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FIOH external quality assurance scheme for organic solvent metabolites

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Summary. - The scheme consists of analyses for phenol, 2,5-hexanedione, and mandelic, trichloroacetic and methylhippuric acids in urine. The present participants are 31 laboratories from 14 countries. Samples are prepared by pooling urine obtained from occupationally exposed workers or by spiking with appropriate pure metabolites. Four sets of samples at two concentration levels for each analyte are distributed annually. The report includes information on the arithmetic means, standard deviations and CVs for overall results and separately for different methods. During the last three years, the CVs have varied rather non systematically, being 21-31% for mandelic acid, 24-26% for trichloroacetic acid, 24-35% for phenol, 55-110% for 2,5-hexanedione and 44-50% for methylhippuric acid.

Key words: Finland, external quality assurance, organic solvent, urine, occupational health, biological monitoring.

Riassunto *(Il programma del Finnish Institute of Occupational Health per la valutazione esterna di qualità nell'analisi di metaboliti dei solventi organici)*. - Lo schema comprende l'analisi di fenolo, 2,5-esandione, acido mandelico, acido tricloroacetico e acido metilippurico nelle urine. Attualmente vi partecipano 31 laboratori di 14 diversi paesi. I campioni sono preparati miscelando urine ottenute da lavoratori professionalmente esposti o per aggiunta degli appropriati metaboliti puri. Ogni anno vengono distribuiti quattro gruppi di campioni a due livelli di concentrazione per ciascun analita. Il rapporto inviato ai partecipanti include le informazioni relative alle medie aritmetiche, deviazioni standard e coefficienti di variazione (CV) per i risultati presi tutti insieme e separatamente per ciascuno dei vari metodi. Negli ultimi tre anni, i CV osservati sono variati in modo non sistematico. I valori osservati variavano tra il 21% e 31% per l'acido mandelico, tra il 24% e 26% per l'acido tricloroacetico, tra il 24% e il 35% per il fenolo, tra il 55% e 110% per l'esandione e tra il 44% e il 50% per l'acido metilippurico.

Parole chiave: Finlandia, valutazione esterna di qualità, solventi organici, urine, medicina occupazionale, monitoraggio biologico.

Introduction

Analyses for the biomonitoring of exposure to chemicals at the workplace are complicated and limited possibilities exist for external quality assurance. It is not therefore surprising that the analytical performance in biological monitoring is not at a satisfactory level [1-8]. On the other hand, it has been repeatedly shown that an improvement may be achieved by continuous external quality assurance even in difficult analyses performed in biological monitoring of exposure to industrial chemicals [3, 7, 9-11]. In the European community and world-wide there are some quality assurance schemes for the analyses of metals in biological specimens but for organic solvents for which biological monitoring is in extensive use, no educational external quality assurance scheme is available. In order to fill this gap, in 1979 the Finnish Institute of Occupational Health (FIOH) started an inter-comparison programme for organic solvent metabolites in urine [4]. The programme has been considered useful, as indicated by the steadily increasing number of participants, and by responses from the participants directed to us and also by views expressed in scientific literature [12].

Organisers, participants and financing

The coordinator of the programme is the Biomonitoring Laboratory, a division of the Department of Industrial Hygiene and Toxicology in the Finnish Institute of Occupational Health. The laboratory performs

FIOH QUALITY ASSURANCE PROGRAMME FOR ORGANIC SOLVENT METABOLITES

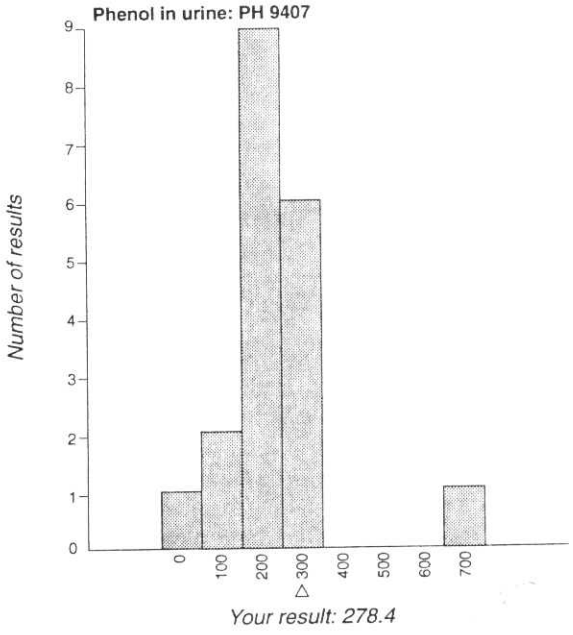
LAB. CODE: 1
RUN: 4
DATE SENT: 28.11.1994

STATISTICS Specimen PH9407

Phenol in urine	No.	(Outliers)	Mean	STD	CV%
Overall	18	(1)	220.76	76.53	34.7
GC	11	(1)	244.19	54.64	22.4
HPLC	4	(0)	151.37	102.92	68.0
SP	2	(0)	261.50	74.25	28.4
Method unknown	1	(0)	159.00		

Your result: 278.4

Pooled urine (sample 9408) collected from nonexposed people was spiked with 250 µmol/l (as phenol) of pheny-β-D-glucuronide



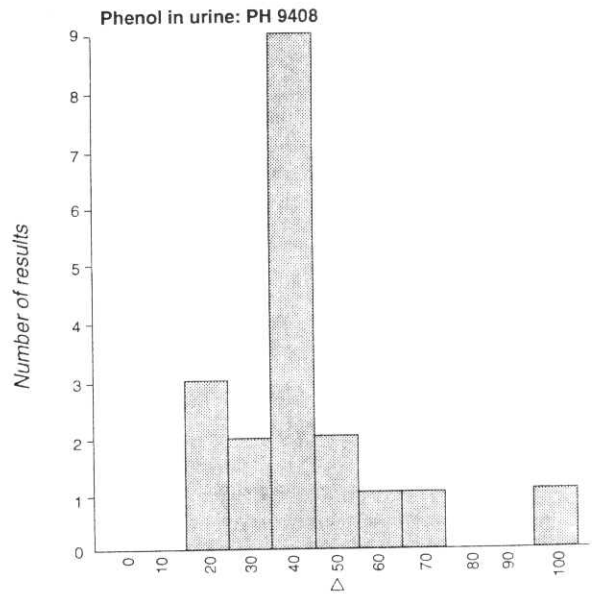
Your result: 278.4

STATISTICS Specimen PH9408

Phenol in urine	No.	(Outliers)	Mean	STD	CV%
Overall	18	(1)	40.49	12.94	32.0
GC	11	(1)	36.78	8.82	24.0
HPLC	4	(0)	39.30	14.70	37.4
SP	2	(0)	61.95	18.31	29.6
Method unknown	1	(0)	43.00		

Your result: 49.90

Pooled urine collected from nonexposed people



Your result: 49.90

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all statistics (N,MEAN,STD,CV%) calculated after exclusion of outliers.

Fig. 1. - Report of an EQAS round to a participant.

annually about 7000 biological monitoring analyses. Altogether 50 different analyses belong to the repertoire, and those most frequently requested include chromium, aluminium, mercury, nickel, mandelic acid and thiothiazolidine carboxylic acid in urine and lead in blood. Two Regional Institutes of Occupational Health contribute to the programme, mainly by providing samples for the analysis of 2,5-hexanedione (Tampere Regional Institute) and methylhippuric acid (Turku Regional Institute), as well as advice on these analyses and the overall operation of the scheme.

At present 30 laboratories in 14 countries participate in the programme (*). Sixteen of them are from seven European Union Member States, six have national analytical responsibilities, and five are universities. The participating laboratories are 2 from Belgium, 6 from Brazil, 2 from Canada, 5 from Italy, 2 from UK, 3 from USA, 3 from Finland and 1 each from Germany, Hungary, Ireland, Israel, Poland, Sweden and Croatia. All participating institutions have extensive experience in biological analysis. The anonymity of the participating laboratories is protected by using code numbers. However, with the agreement of all participants, the names of the participants have been disclosed among the group, to create a possibility of consultation in case problems arise in a specific analysis.

The direct costs of the collection, preparation and distribution of the specimens, and the analysis of the results are charged to the participants. The charge is at present 75 US $/analyte/year (1995). The Institute covers the balance of the programme costs; the working time for the management and coordination of one round is about 14 days.

Samples

At present the analytes covered by the programme are mandelic acid (metabolite of styrene), phenol, trichloroacetic acid (metabolite of trichloroethylene and perchloroethylene), methylhippuric acids (metabolites of xylenes) and 2,5-hexanedione (metabolite of hexane). Four deliveries are done annually, following a schedule distributed in advance to the participating laboratories.

Samples are collected from workers exposed to the chemicals of interest. In case specimens from exposed workers are not available, or also when there are special reasons to verify the accuracy of the results obtained, we use samples from non-exposed subjects spiked with pure chemicals - in the form that the analytes appear in urine of exposed workers (e.g. phenyl-β-D-glucuronide, not phenol).

The following steps for urine collected from exposed workers or non exposed laboratory personnel are used. Urine is stored frozen (- 20 °C) and homogeneous urine pools at two concentration levels of each analyte are prepared. The pools are filtered and the addition of the analytes is performed when appropriate. The concentration level of the pools is determined in the laboratory preparing the specimen, using the routine method. Each laboratory receives two samples at different concentration levels for each required analyte in vials of 25 ml which are frozen before shipment. New pools are prepared for each round.

Express mail is used for the delivery of the specimens, and the samples are packed into cold-packs (- 20 °C). We request the participating laboratories to store the specimens in the refrigerator, not frozen, until analysis.

Target values

Two approaches are used to obtain the target values. For natural samples collected from exposed workers a consensus mean value is determined from the results of all participants (after excluding outliers) and used as a target value. For *spiked samples* the added analyte concentration plus the endogenous concentration of the analyte is used as the target. The endogenous concentration is derived from the consensus mean value if available (both spiked and nonspiked specimen sent for analysis), or from analysis by the organising laboratory.

Reports

The reports are produced within two weeks after the deadline for reporting results. The report for each round is based on a computer-driven analysis of the results of the run, using a SAS-programme. The report includes numeric and graphical analysis of the distribution of the results of each of the two specimens (Fig. 1). After exclusion of outliers (defined as results falling outside the interval mean ± 3 SD) the arithmetic means, standard deviations and coefficients of variation (CV) are calculated for overall results and separately for each analytical method used. The amount of analyte added (if any) to the specimens during preparation is also reported.

So far, no cumulative reports have been issued. However, we are in the process of preparing a long term report for one year's operation of the scheme; this will become a standard feature from 1996 onwards. The long-term report gives a general view of the analytical performance of the laboratory in relation to the target values and analytical recovery calculated from spiked samples and also includes the organiser's view on what is acceptable performance, since it has been shown that such information tends to improve laboratory performance [9]. The deviation of an individual result from a target value is calculated for each sample and

(*) At the time of printing of this article, the number of participants is 41, from 19 countries.

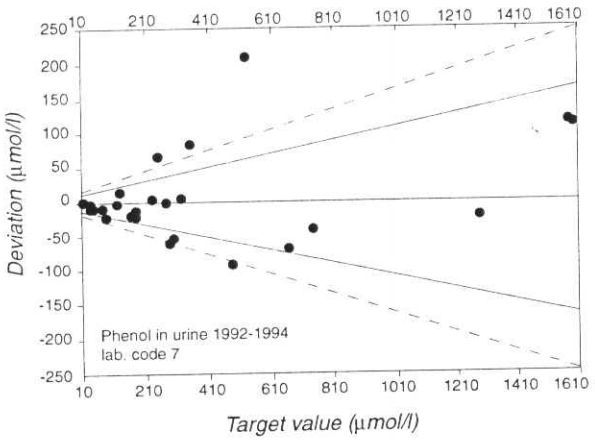

Fig. 2. - Projected cumulative report to a participant. Acceptable results within the bold lines, intermediary results between the bold and dashed lines, unacceptable results outside the dashed lines.

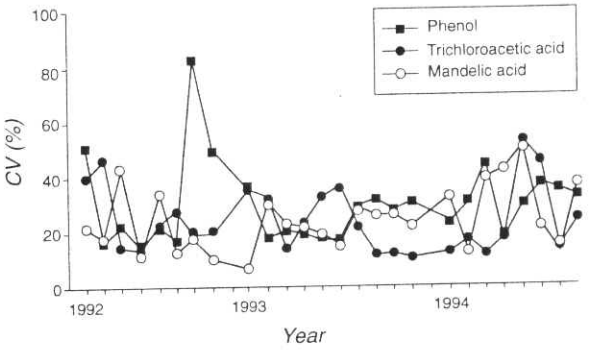

Fig. 3. - Variation of the between-laboratory agreement (CV%) in 13 consecutive rounds for mandelic acid, phenol, and trichloroacetic acid.

plotted using the model of the Robens Institute scheme for trace elements (TEQAS, Robens Institute, University of Surrey, Guildford, UK, Fig. 2) [9, 13]. A larger relative error is accepted at low analyte concentration levels; acceptable, intermediate and non-acceptable ranges are defined.

Results

The coefficients of variation obtained from the 26 samples over 13 consecutive runs have varied in a nonsystematic fashion during the 3 years (Figs 3 and 4). The dependence of the coefficients of variation on the concentration level of the samples, as observed in this study, is shown in Table 1. The largest difference was observed for the analysis of mandelic acid where the CV for samples with concentrations below 2 mmol/l was on average 31% (range 18-50), and for levels above 2 mmol/l, 14% (range 6-21) (Fig. 5).

The trend of the between-laboratory agreement over the years has been evaluated from the comparison of the annual means of the CVs obtained for each analyte and each specimen (Fig. 6). The lowest average variation has been observed for mandelic acid (21-31%), trichloroacetic acid (24-26%) and phenol (24-35%) and the highest for 2,5-hexanedione (55-110%) and methylhippuric acid (44-50%).

Some improvement with time was seen for 2,5-hexanedione (from 110% to 55%) and for methylhippuric acid (from 50% to 44%), although the CVs for these analytes still remain high (Fig. 6).

Because of the small number of participating laboratories, very limited data have accumulated on variation dependent on the analytical methods. Some such information is given in Fig. 7.

Table 1. - Average coefficients of variation (CV%), range (in parenthesis) by concentration levels observed for various analytes between 1992 and 1994 (13 rounds, each round including 2 samples)

Year	2,5-Hexanedione			Mandelic acid			Methylhippuric acid			Phenol			Trichloroacetic acid		
	CV%		no.	CV%		no.	CV%		no.	CV%		no.	CV%		no.
	<5 mc (µmol/l)	>5 mc (µmol/l)		<2 mc (mmol/l)	>2 mc (mmol/l)		<2 mc (mmol/l)	>2 mc (mmol/l)		<150 mc (µmol/l)	>150 mc (µmol/l)		<200 mc (µmol/l)	>200 mc (µmol/l)	
1992	147 (147)	104 (47-176)	4-6	29 (18-43)	13 (10-17)	15-19	64 (39-97)	32 (28-37)	12-14	35 (17-51)	34 (15-82)	11-14	29 (15-47)	20 (14-28)	9-12
1993	38 (36-40)	81 (38-98)	4-7	25 (19-31)	14 (6-21)	14-19	57 (31-114)	36 (28-46)	10-17	28 (17-35)	22 (17-30)	9-16	17 (11-33)	26 (10-37)	11-15
1994	35 (35)	63 (24-106)	7-8	40 (32-50)	15 (11-21)	17-21	48 (29-86)	36 (20-50)	17-21	32 (23-45)	29 (16-37)	14-19	29 (12-52)	20 (11-45)	14-19

no. = number (min-max) of participating laboratories; mc = mean concentration.

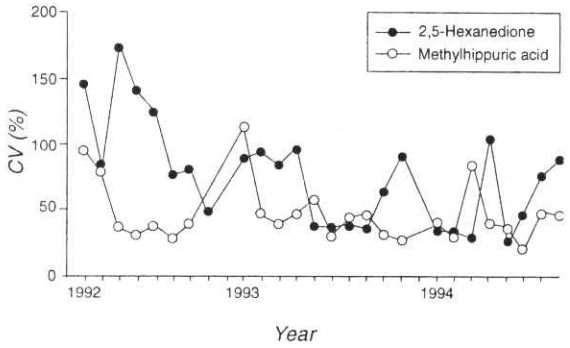

Fig. 4. - Variation of the between-laboratory agreement (CV%) in 13 consecutive rounds for 2,5-hexanedione and methylhippuric acid.

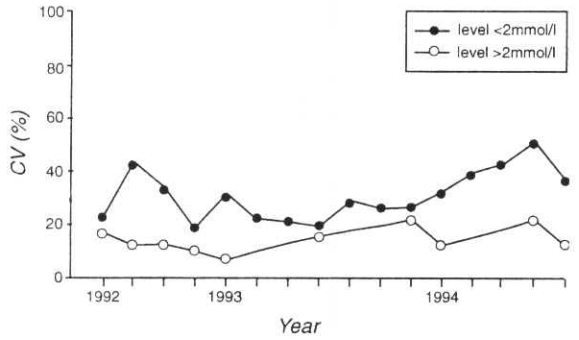

Fig. 5. - Variation of the between-laboratory agreement (CV%) over the years 1992-1994 for the analysis of mandelic acid at concentration levels higher or lower than 2 mmol/l.

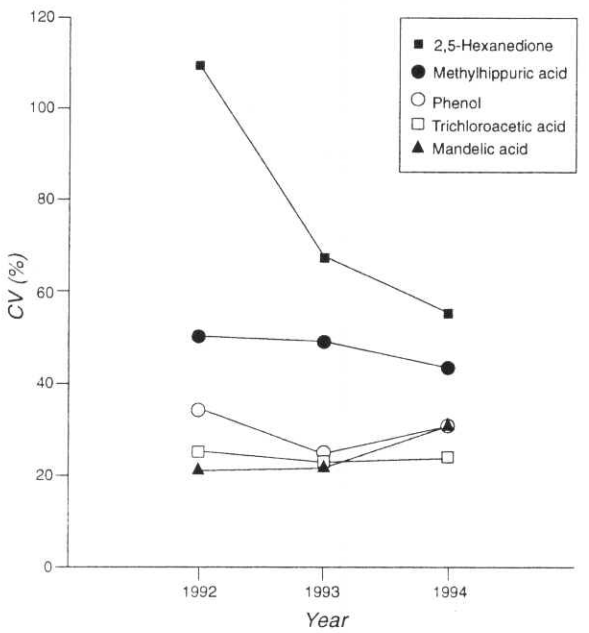

Fig. 6. - Trends in overall agreement over the years 1992-1994 for 2,5-hexanedione, methylhippuric acid, trichloroacetic acid, mandelic acid, and phenol. Each plot represents the annual mean of CV.

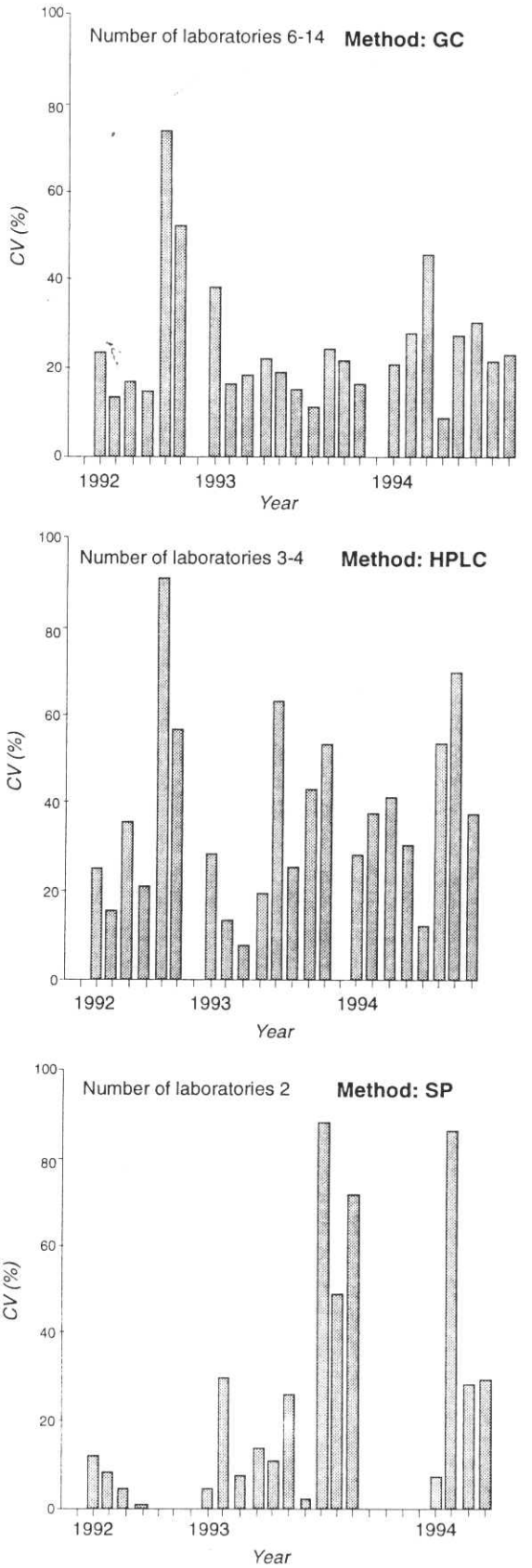

Fig. 7. - Between-laboratory agreement (CV%) by analytical method for phenol in urine.

Stability of the specimens has been a problem for some analytes, especially for phenylglyoxylic acid, 2,5-hexanedione and methylhippuric acid. For that reason we discontinued to send samples for phenylglyoxylic acid analysis. We have tried to shorten the delay in the post by using express mail and also introduced cool packs in our mailings (even this way, the samples will not keep cool for long periods in the mail, but the procedure will at least shorten the time that the samples may be exposed to elevated temperatures). The delay in the post is 5 days on average, including all laboratories. We asked the participants if they were willing to pay the cost of courier service which would guarantee delivery of the samples within 24 h everywhere. However, the laboratories were not willing to accept this added cost.

The large variation in the results of methylhippuric acid analysis is most likely due to hydrolysis to methylbenzoic acid [14]. Methods which measure the sum of methylhippuric acid and methylbenzoic acids (hydrolysis of glycine conjugate before extraction) will give accurate results even in samples where hydrolysis has taken place during transport and storage. In order to further clarify the reasons for the large variation between laboratory results we added 5 mmol/l of pure *ortho-*, *para-* or *meta*-isomers of methylhippuric acid to three samples. The overall recoveries of added isomers were good: 89% (no. =11 labs) for *ortho-*, 99% (no. = 12 labs) for *para-* and 98% (no. = 12 labs) for *meta*-hippuric acid. The overall coefficient of variation was better for spiked isomers (23.8%, *ortho*, 9%, *para-* and 16.2%, *meta*) than for total methylhippuric acid analysis which varied from 32% to 64%. The fact that the recoveries for each isomer were excellent but there was still considerable variation in the individual results, indicates that there is a problem of trueness while the precision is acceptable.

Several recent publications have indicated that there are major problems in the analysis of 2,5-hexanedione [15-18]. The differences in the preparation of the samples may cause over ten-fold variation in results. We tried to assess these problems by sending a questionnaire to the participants concerning the details e.g. use of hydrolysis, prepurification, derivation and separation, detection, standardisation and calibration of the analytical method. The average recovery of spiked 2,5-hexanedione was 90% (SD 42.7) for the laboratories (no. = 3) which did not use any hydrolysis and 188% (SD 150.5) for laboratories (no. = 5) using either enzymatic or acid hydrolysis. This is in agreement with the findings that methods including acid hydrolysis give a positive response for 2,5-hexanedione even in urine of non-exposed subjects [15, 19].

For phenol analysis, 17 of 26 specimens were spiked with phenyl-β-D-glucuronide and the recovery was calculated as the difference of the spiked and endogenous value concentration. The average recovery obtained from all results and separately for each method was: 80% for overall recovery (no. = 20 labs, range: 54-92), 80% for GC methods (no. =14 labs, range: 63-94); 77% for HPLC (no. =4 labs, range: 48-107); 79% for photometric methods (no. = 2 labs, range: 45-105). Deficient recovery may be due to incomplete hydrolysis or lack of stability of the hydrolysed phenol in the acid hydrolysis medium [20].

Future

Because of the demanding analyses and limited number of laboratories carrying out the biological monitoring of exposed workers, it is not economically feasible to organise quality assessment programmes in this field on a national basis in most countries. Rather, it requires international collaboration. At present there is in Europe one extensive external quality assurance programme for the analyses used in the biological monitoring of exposure, run by the German Society for Occupational and Environmental Health. The programme works very efficiently and is most useful to participating laboratories. However, the programme is geared toward accreditation of laboratories. Also, the sample distribution only takes place twice a year. Therefore, there is a need in Europe for an educational EQAS programme with a frequent sample distribution schedule. This is a task that the European Union, perhaps its Standards, Measurement and Testing programme should carry out. Until such a programme is operative, our intention is to continue, and expand our programme to include all important work place chemicals that are biologically monitored by urine analyses.

Submitted on invitation.
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Appendix. - Summary of the scheme

Country	Finland.
Name of scheme	FIOH External quality assurance scheme for organic solvent metabolites.
Status of scheme	World-wide, voluntary and educational. Run by the Finnish Institute of Occupational Health, Helsinki, Finland. *Aims:* improvement and documentation of the quality of the analyses in biological monitoring of occupational exposure to chemicals. *Participants:* 31 laboratories (educational and research institutes, hospitals, labour protection organisations) from 14 countries.
Scheme description	*Control materials:* human urine samples, from both occupationally exposed people and referents, fortified by added chemicals in the form they occur in human urine. One analyte per specimen. Frozen samples (in plastic vials) sent by mail in cold box. Target values determined from the added amount or as a qualified mean of reported results. *Organization of EQA exercises:* four sets of samples, usually two concentration levels sent per year. Reporting by fax/mail. *Elaboration of results:* participant's results compared to target values and the frequency histogram of all participants. *Criteria for evaluation of laboratory performance:* no formal assessment of laboratory performance by the organiser. *Measures taken against poor performers:* no sanctions for poor performance. *Advice* given when requested. *Financial support:* nominal fee for participants, balance from the Institute's budget.
Organization	Antero Aitio/Sinikka Valkonen Biomonitoring Laboratory Finnish Institute of Occupational Health Arinatie 3 FIN-00370 Helsinki, Finland Tel + 358 0 47471. Fax + 358 0 556157 e-mail: sval@occuphealth.fi; aait@occuphealth.fi
Analytes and matrices covered	Urinary hexanedione, mandelic acid, methylhippuric acid, phenol, trichloroacetic acid.