Industrial Health 2011, **49**, 126–132 **Case Report**

Monitoring of Occupational Exposure to Volatile Organohalogen Solvents (VOXs) in Human Urine Samples of Dry-cleaner Workers by TLHS-DAI-GC-ECD Procedure

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Abstract: Chlorinated hydrocarbon solvents are often used for dry-cleaning clothes in the laundry industry. The object of this study was to monitor the occupational exposure of dry-clean employees coming into contact with VOXs. Twenty five workers collected their urine samples before the work shift, after 4 h of work and after the work shift. The analyses of urine samples and solvents used in dry-cleaning were performed using TLHS-DAI-GC-ECD. Chloroform was detected in all urine samples, and dichloromethane and tetrachloroethene in nearly all urine samples collected before and after the work shift. The concentrations of the compounds determined in urine samples were higher at the end of the workday in directly exposed individuals. Concentrations of the compounds determined in urine samples depended mainly on the type of activities carried out at the dry-cleaning establishments.

Key words: Volatile organohalogen compounds, Human urine samples, Occupational exposure, Biological monitoring, Thin layer headspace

Introduction

The biological monitoring of unchanged forms of solvents sampled from exposed workers has been investigated in many laboratories[1–3] but occupational exposure of dry-clean workers is still a pressing problem[4, 5].

Dichloromethane, chloroform and tetrachloroethene (also known also as tetrachloroethylene or perchloroethylene) are chlorinated hydrocarbon solvents widely employed in industry as degreasing agents[6] and are even more commonly used for the dry-cleaning of clothes[4, 7, 8].

The task of employees at a dry-cleaner is to remove dirt and stains from clothes and fabrics using non aqueous solvents. That is why these workers are often exposed to large amounts of dry-cleaning agents which can cause various health problems. Even at very low concentrations, these solvents are suspected of having carcinogenic, mutagenic and teratogenic properties and thus represent a direct health risk to workers. The International Cancer Research Institution has classified tetrachloroethene as a group 2A carcinogen, and chloroform and dichloromethane as group 2B carcinogens[9, 10]. Among other things, they cause liver, kidney and lung dysfunction. Inhaled at high concentrations, tetrachloroethene may be toxic to various human organ systems. The neurological effects include changes in behaviour and coordination, as well as damage to the central nervous system[11]. Research is also being carried out to examine the possible connection between the exposure to tetrachloroethene of parents working at a dry-cleaner and the risk of schizophrenia in their children[12].

Dichloromethane, chloroform and tetrachloroethene

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Table 1. Characteristics of the study population

Dry-cleaner	Total no. of subjects	Age in years		No. of smokers	Length of employment in years	
		mean	range		mean	range
industrial						
	9	37	23–53	3	6.3	0.3–12
commercial						
1	4	37	32–43	4	2	0.1–4
2	2	46.5	37–56	1	2.75	0.5–5
3	2	39	38–40	"0"	15	–
4	4	30.75	26–34	2	2	–
5	1	51	–	1	0.2	–
6	3	44	42–45	2	18	5–25

can enter the human body *via* inhalation (in the form of vapours, gases, fumes and mists), by dermal contact, or by inadvertent ingestion *via* hand-mouth contact. After intake, the chemicals may enter the bloodstream and, once in the body, they may either accumulate or be excreted, usually with the urine (in non-metabolised form)[5, 13].

The determination of chlorinated hydrocarbon solvents in human urine samples constitutes a challenge for analytical chemists because of the complex and often variable matrix composition of the samples. Typical biological samples usually require special pre-treatment prior to analysis by chromatography or related techniques. Special attention has recently been paid to the use of so-called solvent-free analyte isolation and/or preconcentration techniques, which can be attributed to the development of green analytical chemistry[14]. It seems that thin-layer headspace analysis may be the technique of choice for this task[15–18]. The idea of TLHS analysis is described elsewhere[19, 20].

The aim of the present study was to monitor the exposure of dry-clean workers to dichloromethane, chloroform and tetrachloroethene in an industrial environment by determining the unchanged part of those three solvents in urine samples before work, after 4 h' work, and after the work shift. The concentrations of all of these compounds used as dry-cleaning solvents were investigated. The results also confirmed the possibility of quantitatively determining dichloromethane, chloroform and tetrachloroethene in liquid samples by TLHS-DAI-GC-ECD procedures at very low ppt levels.

Methods

Subjects

The exposed group was composed of 25 subjects: 24 women and 1 man, mean age 38 (range 23–56), exposed to dichloromethane, chloroform and tetrachloroethene during dry-cleaning. The solvent-exposed workers were employed at 1 industrial and 6 commercial dry-cleaning establishments. The volunteers from the industrial dry-cleaner were employed in three different workplaces: the cleaned clothes store, the unwashed clothes store, and in direct contact with the washing machines (solvents). The workers at the commercial dry-cleaning establishments were engaged in various activities.

Anonymous questionnaires were distributed among the workers, in order to collect the following information:

- age and sex;
- smoker/non-smoker;
- length of employment at the dry-cleaning establishment.

The data concerning the dry-cleaning establishments and the exposed workers are shown in Table 1.

Sample collection

Urine samples

The research was carried out in two stages. In the first stage, urine samples were collected from industrial dry-clean workers. Collecting urine samples from volunteers working at the industrial dry-cleaner was carried out on the Tuesday and Wednesday of two consecutive weeks (14, 15, 21, 22 February). The samples were taken before the work shift, then after 4 h of work and after the work shift. Employees of the industrial dry-cleaner were exposed to the solvents used on the premises in different ways. During the second stage of the studies, urine samples were collected from employees of six small commercial dry-cleaners. Urine samples from the workers of commercial dry-cleaners Nos. 1–3 were collected before the work shift and after the work shift between February and May. At the other three commercial dry-cleaners (Nos. 4–6), urine samples were collected from volunteers between November and March before the work shift, then after 4 h of work, and again

after the work shift.

The samples were collected in 250 ml flasks for biological tests with ground glass stoppers stored in the stoppered flasks at 4°C and analysed within 12 h.

Analytical procedure

Volatile organohalogen compounds were determined in urine using continuous flow TLHS analysis with autogenous generation of the liquid sorbent. The TLHS apparatus (see Fig. 1) was developed at the Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, (GUT, Gdańsk, Poland) and is described in detail elsewhere (Jakubowska *et al*., 2007a; 2007b).

The liquid samples were delivered through a pre-heating system to the top of a spirally wound, heated glass tube (90°C) with the aid of a peristaltic pump. The sample flowed down the column in the form of a thin, gravity-driven film. At the same time, purified air was supplied countercurrently to the column. VOXs were partitioned from the liquid film to the air and were carried away with it. The warm air leaving TLHS column I, saturated with water vapour, was directed to a second, smaller TLHS column (10°C). The water vapour contained in the gas phase condensed on the walls of the column forming a film of water, to which the volatile analytes partitioned from the air. Thus, the condensed water played the part of liquid sorbent. The condensate from TLHS column II was collected in ice-cooled vials and analysed without any further processing by DAI-GC-ECD.

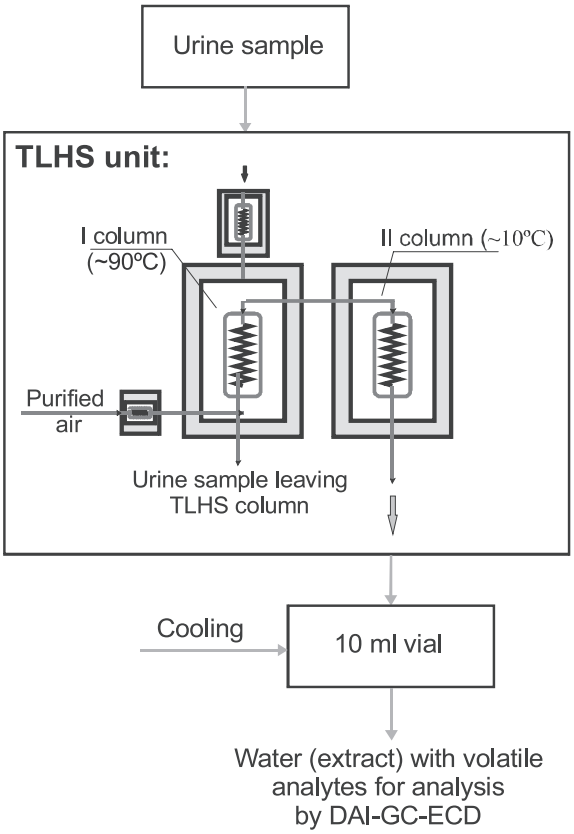

Fig. 1. General scheme of the TLHS-DAI-GC-ECD procedure used for determining the levels of volatile chloroorganic analytes in liquid samples.

The conditions of GC analysis were as follows: GC 8000Top (Carlo Erba Instruments, Milan, Italy); cold on-column injector with secondary cooling; 60 m × 0.32 × 5 μm DB-1 column (J&W Scientific, Folsom (CA), USA); temperature programme: 95–110°C, at 5°C/min, 110°C hold for 5 min, 110–130°C, at 10°C/min, 130°C hold for 10 min; hydrogen carrier gas flow rate 2.2 cm^3/min; pressure P_{const}=130 kPa; detector temperature 350°C; and nitrogen make-up gas flow rate 60 cm^3/min, data acquisition software: Chrom-card.

Stability of VOXs in Urine Samples

The stability of liquid solutions of volatile organic analytes (e.g. dichloromethane, chloroform, tetrachloroethene), which aimed to establish the optimum conditions for the preparation and possible storage of standard solutions used for calibration, were studied and presented in more detail in Kozlowska K *et al*[16]. The following main conclusions can be drawn:

- liquid solutions of volatile organohalogen compounds should be prepared in completely filled containers;
- compounds with higher boiling points and lower vapour pressures have greater stability in liquid samples, according to the following series $C_2Cl_4 > CHCl_3 >>> CH_2Cl_2$
- the loss of analytes from aqueous solutions does not depend on their storage time but on the volume of headspace remaining in the container and on the number of samples taken from the container;
- liquid samples should be kept at ca 4ºC;
- human urine samples should be analysed within 24 h;
- liquid extracts of volatile analytes obtained after TLHS and collected in vials with no headspace display a greater stability of composition during the successive sampling steps.

Results

Table 2 summarises the results of the study, including the range of VOX concentrations (minimum and maximum) in urine.

Table 2. Concentrations of dichloromethane, chloroform and tetrachloroethene in urine samples before, after 4 h and after the work shift

Dry-cleaner	Number of workers	Number of samples	Dichloromethane (μg/l)			Chloroform (μg/l)			Tetrachloroethene (μg/l)		
			before work	after 4 h	after work	before work	after 4 h	after work	before work	after 4 h	after work
Industrial	9	108	0.07 (n.d.–0.18)[a]	0.29 (n.d.–0.95)	1.66 (0.38–6.01)	2.50 (0.42–12.10)	5.46 (0.51–13.40)	15.62 (0.07–50.90)	1.00 (0.18–3.33)	2.73 (0.51–9.12)	6.63 (1.08–17.90)
commercial											
1	4	19	0.12 (n.d.–0.33)	–	0.52 (n.d.–1.27)	5.74 (1.50–14.8)	–	8.61 (0.24–20.0)	1.68 (n.d.–3.85)	–	3.78 (0.06–18.7)
2	2	9	0.34 (n.d.–0.42)	–	1.42 (n.d.–1.42)	4.37 (0.81–11.1)	–	1.99 (0.03–5.00)	1.70 (n.d.–3.64)	–	16.4 (n.d–28.6)
3	2	11	0.02 (n.d.–0.02)	–	0.25 (n.d.–0.25)	10.2 (0.48–28.6)	–	11.1 (0.27–30.8)	2.74 (n.d.–6.32)	–	7.30 (n.d.–12.3)
4	4	51	0.39 (n.d.–2.32)	2.82 (n.d.–29.7)	2.27 (n.d.–8.56)	4.28 (1.21–11.5)	6.37 (1.76–11.9)	6.88 (1.34–12.7)	4.09 (n.d.–8.61)	7.89 (0.89–21.2)	7.34 (0.97–20.5)
5	1	16	2.97 (1.99–5.03)	7.63 (4.07–11.3)	9.9 (3.72–15.5)	4.62 (2.87–6.73)	11.3 (5.46–17.9)	20.3 (15.8–29.8)	4.40 (0.08–7.25)	12.3 (3.54–30.1)	19.9 (10.3–24.9)
6	3	36	4.91 (1.31–13.8)	4.19 (0.92–10.4)	4.38 (0.08–10.6)	9.87 (1.44–41.7)	15.7 (1.21–51.4)	7.81 (0.81–15.1)	15.3 (2.04–60.5)	10.7 (2.19–29.6)	9.98 (2.12–24.4)

[a]Mean and range, n.d. – not detected.

Figure 2 shows changes in the concentrations of volatile organochlorine compounds in urine samples collected from the industrial dry-clean volunteers on the first day before the work shift, after 4 h of work and after the work shift. It was observed that with lengthening duration of exposure, the concentrations of each compound in the urine sample increased. In the case of volunteer No.1, for example, the mean concentration of tetrachloroethene (the main component of the solvent used at the industrial dry-cleaner) was 0.567 μg/l before work, but rose to a level of 9.85 μg/l after the work shift. The mean concentrations of chloroform and dichloromethane in the collective urine sample were also higher after the work shift, 14.8 μg/l (volunteer No.3) and 1.13 μg/l (volunteer No.2) respectively.

The diagrams in Fig. 3 represent the concentrations of tetrachloroethene in the urine samples collected on 14, 15, 21 and 22 February (before work, after 4 h of work and after the work shift), from employees who were directly and indirectly exposed to the volatile organohalogen solution.

For example, employee No. 9 was in direct contact with the solvents (as that worker was operating the washing machines), but the other workers at the industrial dry-cleaner were not directly exposed (e.g. employee No.4 worked in the cleaned clothes store, No.6 worked in the unwashed clothes store). Employees at the industrial dry-cleaner were exposed mainly to volatile organochlorine compounds present in the air from the solvent used at the dry-cleaner, and through contact with cleaned clothes. It was observed that at the end of the workday, the concentrations of tetrachloroethene in urine samples were higher in both directly and indirectly exposed individuals. Concentrations of the target compounds in urine samples were significantly greater in workers directly exposed than in those indirectly exposed: Fig. 4 makes this clear. It can also be seen that employees working in the cleaned clothes store are exposed to a higher concentration of tetrachloroethene than those working in the unwashed clothes store. This is due to the fact that some of this solvent remains on the cleaned clothes and its gradual release causes increased exposure[21)].

Discussion

A total of 250 urine samples from 25 volunteers exposed to chlorinated hydrocarbon solvents used in dry-cleaning were analysed. Dichloromethane and tetrachloroethene were detected in nearly all urine samples collected before work and after the work shift at dry-cleaning establishments. Chloroform was detected in all urine samples collected before and after work. Volatile organohalogen solvents were detected in urine samples collected before work. This means that compounds absorbed by the volunteers the previous day were not completely eliminated from the body. The content of the target analytes in urine samples depends on the time spent at work by the volunteers. The concentrations of these compounds in urine samples were observed to increase monotonically according to the number of hours worked.

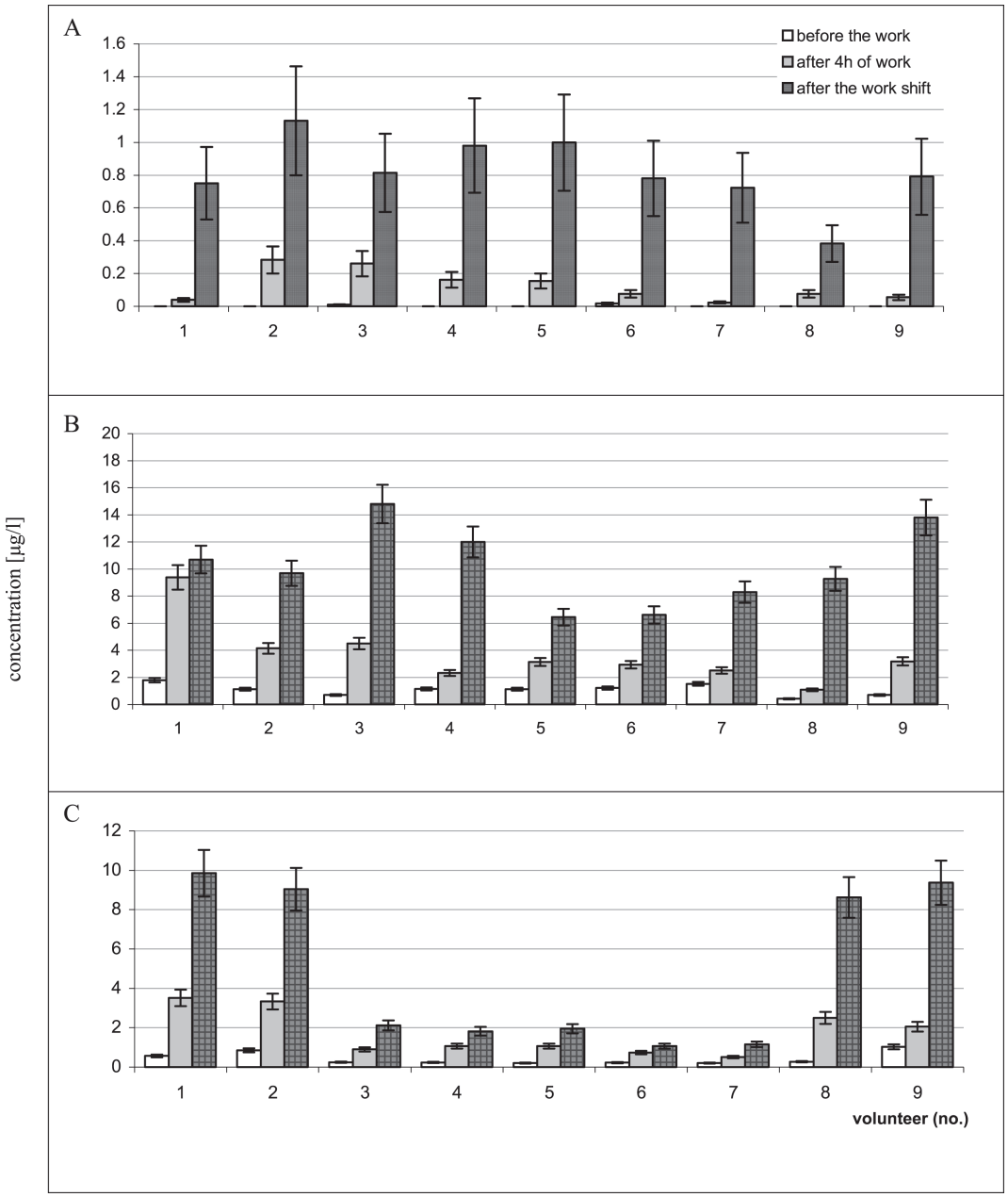

Fig. 2. Changes in the concentrations of volatile chloroorganic compounds in urine samples collected from all volunteers on the first day of research, before the work shift, after 4 h of work and after the work shift at the industrial dry-cleaning establishment.

A- dichloromethane, B-chloroform, C-tetrachloroethene.

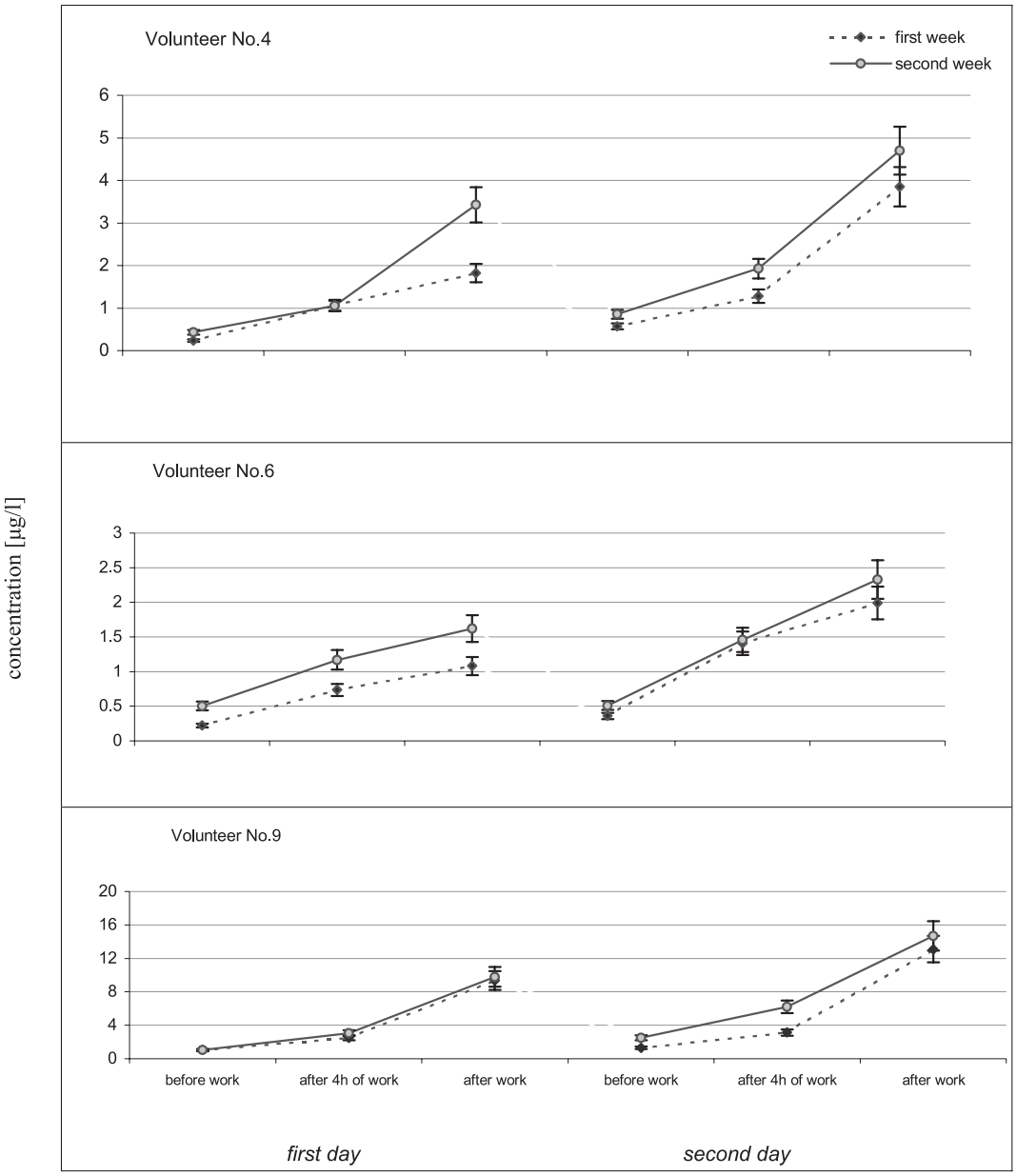

Fig. 3. Changes in the concentrations of tetrachloroethene in urine samples collected from industrial dry-cleaner workers (Volunteer No. 4 – cleaned clothes store, Volunteer No. 6 – unwashed clothes store, Volunteer No. 9 – direct contact with solvents).

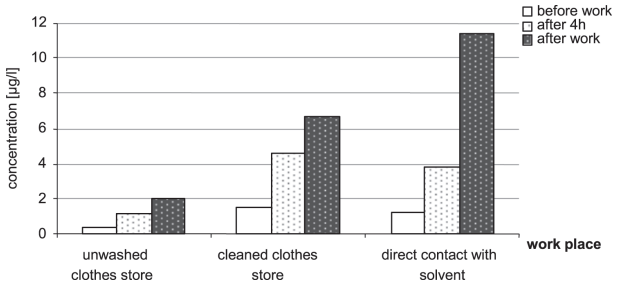

Fig. 4. Changes in the mean concentrations of tetrachloroethene in urine samples collected from industrial dry-cleaner employees working at different workplaces.

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