistically significant differences among groups.

Finally, in Table 7, a number of molecules to be considered for the future are listed. They do not figure in Tables 1 to 3 because either their uremic concentration is not exactly known and/or because their uremic retention has not been proven.

Table 7. Solutes to be considered for the future

1-alkyl-2-formyl-3,4-glycosyl-pyrrole
2-(2-fuoryl)-4(5)-(2-furanyl)-1H-imidazole
3-deoxyfructosone
3-hydroxykinurenine
4-hydroxynonenal
Advanced oxidation protein products (AOPP)
Advanced glycation end products-β_2-microglobulin
Anthranilic acid
β_2-microglobulin fragments
Cadaverine
Crossline
Dimethylamine
Guanosine
Imidazolone
Malonaldehyde
Malondialdehyde
Methylamine
N^{ε}-carboxyethyllysine
Organic chloramines
Oxidized low-density-lipoprotein (oxLDL)
Parathyroid hormone fragments
Pyrraline
Pyrrole aldehyde
Trimethylamine

DISCUSSION

This literature search essentially offers an overview of the currently known uremic retention solutes, together with their reported concentrations. Furthermore, a number of physicochemical characteristics and relative differences between uremic and normal concentrations and between maximal and mean uremic concentrations are considered as well.

The resulting lists are a striking illustration of the complexity of uremic retention. Obviously, there is more than the retention of a single compound or even a group of compounds, and also, there is more than the retention of the current markers, urea and creatinine. Without any doubt, clinical uremic toxicity is even more complex, since a host of retained compounds remain unidentified.

In the present inventory, uremic solutes are listed and classified according to the characteristics that potentially influence their removal pattern during dialysis or other methods of extracorporeal elimination (i.e., molecular weight and lipophilicity/hydrophilicity resulting in pro-

tein binding). The publication does not deal with biochemical or clinical relevance of the listed compounds, which has been presented by the same group of authors elsewhere [3]. The major practical impact of this work is as a tool for the future for any effort of in vitro or in vivo evaluation of uremic toxicity. Furthermore, the information collected here might also be of help for kinetic evaluation and modeling of uremic toxin time courses for generation rate and their appearance in different compartments.

Our data clarify the need to approach the problem of uremic toxicity in a different way than has been done in the past, as analyses were essentially restricted to the evaluation of one or a few molecules regarding their impact on one or a few biologic systems. The consideration of several compounds or groups of compounds at a time might conform more with reality. Hence, to solve the problem of uremic toxicity, collaborative studies involving different research groups with different scopes of interest will be needed. Another aspect to be considered for the future is the intermutual interference of uremic compounds, which might necessitate the study of solutes in a "uremic" rather than a "normal" milieu.

It is of note that molecules with a molecular weight exceeding 60,000 D (cutoff of the glomerular basement membrane) and anorganic substances were excluded from the analysis. This, for example, eliminated lipoprotein (a) (molecular weight ~100,000 D), despite an enhanced concentration in uremia [69, 70], and a potential for biologic activity. Likewise, phosphate was excluded, despite a potential link to cardiovascular morbidity/mortality [9, 71].

Essentially, we discerned small water-soluble compounds with a low molecular weight (<500 D), small protein-bound solutes, and middle molecules (≥500 D). The two latter types of molecules are characterized by a reduced removal during standard dialysis with small pore, low-flux membranes [72, 73], compared to the small water-soluble compounds. Although not definitely conclusive, circumstantial evidence suggests that membranes with a larger pore size are not only linked to a more efficient removal of larger molecules [73–75], but also to an improvement of clinical status and/or survival [4, 76–78]. It is of note that a recent prospective study could not demonstrate, however, any difference in survival outcome in patients treated with large pore vs. small pore dialyzers [79].

Protein-bound molecules, on the other hand, are not very efficiently removed by any dialytic procedure [72]. As a consequence, their concentration can hardly be influenced in ESRD, so that their clinical impact could never be studied appropriately in dialyzed uremics.

AGEs were classified among the protein-bound solutes. AGEs have been shown to be bound to albumin and other proteins and may even change albumin structure and, hence, also its binding capacity for other ligands [80]. Nevertheless, the relation with the binding proteins is probably different from that of other classical protein-bound solutes, such as indoxyl sulfate or *p*-cresol. The binding should be considered as more long-lived than for the other ligands, which display a continuous and dynamic competition among each other and with drugs for the protein-binding sites [81–83]. In some publications, not only total concentrations but also free concentrations are reported for the AGEs [11, 56]. These values are by definition much lower than the total concentration. More than 250-fold lower values were found for furosine, the free fraction of fructoselysine (201.1 ± 73.1 μg/L vs. 58.1 ± 10.8 mg/L) [11]. The tables only contain total (free + bound) concentrations.

One should be aware of the fact that the biologic action of protein-bound compounds is exerted by their free fraction. Hence, the total concentrations, as depicted in the present tables, are only valid if the experimental medium contains a sufficient quantity of albumin (or plasma protein). For in vitro experiments, it might be worthwhile to measure the free fraction in the final medium; however, usually only one solute will be added at a time, whereas in vivo many ligands will be present together, competing for the protein-binding sites and increasing the free fraction.

Overall, molecular weights of the listed compounds extended from 60 D (urea) to 32,000 D (IL-1β). Also concentrations extended over a broad range with as highest value urea, in the g/L range, and as the lowest methionine-enkephalin, in the ng/L range. A high concentration is not necessarily related to a strong biologic activity. The two molecules with the highest concentrations (urea and creatinine) are known for their relatively limited biologic activity [1, 3]. Addition of urea to the dialysate at concentrations exceeding those encountered in uremia had relatively little impact on the clinical status of the patients submitted to this regimen [84].

The index C_U/C_N offers an indication on the differences between uremic and normal values. The larger the gap between the two values, and the larger the index, the larger the range over which biologic activity can be exerted, and the bigger the chance that biologic effects become apparent in vivo or in vitro. It is of note that a high C_U/C_N is to a large extent characteristic of guanidines, protein-bound solutes, and middle molecules (altogether 13/20 of the solutes scoring >15), whereas many of these compounds exert substantial biologic activity [1, 3]. Furthermore, all these molecules are characterized by a different intradialytic behavior compared to the classical marker urea [27, 72, 73].

For protein-bound molecules the ratio C_U/C_N might even become more important for the unbound and biologically active fraction, than for total concentration, as has been observed for *p*-cresol [85].

Hence, removal strategies should be designed in a way that not only the standard molecules, but also other molecules that might be more important in the deterioration of the clinical condition, can be removed efficiently. A typical example is cardiovascular morbidity and mortality, at present one of the most worrying complications of the uremic condition [3]. Careful analysis of the literature shows that most molecules held as yet responsible for uremic toxicity are removed in a different way than urea and creatinine, either due to their molecular weight, protein binding, and/or multicompartmental behavior [1, 3]. Subsequently, dialytic removal strategies should be adapted accordingly, based on a global knowledge of the compounds playing a role in any complication of uremia.

The index C_{MAX}/C_U indicates which solutes follow a non-Gaussian distribution. The higher the value of this index, the more abnormal the distribution of concentrations. This might be the consequence of various interfering factors that are not necessarily related to the uremic status (e.g., gender, genetic predisposition, general condition, body composition, or nutritional status) [46, 86–88]. Several guanidines and middle molecules show a strong trend for abnormal distribution. Among these, IL-6 and leptin may have an important potential for biologic activity [3].

It is of note that for certain solutes a substantial gap exists between the highest reported mean concentrations [13, 34, 63] and the other reported concentrations [15, 36, 67]. This is the case for ADMA, IL-6, and 3-DG (Table 4). We considered it beyond the scope of this work to offer a critical analysis on the origin(s) of these differences in concentrations. Overestimation of true values is possible if tests are too sensitive [e.g., for certain types of enzyme-linked immunosorbent assay (ELISA)], if in chromatographic analysis [e.g., high-performance liquid chromatography (HPLC)] peak heights are overestimated because several compounds elute at the same moment or if external contamination has taken place. On the other hand, true values might be underestimated if extraction or derivatization is inefficient or if compound is lost during the preparative steps or analytic procedures (e.g., absorption on chromatographic columns or evaporation). Whether the discrepancies reported in Table 4 are attributable to technical reasons, or to differences in patient populations or removal strategies, is left open for discussion. If these concentrations are to be used for in vivo or vitro experiments, it is left to the reader which concentration to use, but he or she should be aware of these discrepancies and take them into account when interpreting the results.

Although we tried to make this database as complete as possible, it might be not exhaustive. Also in the future, new publications will appear on newly discovered compounds and/or known compounds will be presented with different mean uremic or highest values than the ones reported here. For that matter, this database will need continuous updating (see **Acknowledgments**). Nevertheless, the present data offer, in our opinion, a broad overview of the present state of the art, and such an overview has, to our knowledge, never been published before. The authors will appreciate receiving additional information about new solutes to be added to the lists, or about enlisted molecules with reported concentrations that are different from the ones that are cited at present, enabling a continuous update.

CONCLUSION

An overview is given on the reported range of concentration of uremic retention solutes. Most known uremic retention solutes have a low molecular weight, although these not necessarily exert toxicity, especially if they are not protein bound. Concentrations of 90 solutes are reported, ranging from 2.3 g/L (urea) to 0.32 ng/L (methionine-enkephalin). Low concentrations are found especially for the middle molecules. The ratio C_U/C_N is high for several of the guanidines, protein-bound molecules, and middle molecules, and many of these solutes are toxic. These data can be of use as a guideline for future in vivo and in vitro experiments. Uremic retention appears to be a complex kinetic and multifactorial problem concerning a larger amount of solutes than those currently used as markers, such as urea and creatinine.

ACKNOWLEDGMENTS

The European Uremic Toxin Work Group (EUTox) has been created within the European Society for Artificial Organs (ESAO) to discuss and analyze matters related to the identification, characterization, analytic determination, and evaluation of biologic activity of uremic retention solutes. More information about the European Uremic Toxin Work Group, which is responsible for this publication, can be obtained at the website: *http://uremic-toxins.org*.

The authors would appreciate being contacted about uremic toxins or concentrations that are not listed in this publication. Please use the following e-mail address: *raymond.vanholder@rug.ac.be*.

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