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Finnish quality assurance programme for biological monitoring of organic solvents

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Abstract

The FIOH quality assurance programme for organic solvents and their metabolites consists of analyses for 2,5-hexadione, phenol, mandelic acid, methylenedianiline, methylhippuric acid, *trans*,*trans*-muconic acid and trichloroacetic acid in urine, and creatinine and relative density for standardisation. Four times a year two levels of spiked urine or urine specimens collected from occupationally exposed workers are distributed to the participants in 22 countries. RSD and recovery were studied during 1997–2000. Average RSDs of all participants varied between 23 and 56% and were clearly dependent on the analytical method used and the concentration level of the samples. Since 1997 the target values have been determined in reference laboratories for five of the analytes. Lower RSDs (9–21%) and good recoveries were obtained for all analytes in these laboratories, indicating that good performance can be achieved even in the complex analyses performed in biological monitoring of exposure to industrial chemicals.

Keywords: Quality assurance; Biological monitoring; Organic solvents

1. Introduction

Biological monitoring is an important approach in assessing exposure to chemicals in the work place and evaluating the health risks of exposed workers. The main objective of the programme is to improve and harmonise the analytical performance in the laboratories of organic solvent analyses. Several studies have indicated that biological monitoring analyses are not at a satisfactory level. The analyses are complicated, and there are limited possibilities for both external and internal quality assurance. Certified reference materials are not available for organic solvents or their metabolites, and only few quality assurance schemes operating worldwide are reported for the analysis of urinary metabolites of organic solvents [1–6].

2. Experimental

2.1. Operation of the programme

The Biomonitoring Laboratory of the Finnish Institute of Occupational Health (FIOH) started an external quality assurance programme for organic solvent metabolites in 1979 [1]. The programme operates on an educational basis, and the aim is to give reliable and useful information to the participants. Today 42 organisations, universities, government institutions and private companies in 22 coun-

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Table 1
Participating countries and number of participants registered in the programme in 1997–2000

Country	No. of laboratories	Country	No. of laboratories
Argentina	1	Italy	4
Belgium	2	Poland	1
Brazil	3	Portugal	2
Canada	2	Singapore	2
Chile	1	Slovenia	1
Finland	3	Spain	1
France	3	Sweden	2
Germany	1	Thailand	1
Hungary	2	Turkey	1
Ireland	1	United Kingdom	2
Israel	3	USA	3

tries participate in the programme (Table 1). The coordinating laboratory is an accredited testing laboratory performing annually about 6000 biological monitoring analyses. A Regional Institute of the FIOH in Turku contributes to the programme by providing samples for methylhippuric acid analysis. Seven institutions in Europe having extensive experience in biological analysis serve as reference laboratories, providing reference values on test items for the programme. Since 1998 the analytes involved in the study are urinary mandelic acid (metabolite of styrene), methylenedianiline, *trans*,*trans*-muconic acid (metabolite of benzene), methylhippuric acids (metabolites of xylene), phenol, trichloroacetic acid (metabolite of trichloroethylene and perchloroethylene), 2,5-hexanedione (metabolite of *n*-hexane), creatinine and relative density (Table 2). Samples are distributed to the participants quarter-yearly. The policy of the scheme is to maintain confidentiality of the identity of the participants. The participants are identified by given laboratory code numbers and the code number is known only by a minimum number of experts involved in coordinating the programme. Communication with the participants is done via email, fax or the Internet (http://www.occuphealth.fi/ttl/osasto/tt/bio/fioh.htm).

2.2. Specimens

Preparation of urine specimens by the coordinator involves material collection, preparation, measuring and testing, verifying of accuracy of spiking, storage, packaging and labelling of the vials. Stability tests for each compound have been performed under conditions similar to those in which the samples are stored during the rounds. Two types of urine specimens, either natural or spiked, are prepared separately for each round. During the study period 1997–2000 all specimens for 2,5-hexanedine, phenol and methylenedianiline were spiked. Natural specimens

Table 2
Analytes, number of participants and target concentration levels in 1997–2000

Analyte	No. of laboratories	Concentration level
2,5-Hexanedione	20	1.1–25.1 μmol/l
Mandelic acid	33	0.5–5.4 mmol/l
Methylhippuric acid	34	0.2–9 mmol/l
Methylenedianiline*	8	50–993 nmol/l
trans,*trans*-Muconic acid*	21	5–75 μmol/l
Phenol	27	74–1150 μmol/l
Trichloroacetic acid	30	61–444 μmol/l
Creatinine*	22	Natural specimens
Relative density*	12	Natural specimens

*Included in the scheme in 1998.

were mostly used for mandelic acid (28 of 30 specimens) and methylhippuric acid (18 of 30). If urine from exposed workers is not available, urine of nonexposed laboratory personnel is used. Known concentrations of pure chemicals are added to it in the form the analytes appear in the urine of exposed workers (e.g., phenyl-β-glucuronide for phenol exposure and a solution containing 25% of *ortho*-, 25% of *para*- and 50% of *meta*-isomers of hippuric acid for methylhippuric acid exposure). Sulfamic acid is added as preservative in samples containing methylenedianiline. The levels of analytes are chosen to be close to those routinely analysed in the participating laboratories (Table 2). Natural urine specimens collected from exposed workers are stored frozen (−20°C) until homogenous pools with different concentration levels are prepared for each analyte. The pools are filtrated and spiked when appropriate, mixed and dispensed to the labelled transfer vials and stored at −20°C. The concentrations of test items are measured in the coordinating laboratory using validated routine methods and instruments before the samples are distributed to the participating laboratories [7–13]. Frozen samples are packed into cool packages and despatched to the participating laboratories by express mail. Courier service is used for some overseas laboratories. The delay due to transportation is 2–5 days. The coordinator completes the appropriate customs declaration forms to ensure that the delays in customs are minimised, and requests the laboratories to store the specimens in the refrigerator until analysis.

2.3. Target values

The assigned value for each specimen is obtained either by the reference laboratories (2,5-hexanedione, mandelic acid, methylhippuric acid, phenol and trichloroacetic acid), from the added analyte concentration (*trans,trans*-muconic acid and methylenedianiline) or it is the mean of all results (creatinine and relative density). Since 1997 four or five laboratories per analyte were asked to serve as reference laboratories based on their long-term good performance in the scheme. The assigned values produced by the reference laboratories are the means of their results, analysed as duplicates on three separate days, after excluding the outliers (±2SD). The analytical method used in the reference laboratories is free of choice. Before acceptance and to further confirm the accuracy of spiking and homogeneity of the samples, the target concentration for each specimen is checked by comparing it with the added amount of the analyte and with the mean of the results of all participants.

2.4. Reports

The laboratories are obligated to analyse and send the results in about 1 month. Reports for each round are available to the participants within 3 weeks and assigned values within 2 weeks after the deadline for reporting results. A computer-driven scheme report using the SAS-programme is provided to the par-

STATISTICS Specimen: MA0004

Mandelic Acid	N (Outliers)	MEAN	STD	CV%
OVERALL	27 (0)	1.5	0.5	32.2
GC	4 (0)	1.5	0.4	27.9
HPLC	20 (0)	1.4	0.5	33.4
SP	3 (0)	1.8	0.6	31.0

Your result: 1.5

Pooled urine collected from exposed workers.

Target value is 1.5 mmol/L, determined by the reference laboratories.

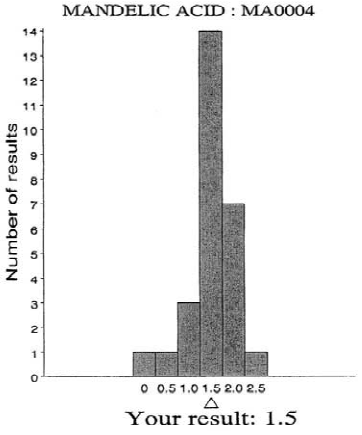

Fig. 1. Statistical and graphical distribution of laboratory results of a single specimen.

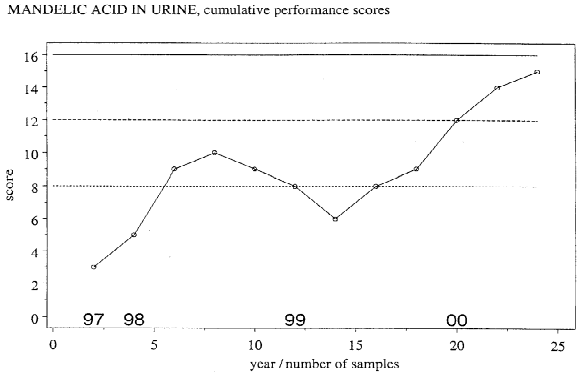

Fig. 2. Cumulative analytical performance by scoring over the years 1997–2000 of a single laboratory. Each plot represents sum of scores of last four rounds.

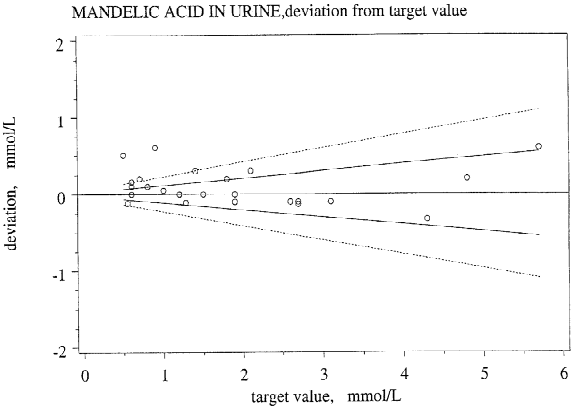

Fig. 3. Deviation from the assigned values. A chart for plotting the results of an individual laboratory for mandelic acid.

ticipants and it consists of overall and individual information on the results.

The summary data of all participants includes arithmetic means, standard deviations (SDs) and interlaboratory variations (RSDs) for overall results, and by method groups after exclusion of outliers (±3SD), and a frequency histogram for the results of each specimen is produced (Fig. 1). Individual data include concentration-based scoring and long-term cumulative indication of analytical performance in relation to the assigned values and acceptable limits. The deviation % of an individual result from an

Table 3
Limits of deviation of scores 1 and 2 at two concentration levels representing Finnish upper reference limit (URL) and Finnish biomonitoring action level (BAL)

Analyte	Concentration level URL and BAL		Deviation score 1	Deviation score 2
2,5-Hexanedione (2,5-HD)	URL	1 μmol/l	±0.4	±0.2
	BAL	5 μmol/l	±1.0	±0.5
Mandelic acid (MA)	URL	0.2 mmol/l	±0.08	±0.04
	BAL	2.9 mmol/l	±0.58	±0.29
Methylenedianiline (MDA)	URL	10 nmol/l*	±4	±2
	BAL	400 nmol/l*	±80	±40
Methylhippuric acid (MHA)	URL	0.2 mmol/l	±0.08	±0.04
	BAL	10 mmol/l	±2	±1
trans,*trans*-Muconic acid (*t*,*t*-MCA)	URL	0.5 μmol/l	±0.2	±0.1
	BAL	40 μmol/l	±8	±4
Phenol	URL	200 μmol/l	±80	±40
	BAL	3200 μmol/l	±640	±320
Trichloroacetic acid (TCA)	URL	50 μmol/l	±20	±10
	BAL	360 μmol/l	±72	±36

*Values are converted from Finnish URL (1 μmol/mol creatinine) and BAL (50 μmol/mol creatinine).

assigned value produced by reference laboratories or spike is calculated for each specimen. A larger deviation for all analytes is accepted at low concentration levels, while a higher accuracy is required at levels close to the biomonitoring action levels. To obtain a maximum score 2, the deviation from the assigned value has to be ≤20% at the Finnish upper reference limit and ≤10% at the Finnish biomonitoring action level (Table 3). To obtain an intermediate score 1, the corresponding deviations were set at 40 and 20%, respectively. For concentrations between these values, or higher, linear extrapolation is used. Deviations outside the fixed limits are considered as not acceptable, yielding a score of 0. In cumulative figure the sum of scores for the last four rounds, over eight specimens, is plotted in the chart (Fig. 2). The maximal score in the figure is 16, score 12 represents a 75% and score 8 a 50% success in achieving an acceptable result every time. Missing analytical results in the cumulative report will yield a score of 0, which is an unacceptable result (Fig. 2). Once a year the deviation of single results from the assigned value is provided as a figure, where the inner zone represents acceptable results, the outer zone intermediate results, and results outside the broken lines were not acceptable (Fig. 3).

3. Results

The dependence of between-laboratory agreement on the concentration levels of the specimens and on different analytical methods has been evaluated from 11 to 15 consecutive rounds in the years 1997–2000.

3.1. Average RSD by concentration level

The lowest average annual RSD over the years was observed for trichloroacetic acid (range 19–25%) and *trans*,*trans*-muconic acid (23–26%), and the highest for 2,5-hexanedione (28–51%) and methylenedianiline (32–93%). Average RSDs of the results for each analyte at high and low concentration levels are shown in Fig. 4. RSD is smaller for higher concentrations in all cases except for phenol. In general, the RSD varied nonsystematically over the study period, but decreasing trends of RSD were observed for 2,5-hexanedione, and for mandelic acid

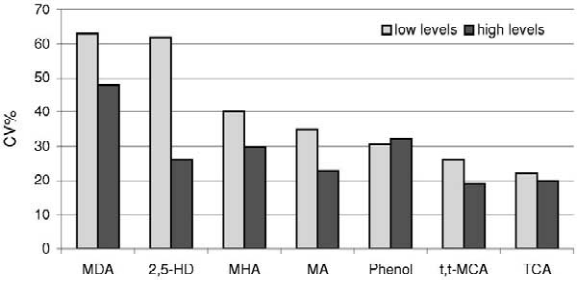

Fig. 4. Average interlaboratory variation (RSD) at low and high concentration levels for methylenedianiline (MDA), 2,5-hexanedione (2,5-HE), methylhippuric acid (MHA), mandelic acid (MA), trichloroacetic acid (TCA), phenol and *trans*,*trans*-muconic acid (*t*,*t*-MCA) in 1997–2000.

at levels below 2 mmol/l when analysed using high-performance liquid chromatography (HPLC) technique. RSDs of the results produced by the reference laboratories over the last 15 rounds were lowest for trichloroacetic acid and methylhippuric acid and highest for phenol and 2,5-hexanedione. Interlaboratory variations of the reference laboratories were lower, 43–69% depending on the analyte, than RSDs of all participants (Fig. 5).

3.2. Average RSD by analytical techniques

Average RSD by analytical techniques, gas chromatography (GC), HPLC and spectrophotometry (SP) for the analytes over the study period are shown at low and high concentration levels in Fig. 6. For methylhippuric acid and phenol lowest RSDs were observed in HPLC. RSDs for mandelic acid, methylenedianiline and trichloroacetic acid were lowest

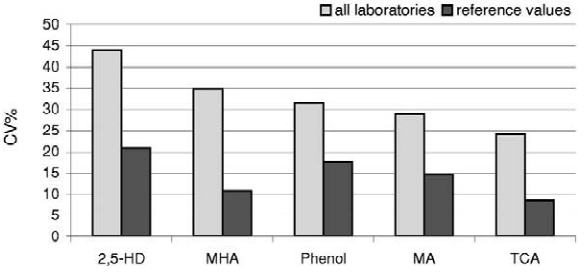

Fig. 5. Average interlaboratory variation (RSD) between all participants and reference laboratories for 2,5-hexanedione (2,5-HD), methylhippuric acid (MHA), phenol, mandelic acid (MA) and trichloroacetic acid (TCA) in 1997–2000.

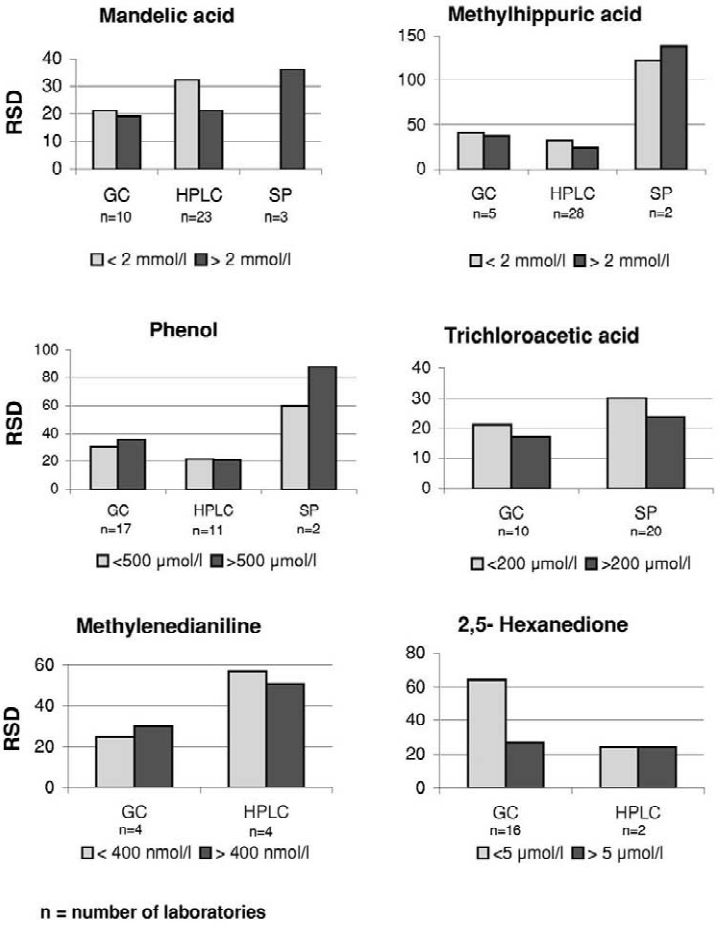

Fig. 6. Average interlaboratory variation (RSD) for mandelic acid, methylhippuric acid, phenol, trichloroacetic acid, methylenedianiline and 2,5-hexanedione obtained by different analytical techniques at low and high concentration levels in 1997–2000.

when analysed by GC. Interlaboratory variations were high for mandelic acid, methylhippuric acid and for phenol when spectrophotometric methods were used.

3.3. Accuracy of the results

Accuracy of laboratory results was evaluated by calculating the recovery of spiked specimens analysed during 1997–2000. Average recoveries of added the chemicals with low and high concentration levels obtained for different analytical techniques are shown in Table 4. In the calculations of recoveries, the possible low levels of endogenous concentrations were not subtracted from results. The best recoveries were obtained for trichloroacetic acid and *trans*, *trans*-muconic acid, and the worst for low levels of 2,5-hexanedione.

The biggest difference in recoveries depending on chromatographic method was obtained for methylenedianiline. Clearly higher results were obtained by HPLC, and lower by GC, than expected. Poor recoveries were obtained for methylhippuric acid and mandelic acid when SP methods were used. The average recovery and RSD for 2,5-hexanedione was poor by all analytical techniques at levels below 5 μmol/l. The reason for this was evaluated by studying the preparation procedures of the samples.

Table 4
Average recovery by different analytical techniques at low and high concentration levels of spiked specimens in 1997–2000 for all laboratories

Analyte	Concentration level	Recovery (%)		
		HPLC (*n*)	GC (*n*)	SP (*n*)
2,5-HD	≤5 μmol/l	172 (16)	144 (138)	212 (6)
	>5 μmol/l	121 (19)	101 (273)	95 (12)
MA	≤2 mmol/l	88 (38)	88 (16)	
	>2 mmol/l	91 (81)	98 (29)	68 (9)
MDA	≤400 nmol/l	135 (33)	86 (21)	
	>400 nmol/l	136 (49)	83 (21)	
MHA	≤2 mmol/l	97 (53)	82 (7)	56 (2)
	>2 mmol/l	96 (252)	86 (35)	25 (12)
t,t-MCA	≤40 μmol/l	100 (224)	96 (4)	
	>40 μmol/l	101 (103)	95 (2)	
Phenol	≤500 μmol/l	97 (131)	99 (236)	109 (15)
	>500 μmol/l	83 (102)	80 (174)	82 (11)
TCA	≤200 μmol/l		99 (115)	104 (221)
	>200 μmol/l		98 (104)	92 (239)

n = Number of results.

At a low 2,5-hexanedione level the average recovery for the laboratories ($n=11$) which used acid hydrolysis was 160%, and for laboratories ($n=8$) which did not, was 113%. With concentrations over 5 μmol/l the recovery was not dependent on the use of hydrolysis. The high recovery, especially at low levels, is in agreement with the previous findings of background level in the urine when acid hydrolysis is used [14–16].

3.4. Success by method used

The success of individual scheme results is obtained by scoring. The averages of the scores for the reported results of six specimens in the year 2000 by different analytical techniques are shown in Fig. 7. The highest average score has been achieved by HPLC when compared to other techniques for phenol score (1.6), methylhippuric acid (1.1), methylenedianiline (1.0) and 2,5-hexanedione (0.9), and the lowest scores by SP methods for phenol (0.8), mandelic acid (0.5), methylhippuric acid (0) and 2,5-hexanedione (0.3). For trichloroacetic acid the highest average score, 1.4, was observed by SP. For mandelic acid an equal score (1.2) was observed by HPLC and GC.

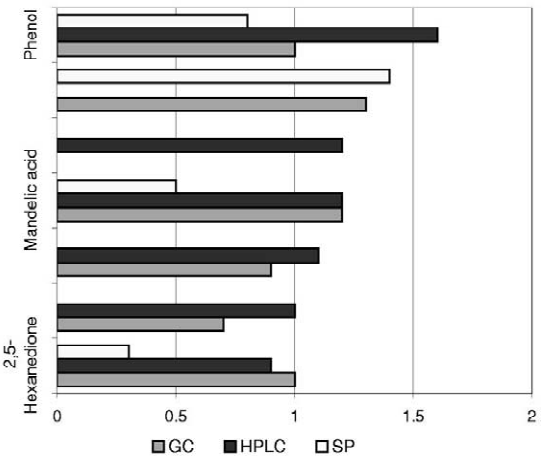

Fig. 7. Average score obtained by different analytical techniques, gas chromatography (GC), high-performance liquid chromatography (HPLC) and spectrophotometry (SP) over three rounds in 2000.

Correspondingly, the scores obtained by reference laboratories including all used methods were 1.3 for methylhippuric acid, 1.4 for phenol and 2,5-hexanedione, 1.5 for mandelic acid and 1.9 for trichloroacetic acid.

4. Discussion

Since 1997, the FIOH QA Programme has used reference laboratories for assignment of the target values for mandelic acid, phenol, trichloroacetic acid, methylhippuric acid and 2,5-hexanedione. During the follow-up period, 1997–2000, it can be noted that the agreement, indicative of good performance, has become even better among the reference laboratories. For example, the average RSD of the reference laboratories for 2,5-hexanedione was 30% during 1993–1996 [1] and 10% during 1997–2000. The mean values of reference laboratories have been very close to the anticipated, spiked concentrations, the analytical recoveries of the added amounts of the chemicals were close to 100% (98–101%).

The reference laboratories continued to obtain high scores in the FIOH QA Programme. For example in the case of trichloroacetic acid, in 80% the results yielded a maximum score of 2 for the reference laboratories during the 4-year follow-up period. The results indicate that the reference laboratory system is a good way to determine the assigned values for natural and spiked specimens in our programme. Poor performance in the quality control scheme may indicate both preanalytical (stability, hydrolysis, etc.) and analytical problems in the analysis. For most of the analytes, the RSDs were dependent on the method or pretreatment of the specimen used. For the analytes included in the programme chromatographic methods yielded higher scores and lower RSDs than SP methods except in the case of trichloroacetic acid. As expected, RSD is also clearly dependent on the concentration, higher RSDs are obtained with low levels of the compounds except in the case of phenol. For 2,5-hexanedione acid, hydrolysis as a pretreatment should be avoided because it increases the amount of the analyte by detecting total, conjugated metabolites of 2,5-HD [16]. In addition, it has been shown by others that the level of *n*-hexane in the air correlates better with results obtained from urine samples analysed with methods which do not use hydrolysis [11,15].

Proficiency testing schemes are used by the accreditation bodies as part of the process to assess the ability of laboratories to perform competently tests for which accreditation is held [17–20]. This means high demands for the providers of the schemes. Our intention is to improve the instructions and scheme reports of the FIOH QA Programme by further evaluating the methods and studying the stability and homogeneity of the samples in more detail. We aim to help the poor performers to improve their analytical quality, and to give more precise advice to the participants. In future we will set up the reference laboratory system for those analytes which do not yet have it, and an advisory board to ensure that all tasks involved in the provision of such a quality assurance programme have been performed competently.

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Arja Kallio studied biochemistry at the University of Helsinki and completed her Ph.D. in 1979 in the Department of Biochemistry. To continue her studies in cancer research she worked as a postdoctoral fellow at the Merrell Research Centre in Cincinnati, OH, USA, in the years 1980–1991. In 1988 she joined the Institute of Gene Technology at the University of Helsinki to study the mammalian cell expression vectors and later worked as the head of the hybridisation laboratory until 1993. In 1996 she moved to the Finnish Institute of Occupational Health and works as a chief of the Biomonitoring Laboratory in the Department of Industrial Hygiene and Toxicology.