MEASUREMENT OF URINE CONTAINED ORGANIC HALOGEN COMPOUNDS AN ASSAY FOR HUMAN ENVIRONMENTAL EXPOSURE

M.S. Salkinoja-Salonen*, K. Al-Soufi*, P. Jäppinen** and J.K. Jokela*

*University of Helsinki, Department of General Microbiology
Mannerheimintie 172, 00300 Helsinki FINLAND
**Enso Gutzeit OY, Occupational Health Centre, SF 55800 Imatra Finland

ABSTRACT

The present paper describes the measurement of urine contained organic halogen compounds. The method is an application of the adsorbable organic halogen (AOX) assay which is widely utilized for analysis of industrial waste water and drinking water. We found that this assay can be applied to human urine if the urine is pretreated to hydrolyze the mucins so as to cleave the neuraminic acid residues, responsible for the high viscosity of these slimy proteins. The method was found sensitive down to 1 ug of organic halogen /100ml of urine. We found 50 to 260 ug of AOX in night urine of healthy, occupationally unexposed volunteers. The urine contained AOX correlated positively to that of the local drinking water of which the AOX contents varied from 15 to 220 ug per l. Since many toxic chemicals to which man may be exposed environmentally or occupationally, are in fact halogen compounds, this assay may be used to monitor for human exposure. We measured the urine contained AOX of workers of pulp bleaching plant and found somewhat elevated levels as compared to a reference group from the same region.

INTRODUCTION

The majority of all hazardous man-made chemicals are halogenated (Cl, Br) compounds. This group covers most pesticides and herbicides, many antimicrobial agents as well as the widely utilized industrial solvents, frequently causing occupational poisoning and pollutes ground water. Large quantities of organochlorine compounds are discharged into the environment during bleaching of pulp and chlorine disinfection of low quality raw water for drinking water.

The extent to which man actually gets exposed to chemicals from the environment, is poorly documented. Suitable methods are lacking to analyse the diverse chemicals in any large group of persons. Modern analytical methods, like gas chromatography-mass spectrometry, give accurate results but are costly and laborious. This paper describes the measurement of organic halogen compounds in human urine by microcoulometric titration after adsorption to activated carbon and combustion into hydrogen halides. It is not labour intensive and can be applied to analyse large numbers of urine samples. It is group specific rather than compound specific and may thus have potential use for monitoring human exposure to a wide spectrum of chemicals in the environment and in the workplace.

EXPERIMENTAL

Collection and pretreatment of urine samples
Night urine was collected in toto. Before analysis the urine was pretreated to desialidate the urine contained mucins (sialic acid containing mucoproteins) either with neuraminidase (0.01 U/50ml of urine, >2h at 37 C, pH 4 to 5, adjusted with nitric acid) or mild acid hydrolysis at pH 1.5 (nitric acid) for 1 to 2 h at room temperature. The urine was then diluted to 1000ml or desired density (1.024g/cm3) with pure water.

Analysis of organic halogen
Aliquots of 50ml in triplicate of the pretreated, (diluted) urine were processed as described in the standard protocols for determining AOX (adsorbable organic halogen) in water or waste water (Anonymous 1985) The microcoulometric analyzer of Euroglass B.V. (Delft, the Netherlands), equipped with an automated sample feeder, was used. Activated carbon was from Euroglass B.V. (Delft, the Netherlands), polycarbonate filters (0.2 um) from Nuclepore and neuraminidase (EC 3.2.1.18) from Sigma.

RESULTS

We studied the suitability of the AOX procedure for determining organic halogen compounds in human urine. The method is based on the adsorption of the halogen compounds from acidified solution to activated carbon which, after washing with dilute acidified nitrate solution, is combusted and the emerging hydrogen halogenide quantified microcoulometrically (Anonymous, 1985). We found that the filterability of human urine through the polycarbonate was poor, leading to incomplete removal of inorganic halides at the nitrate wash, and thus to unreliable results (too high).

We found further that the filterability of urine was dramatically improved upon preincubation of the urine with neuraminidase. Filtration time required for 50ml of urine was decreased by this treatment from 2..5 hours down to 10 minutes or less, i.e. a value comparable to what is found for clear water samples. The same effect was achieved by mild acid hydrolysis of the urine (pH $\leq$1.5).

We tested the halogen recovery of a variety of different organic halogen compounds in urine, added to 0.2...10uM concentration: aldrin, quintozene, MCPA, 2,4-D, pentachlorophenol, 2,4,5-trichlorophenol, 3,5-dichloroaniline, trichloroacetic acid (pesticides and their parent compounds); 1,2-dichlorobenzene and 1,1,1-trichloroethane (solvents); Irgasan DP-300, Kathon 886MW, DBNPA and 1,4-bis bromoacetoxy- 2-butene (disinfecting agent and industrial antimicrobials). The results (not presented) showed most of them measured with a recovery of approx. 70% and higher, the exceptions being volatile solvents (trichloroethane), which gave recovery of 20 to 50% and trichloroacetic acid (recovery 10 to 30%). The results thus show that other urine contained organic compounds (urea, uric acid, creatinine, bilirubins) did not seriously interfere with the assay of the organic halogen compounds.

We measured the AOX contents of urine from occupationally unexposed volunteers (n= 51, aged 2 to 79 years) in Finland. We found no clearcut correlation of the urine AOX contents to age, sex or the body weight. Unexpectedly, our results suggested regional variation. Figure 1 shows examples of results obtained with residents of Helsinki metropolitan area and those from a small, heavily industrialized town 300km east of Helsinki. The individual variation of the AOX contents of night urine from residents of Helsinki ranged from 50 to 90 micrograms (average for males 71 ug, females 62 ug) and at Imatra from 60 to 190 ug (average for males 132 ug, females 105 ug).

One very obvious exposure route of humans to organic halogen compounds is drinking water. We measured both the urine and tapwater AOX contents at 6 different localities in Finland. The results, depicted in Fig. 2, indicate positive correlation (r = 0.93) between these.

We studied te urine excretion of AOX by the bleach plant workers of one large pulp mill, 13 persons in total, all males, aged from 28 to 59 years. We found an average of 203 ug of AOX per night urine (S.E.= 85ug), while the average of non-exposed males (laboratory and office workers) from the same region was 132 ug (S.E. = 31 ug).

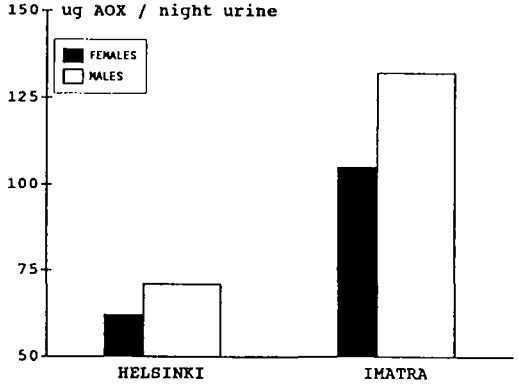

Fig.1. Levels of organic halogen compounds in the urine of occupationally unexposed volunteers measured as the AOX.

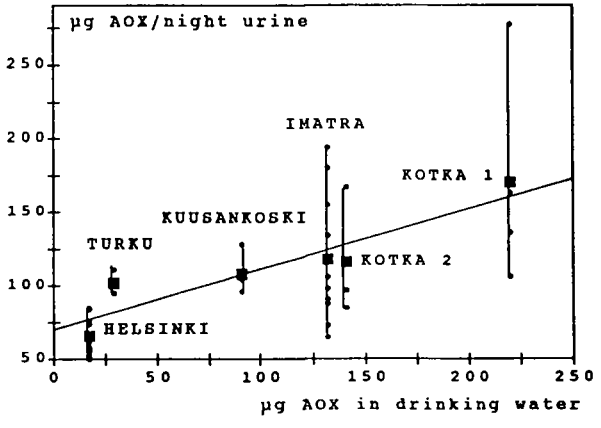

Fig. 2. Correlation between the adsorbable organic halogen (AOX) concentration in the urine and drinking water (ug per litre) at different localities. The samples were collected in January-March 1990 in five different towns.

DISCUSSION

The measurement of total organic halogen compounds has been shown a valuable tool for monitoring potable water quality and undesirable wastewater discharges from the industry (Anonymous 1985, 1989). In this paper we show that it was possible to adapt this method to monitor organic halogen compounds in human urine. We found the the poor filterability of urine, causing the AOX assay to fail, was caused by mucins excreted by the urinary tract mucous membranes. These could be removed by either neuraminidase or mild acidolysis. Both treatments are known to remove neuraminic acid residues from the mucins (Neuberger & Marshall, 1966), leading to loss of mucin viscosity (Sharon, 1975).

There seemed to exist a positive correlation between the urine AOX contents to that of the residential drinking water (fig. 2), indicating that drinking water AOX may hhave been resorbed by the gastrointestinal tract and then renally excreted. In Finland the AOX contents of drinking water is very high, the reason being chlorine disinfection of humic raw water (Vartiainen, 1988).

Human metabolism is not known to synthesize organic halogen compounds, apart from the iodine containing hormone thyroxine. If the data in Fig. 2 are extrapolated to 0 ug of AOX per 1 of drinking water, there remains a residual AOX in the urine of around 70 ug. Some of this amount may originate from thyroxine turn-over, some be be of air-born exposure and food. It was interesting to note, that bleach plant workers had somewat elevated levels of urine AOX than other residents of the same region. Because of the small number of individuals studied, and the fact that some samples were taken after the working shift, others after return to work after a 5-day free period, no statistical evaluation of the results is possible. The results however warrant further study.

CONCLUSION The analysis of urine contained AOX is a powerful tool for monitoring human exposure to chemicals at the workplace and from the environment.

ACKNOWLEDGEMENTS

This study was made possible by the financial support from the Foundation for Work and Environment (MSS, grant 88116), Maj and Tor Nessling Foundation (MSS) and the Academy of Finland (JJ), Environmental Research Council.

REFERENCES

Anonymous (1985). Deutsche Einheitsverfahren. Bestimmung der adsorbierbaren organisch gebundenen Halogene (AOX). DIN 38409 (Teil 14).

Anonymous (1989). Effluents from pulp mills. Organically bound chlorine by the AOX method, Scandinavian pulp, paper and board testing committee SCAN-W 9:89.

Neuberger, A., Marshall, R.D. (1966). Methods for the qualitative and quantitative analysis of the component sugars. In: Glycoproteins. (ed. Gottschalk A). Elsevier Publishing Company, Amsterdam, 190-234.

Sharon, N (1975). Complex Carbohydrates. Their Chemistry, Biosynthesis and Functions. Addison-Wesley Publishing Company, London & Amsterdam. pp.49-63, 177-192.

Vartiainen T. (1988). Mutagenicity of drinking waters in Finland. Publications of the National Public Health Institute, Helsinki, A2/1988.