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Announcement by the German Federal Environment Agency

Standardisation of Substance Concentrations in Urine – Creatinine

Opinion of the Human Biomonitoring Commission of the German Federal Environment Agency

Introduction

Analysis of urine samples in biological monitoring is a preferred method to verify human exposure to a contaminant, as urine sampling is relatively easy to do for anybody and because sufficient amounts of sample material are available.

On the other hand, urinary concentrations of xenobiotics generally vary broadly as a function of urine excretion. Therefore, for urine investigations aimed at determining individual systemic exposure to a substance, it seems desirable to obtain diuresis-independent values. These can be achieved by the use of standardisation procedures that compensate differences in urine dilutions.

There have been several efforts in the past to define uniform standardisation criteria for quantitative assessment of the renal excretion of xenobiotics.

For example, in 1984, the “Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area” of the German Research Foundation (DFG) established assessment principles for the formulation of biological tolerance values for occupational exposures (BAT values) which give preference to expressing substance concentrations in relation to creatinine. However, in individual cases it continued to be necessary to select a suitable other reference parameter for a substance to be determined, namely when a 24-hour composite urine sample or morning urine is not available and simple reference to volume does not appear to be equivalent [1].

Need for standardisation

The issue of standardisation arises in the establishment of reference and human biomonitoring values and in evaluating the results of urine sample analysis on the basis of these values. This is true for the evaluation of individual results [2] as well as for epidemiological investigations into pollutant exposure of adults [3] and, especially, children and adolescents [4].

Creatinine as normalisation parameter

As a scale basis for renal excretion parameters are generally preferred that represent a measure of the renal glomerular filtration rate (GFR). This is based on the assumption that xenobiotics or environmental toxins undergo a simple renal elimination process which is proportional to the GFR without any significant tubular reabsorption mechanism being involved.

These parameters, however, were not originally intended for use as a scale basis for the renal excretion of xenobiotics, but mainly serve as clinical-chemical parameters in renal

function diagnostics. They need not necessarily be concentration-proportional to the measured xenobiotic. This is particularly the case if individual substances with specific elimination processes are referred to creatinine.

The current consensus considers creatinine to be an acceptable reference parameter for the assessment of a xenobiotic in urine. Nevertheless, in the opinion of the Commission, standardisation parameters such as osmolality, density or electric conductivity should be taken into account in the assessment, particularly when the co-action of renal glomerular filtration, tubular secretion and reabsorption needs to be considered and if the sum of all renally excreted substances rather than an individual substance poses the basis for standardisation.

Reference to the creatinine content of the urine sample is considered as a common standardisation method to compensate the interfering influence of differently concentrated urine in the evaluation of urinary concentrations of xenobiotics. This method is not undisputed, however [5, 6, 7, 8]. The criticism in effect refers to the limiting factors resulting from the physiological principles of creatinine excretion.

Physiology of creatinine excretion

Creatinine is a metabolic by-product of protein metabolism which is normally contained in urine. It is mostly filtered glomerularly in the kidney and is not reabsorbed. In healthy persons, physiological creatinine production is largely proportional to muscle mass, and therefore individually its excretion over 24 hours is relatively constant. In adults, excretion rates differ between the sexes and are distinctly reduced in old age. Creatinine production in children is also markedly lower, and moreover, strongly age-dependent [9, 10]. Therefore, in a direct comparison between adult and infantile populations, reference to creatinine generates artificially increased pollutant exposures in children [10, 11, 12].

The individual production rate and hence renal excretion is not constant over the day and is, moreover, dependent upon urine excretion. The shorter the urination intervals, the larger the short-term diurnal variations: Therefore the reliability as individual reference parameter is correspondingly lower [8]. In addition, meat consumption is an external factor contributing to the creatinine pool. It increases total creatinine excretion so that it exceeds endogenous metabolic creatinine production, which is individually relatively constant.

Therefore, especially in comparative group studies and statistical correlation analyses, consideration must be given to the fact that the physiology of creatinine production and excretion is an important co-factor influencing urine analysis values and that the results strongly reflect the physiological conditions of creatinine excretion.

In environmental epidemiological studies with large sample sizes, attempts are made to eliminate these interfering factors by the use of adequate calculation methods [9].

Reference to creatinine and overcompensation

Irrespective of these objections to the use of creatinine as a scale basis , the review of the validity has shown that measured urine parameters are not always, as demanded, independent of the standardisation parameter, but are instead found to be significantly negatively correlated with it after standardisation. Presumably, most xenobiotics are – unlike creatinine – reabsorbed to a considerable extent in the tubular section of the kidneys, so that the ideal of a direct concentration-proportionality to creatinine cannot generally be expected.

This overcompensation found with creatinine as a scale basis could be formally addressed by dividing the substance concentration not by the creatinine concentration itself, but by a power of it to be chosen accordingly, the exponent being a positive number smaller than 1. This parameter, however, has to be determined empirically for each substance from the gradient of the regression line of the relevant study group.

Apart from the fact that these parameters are generated using the very study groups to whom they are generally applied to, it remains problematic to transfer grandients that were determined in this way to other study groups and even to the individual case.

Conclusions relating to urinary measurements

For these reasons, the Commission considers that the standardisationmethod commonly applied to date – that is dividing the urinary concentration of the substance of interest by the urinary creatinine concentration – may not generally be sufficient for gaining a better assessment basis for substance concentrations in urine. Standardisationmethods based on a combination of creatinine and flow rate [13, 14] do not seem to be fundamentally better, either. First of all, such combination methods are complicated to execute und generally require further auxiliary parameters that have to be determined empirically; secondly, they do not exclude the influence of creatinine physiology; and thirdly, determination of the flow rate requires additional information on sample volume and miction interval.

In view of this situation, the Commission has decided to regard the creatinine content almost exclusively as a criterion for urine sample validity intended to exclude the use of extremely dilute or highly concentrated urine samples for environmental medicine assessment.

Establishment of exclusion criteria

In its 1996 guidelines on biological monitoring at the workplace, the WHO recommended that only urine samples with a creatinine concentration of 0.3 – 3 g/l should be used for case-by-case assessment [15]. This essentially reflects the creatinine excretion rate of the adult working population and is suitable mainly as a criterion for assessing occupational exposure. Creatinine excretion is significantly lower particularly in children and the elderly which makes it likely that urine samples with creatinine concentrations below 0.3 g/l occur very frequently.

In analogy to the WHO's recommendations, for deriving reference values for environmental medicine purposes the Commission considers urine samples with a creatinine content between 0.3 and 3 g per litre as appropriate. When sampling is repeated because of an exceeded reference value – for better evaluation of the result - the creatinine concentration of the sample should be in a narrower range. For this purpose the Commission recommends the range of 0.5 - 2.5 g creatinine per litre. This limitation ensures the exclusion of highly diluted samples which otherwise might be assessed as "within the normal range" in spite of indicating an existing exposure.

Recommendations

The creatinine content of the urine samples should be indicated additionally, mainly for the purpose of orientation. This also provides the possibility to obtain creatinine-related results in case both volume-related and creatinine-related reference values are available or only creatinine-related data have been published due to previous conventions.

In the opinion of the Commission a 24-hour composite urine sample is most suitable for determining corporal pollutant exposures via renal excretion investigations; this is true in particular for both epidemiological and smaller environmental medicine studies. However, the

Commission does not underestimate the difficulties associated with obtaining a complete 24-hour composite urine sample. If a 24-hour urine sample is not available, a first-morning-urine sample is recommended as reliable and comparable analysis material for both individual environmental medicine analyses and epidemiological environmental health studies.

References

1. Norpoth K, Heger M (1984) Kreatinin als Bezugsgröße bei der Angabe von Stoffkonzentrationen im Harn. In: Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte), Arbeitsmedizinisch-toxikologische Begründungen, Bd. 1 Herausgeber: D. Hentschler u. G. Lehnert; Kommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe der DFG
2. Kommission Human-Biomonitoring des Umwelbundesamtes (2000) Zur umweltmedizinschen Beurteilung von Human-Biomonitoring-Befunden in der ärztlichen Praxis, Umweltmed. Forsch Prax. 5 (3): 177-180
3. Becker K, Schulz C, Kaus S, Seiwert M, Seifert B (2003) German Environmental Survey 1998 (GerES III). Environmental pollutants in urine of the German population. Int. J. Hyg. Environ. Health 206:15-24.
4. Schulz C, Becker K, Seiwert M (2002) Kinder-Umwelt-Survey. Gesundheitswesen 64 Sonderheft 1:69-79.
5. Alessio L, Berlin A, Dell`Orto A, Toffoletto F, Ghezzi I (1985) Reliability of urinary creatinine as a parameter used to adjust values of urinary biological indicators. Int. Arch. Occup. Environ. Health 55:99-106.
6. Greenberg GN and Levine RJ (1989) Urinary creatinine excretion is not stable: A new method for assessing urinary toxic substance concentrations, J. Occup. Med. 31:832-838
7. Araki S, Sata F, Murata K (1990) Adjustment for urinary flow rate: An improved approach to biological monitoring, Int. Arch. Occup. Environ. Health 62:471-477
8. Boeniger MF, Lowry LK, Rosenberg J (1993) Interpretation of urine results used to asses chemical exposure with emphasis on creatinine adjustment: a review. Am. Ind. Hyg. Assoc. J. 54:615-627.
9. Barr D.B. et al. Urinary Creatinine Concentrations in the US Population: Implication on Urinary Biological Monitoring measurements, in press
10. Heudorf U, Angerer J (2001) Metabolites of organophosphorous insecticides in urine specimens from inhabitants of a residential area, Environ. Res. 86: 80-87.
11. Mills PK, Zahm SH (2001) Organophosphate pesticide residues in urine of farmworkers and their children in Fresno County, California. Am. J. Ind. Med. 40:571-577.
12. Krause C, Babisch W, Becker K, Bernigau W, Helm D, Hoffmann K, Nöllke P, Schulz C, Schwabe R, Seiwert M, Thefeld W (1996): Umwelt-Survey 1990/92 Band Ia: Human-Biomonitoring. Deskription der Spurenelementgehalte in Blut und Urin der Bevölkerung in der Bundesrepublik Deutschland. WaBoLu Hefte 1/96, Umweltbundesamt, Berlin
13. Elkins H.B. and Pagnotto, L.D (1965) Is the 24-hour urine sample a fallacy?. Am. Ind. Hyg. Assoc. J. 26:456-460
14. Hwang YH, Bornschein RL, Grote J, Menrath W, and Roda S (1997) Urinary arsenic excretion as a biomarker of arsenic exposure in children, Arch. Environ. Health 52:139-147
15. WHO (1996). Biological Monitoring of Chemical Exposure in the Workplace. Vol. 1. Geneva: World Health Organization