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Kidney and liver biomarkers in female dry-cleaning workers exposed to perchloroethylene

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Blood and urine perchloroethylene and urine trichloroacetic acid, as markers of exposure, and serum AST, ALT, GGT and creatinine, urine total solutes and proteins, angiotensin converting enzyme, *N*-acetyl-ß-D-glucosaminidase and glutamine synthetase, as markers of effect, were measured in 40 dry-cleaning and 45 ironing-shop female workers. Average perchloroethylene air level in the dry-cleaning shops was 59.7 mg m^{-3}, i.e. three-fold lower than the current A.C.G.I.H. TLV-TWA (170 mg m^{-3}). No statistically significant difference in the mean values of any of the effect markers was observed between the two groups, except for AST which was significantly higher in dry-cleaners. In addition, a statistically significant correlation was observed in dry-cleaners between environmental perchloroethylene and total urinary solutes ($r = 0.308$, $p < 0.05$) or urine glutamine synthetase ($r = 0.469$, $p < 0.01$), between glutamine synthetase and blood perchloroethylene in post-shift ($r = 0.406$, $p < 0.01$) or urinary perchloroethylene in post- ($r = 0.571$, $p < 0.001$) or pre-shift ($r = 0.586$, $p < 0.001$), and between urinary perchloroethylene in pre-shift and GGT ($r = 0.407$, $p < 0.05$). Interestingly, some statistically significant correlations between exposure and effect indices were found in ironing-shop workers alone, as in all subjects. Finally, transaminases, GGT and total urinary proteins were influenced by age and alcohol consumption which were significantly higher in dry-cleaners, thus providing an explanation for some of the correlations observed. In conclusion, our results show a dose-related increase of glutamine synthetase activity, a marker of damage of the *pars recta* of the kidney proximal tubule, in the urine of female subjects exposed to perchloroethylene concentrations in the work environment lower than current A.C.G.I.H. TLV-TWA.

Keywords: perchloroethylene, trichloroacetic acid, glutamine synthetase, angiotensin converting enzyme, *N*-acetyl-ß-D-glucosaminidase.

Introduction

Tetrachloroethylene (perchloroethylene, PER) was introduced as a solvent in dry-cleaning from the mid 1960s, and is now the main solvent used for this application. After inhalation in humans, only 1–3% of absorbed PER is metabolized (Fernandez *et al.* 1976, Monster *et al.* 1979), trichloroacetic acid (TCA) being the main urinary metabolite. Another pathway of the solvent metabolism is via glutathione conjugation, followed by further metabolism of the conjugate to *N*-acetyl-*S*-(trichlorovynil)-L-cysteine, as shown *in vivo* in rats and humans (Völkel *et al.* 1998) and *in vitro* in rats and mice (Lash *et al.* 1998). Although the acute toxicity of PER in experimental animals is low, liver (Buben and O'Flaherty 1985) and kidney (Bergamaschi *et al.* 1992) being the main target

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organs, some recent studies have raised concern about potential carcinogenic effects of PER (IARC 1995). These effects are probably related to nephrotoxic and nephrocarcinogenic tioketene metabolites formed from PER after conjugation with glutathione and further metabolism of the conjugate by ß-lyase, a pathway which is fortunately little used in humans (Völkel *et al.* 1998). However, a higher prevalence of oesophageal cancer was reported in dry-cleaners exposed to PER (Ruder *et al.* 1994). Another controversial question is the higher frequency of abortions and foetotoxic effects reported by some authors in dry-cleaning workers exposed to PER (Doyle *et al.* 1997). These findings, however, were not confirmed by others (Bosco *et al.* 1987, Ahlborg 1990).

Although human exposure to PER during dry-cleaning is generally low, several studies were performed to evaluate the subclinical effects of the solvent on target organs. No severe effects on liver (Lauwerys *et al.* 1983) or kidney (Lauwerys *et al.* 1983, Solet and Robins 1991, Abo el Ata *et al.* 1996) were observed in humans. Isolated increases of the urinary excretion of lysozyme (Vyskocil *et al.* 1990) or retinol binding protein (Verplanke *et al.* 1999) and generalized membrane disturbances such as an increased release of laminin fragments, fibronectin, glycosaminoglycans and epithelial membrane components from tubular cells, such as brush-border antigens and Tamm–Horsfall glycoprotein (Mutti *et al.* 1992), were the only important changes observed in humans.

The present paper reports the results of a clinical-biomonitoring study aiming to assess liver and kidney function in 40 female dry-cleaners exposed to mean PER vapour concentrations lower than a half of A.C.G.I.H. TLV-TWA and 45 female ironing workers with a very low exposure. Various indices of exposure were measured in these subjects and the possible correlation between PER exposure and liver and kidney function tests was investigated. In particular, the measurement of urinary glutamine synthetase activity, a new S_3 segment-specific marker of tubular impairment recently introduced (Trevisan *et al.* 1999a), was used.

Subjects and methods

Subjects

Forty female dry-cleaning workers employed in 38 laundry shops in north-eastern Italy were investigated. Forty-five female ironing workers were also examined. All the subjects lived in the same geographical area and showed the same socio-economical conditions. None of them declared abuse of drugs. Dry-cleaners, however, had a statistically significantly higher age than ironing workers (41.3 ± 10.3 versus 28.8 ± 10.2 years, respectively, $p < 0.001$), and declared a higher daily alcohol consumption (8.4 ± 11.3 versus 3.4 ± 7.6 grams, $p < 0.01$). For this reason, in the statistical analysis, the values of the biological parameters studied were adjusted to age and drinking habits.

Methods

The concentration of PER in the work environment was monitored by means of individual passive samplers (TK200) throughout the entire duration of the work-shift (approx. 8 h). Samplers were then eluted in 5 ml *n*-hexane and analysed gas-chromatographically. PER in blood (PCB) and in urine (PCU), and TCA in urine were measured in biological fluids collected on Thursday evening, at the end of the work-shift, and on Friday morning, before the week's last shift. The urine of the ironing-shop workers was collected on Friday morning, before the last shift. PER was analysed in 0.2 ml of blood or 0.1 ml of urine by means of head-space gas-chromatography. Analysis conditions were as follows: $T_{\text{injection}} = 200°C$, $T_{\text{detector}} = 300°C$, $T_{\text{column}} = 150°C$, in isotherm for 7 min. TCA in urine was determined after esterification, by chromatographic analysis according to the following conditions: $T_{\text{injection}} = 200°C$, $T_{\text{detector}} = 250°C$, $T_{\text{column}} = 80°C$, in isotherm for 12 min. Liver function was investigated by the determination of serum aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2) and γ-glutamyltransferase (GGT, EC 2.3.2.2) with a commercial kit (Boheringer,

Table 1. Biological parameters measured in subjects occupationally exposed to PER (dry-cleaners, $N = 40$) and ironing-shop workers ($N = 45$). Results are expressed as mean (m) ± standard deviation (SD). Range in parentheses. See text for abbreviations.

	Ironing-shop m±SD		Dry-cleaners m±SD	
Age (years)	28.8±10.2	(17–65)	41.3±10.3*	(20–67)
Exposure (years)			15.3±9.2	(2–38)
Alcohol (grams per day)	3.4±7.6	(0–24)	8.4±11.3*	(0–36)
TUS (g $mmol^{-1}$ creat.)	4.2±1.3	(2–8.2)	3.8±0.8	(2.6–5.7)
TUP (mg $mmol^{-1}$ creat.)	18.3±10.5	(5–57)	17.8±11.2	(4–46)
ACE (μmol $mmol^{-1}$ creat.)	3.32±1.38	(0.6–6.6)	3.25±1.56	(0.5–9.4)
NAG (μmol $mmol^{-1}$ creat.)	0.50±0.24	(0.24–1.45)	0.48±0.22	(0.24–1.13)
GS (μmol $mmol^{-1}$ creat.)	1.08±0.51	(0–2.2)	1.05±0.69	(0.21–3.82)
AST (U l^{-1})	14.2±4.0	(8–25)	17.9±6.3*	(10–38)
ALT (U l^{-1})	29.6±5.7	(20–45)	32.5±7.8	(23–70)
GGT (U l^{-1})	21.7±7.5	(13–49)	22.7±11.7	(11–67)
CRS (mg dl)	0.75±0.09	(0.56–1.03)	0.76±0.11	(0.58–1.08)
PER (mg m^{-3})			59.7±61.7	(0.3–243.4)
PCB (μg dL^{-1})				
Post-shift			69.3±54.4	(2.8–194.3)
Pre-shift	2.0±1.0	(0–3.9)	34.8±28.3*	(1.9–140.7)
PCU (μg g^{-1} creat.)				
Post-shift			32.4±51.2	(2–267)
Pre-shift	5.4±5.5	(0–33)	9.1±8.4*	(2–53)
TCA (mg g^{-1} creat.)				
Post-shift			4.1±4.8	(0.3–26.7)
Pre-shift	0.2±0.1	(0.06–0.48)	3.7±4.0*	(0.3–21.4)

*$p < 0.01$ or lower with respect to the ironing-shop workers.

Mannheim, Germany). Kidney function was monitored by the determination of serum creatinine (CRS) with a commercial kit (Boehringer, Mannheim, Germany), urinary excretion of total solutes (TUS) as calculated by the last two digits of the specific gravity multiplied by 2.6 (Haeser's formula), total proteins (TUP) according to Pesce and Strande (1973), angiotensin converting enzyme (ACE, EC 3.4.15.1) using glycyl-L-histidyl glycine (Sigma Chemical Co., St Louis, USA) as the substrate according to Summary (1976), *N*-acetyl-ß-D-glucosaminidase (NAG, EC 3.2.1.29) using γ-nitrophenyl-*N*-acetyl-ß-D-glucosaminide (Fluka, Buchs, Switzerland) as the substrate according to Lockwood and Bosmann (1979), and glutamine synthetase (GS, EC 6.3.1.2) using L-glutamic acid (Sigma Chemical Co., St Louis, USA) as the substrate according to Trevisan *et al.* (1999a). All the urinary parameters of exposure and effect were adjusted to urinary creatinine concentration.

Apparatus

A Perkin-Elmer lambda 5 dual beam spectrophotometer was used for protein and enzyme determinations. Gas-chromatographic determination of the biological samples was performed by means of a Perkin-Elmer 8500 gas-chromatograph equipped with an electron-capture detector. Environmental PER was determined with a Perkin-Elmer Autosystem XL gas-chromatograph.

Statistics

Analysis of variance, regression coefficient and multivariate analysis were performed for the statistical evaluation of the results; statistical significance was assumed for $p < 0.05$.

Results

Table 1 shows the results obtained in dry-cleaning and ironing-shop workers. The dry-cleaning workers were exposed to PER concentrations of 0.3–243.4 mg m^{-3}, i.e. about three times lower in average (59.7 mg m^{-3}) than the current A.C.G.I.H. TLV-TWA (170 mg m^{-3}). The TLV value was exceeded in three

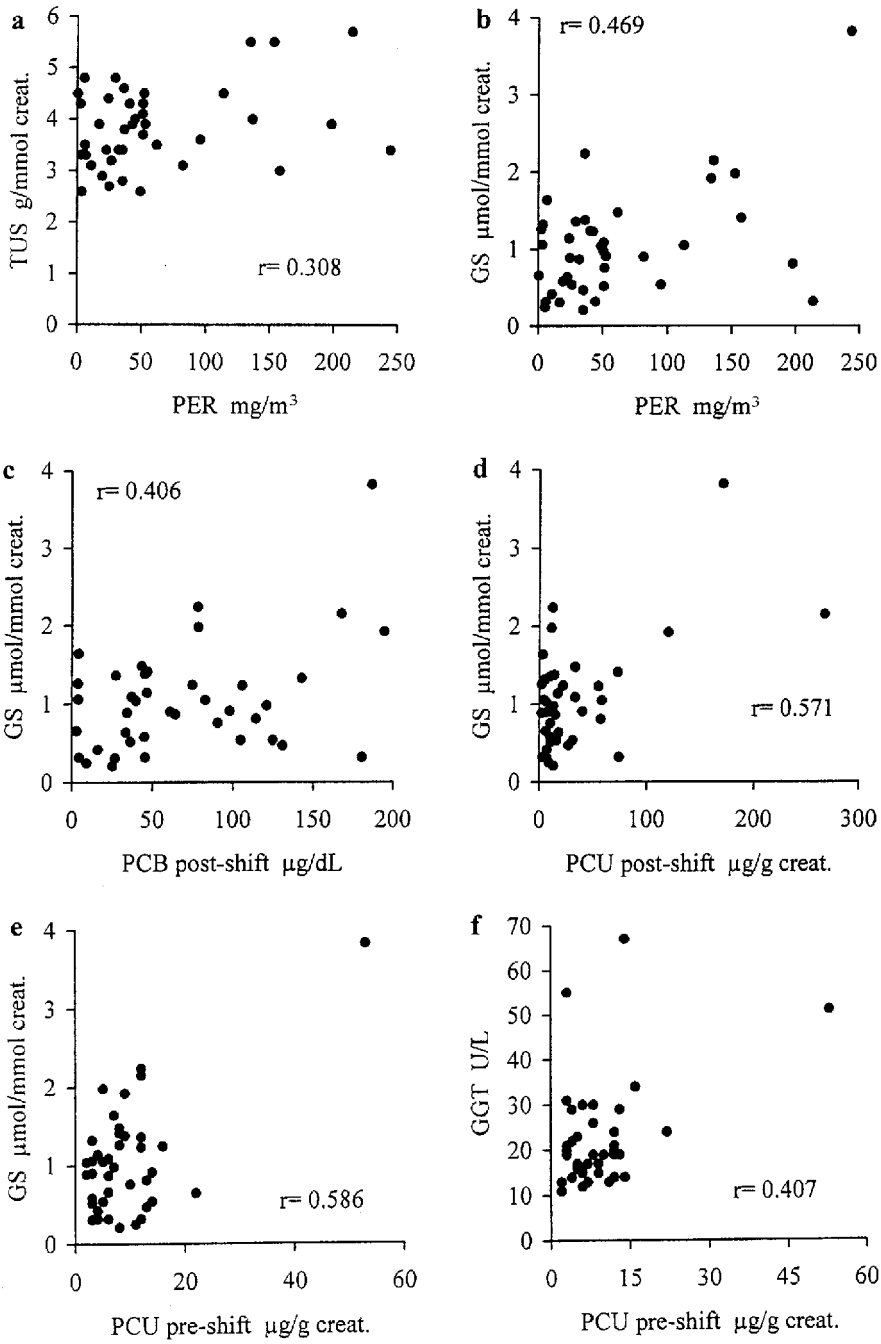

Figure 1. Relationship between work environment PER concentration and TUS (**a**, $y = 0.004x + 3.609$, $p < 0.05$) or GS (**b**, $y = 0.005x + 0.736$, $p < 0.01$), PCB determined in post-shift and GS (**c**, $y = 0.005x + 0.691$, $p < 0.01$), PCU determined in post-shift and GS (**d**, $y = 0.008x - 0.800$, $p < 0.001$), PCU determined in pre-shift and GS (**e**, $y = 0.048x + 0.613$, $p < 0.001$) or GGT (**f**, $y = 0.567x + 17.583$, $p < 0.05$) of dry-cleaning workers (40 subjects).

Table 2. Equation, correlation coefficient and statistical significance among environmental and biological indices of exposure to PER in dry-cleaners (only statistically significant correlation are reported).

			Equation	r	p
PER	vs	PCB post-shift	$y = 0.589x + 34.149$	0.669	< 0.001
	vs	PCB pre-shift	$y = 0.298x + 17.017$	0.650	< 0.001
	vs	PCU post-shift	$y = 0.558x - 0.960$	0.673	< 0.001
	vs	PCU pre-shift	$y = 0.066x + 5.120$	0.485	< 0.01
	vs	TCA post-shift	$y = 0.029x + 2.047$	0.373	< 0.05
PCB post-shift	vs	PCU post-shift	$y = 0.623x - 10.800$	0.661	< 0.001
	vs	PCU pre-shift	$y = 0.071x + 4.184$	0.455	< 0.01
	vs	TCA post-shift	$y = 0.042x + 1.192$	0.483	< 0.01
PCB pre-shift	vs	PCU post-shift	$y = 0.722x + 7.241$	0.399	< 0.01
	vs	PCU pre-shift	$y = 0.100x + 5.583$	0.337	< 0.05
	vs	TCA post-shift	$y = 0.128x - 0.346$	0.763	< 0.001
	vs	TCA pre-shift	$y = 0.071x + 1.192$	0.512	< 0.001
PCU post-shift	vs	TCA post-shift	$y = 0.029x + 3.181$	0.313	< 0.05

subjects. Pre-shift PCB mean values were 34.8 μg dl^{-1}; when the PCB values were related to PER levels, a Biological Exposure Index (BEI) equivalent of 67.7 μg dl^{-1} was calculated from our data, a value which is slightly higher (1.34-fold) than the recommended A.C.G.I.H. BEI (50 μg dl^{-1}). Post-shift urinary TCA mean value was 4.1 mg g^{-1} creat. (or 5.5 mg l^{-1}); when related to PER air levels, the value corresponded to the TWA value of 6.98 mg g^{-1} creat. (or 10.3 mg l^{-1}), about three-fold higher than the A.C.G.I.H. BEI (3.5 mg l^{-1}). On the other hand, very low although measurable air concentrations of trichloroethylene (TRI) were detected in some dry-cleaning shops. Interestingly, ironing workers showed measurable levels of PCB and PCU, although values were in average at least one order of magnitude lower than those of dry-cleaners.

When the values of markers of exposure were compared with each other (table 2), a highly statistically significant correlation was observed between PER and PCB of post- ($r = 0.669$, $p < 0.001$) and pre-shift ($r = 0.650$, $p < 0.001$) or PCU of post- ($r = 0.673$, $p < 0.001$) and pre-shift ($r = 0.485$, $p < 0.01$). A slight, but statistically significant correlation between PER and TCA of post-shift ($r = 0.373$, $p < 0.05$) was also observed. Finally, PCB and PCU in post- and pre-shift were also strongly correlated with urinary TCA and environmental PER.

No difference was observed between the two groups of workers in the mean values of the effect markers, except for a statistically significant increase of AST ($p < 0.001$) in dry-cleaners. On the other hand, dry-cleaners showed a statistically significant positive correlation between environmental PER and TUS ($r = 0.308$, $p < 0.05$, figure 1(a)) or GS ($r = 0.469$, $p < 0.01$, figure 1(b)), and between GS and PCB of post-shift ($r = 0.406$, $p < 0.01$, figure 1(c)), PCU of post- ($r = 0.571$, $p < 0.001$,figure 1(d)) and pre-shift ($r = 0.586$, $p < 0.001$, figure 1(e), and also between GGT and PCU of pre-shift ($r = 0.407$, $p < 0.05$, figure 1(f). Ironing-shop workers showed a statistically significant correlation between PCB and GGT ($r = 0.289$, $p < 0.05$, figure 2(a), between PCU and TUS ($r = 0.584$, $p < 0.001$, figure 2(b)), TUP ($r = 0.382$, $p < 0.01$, figure 2(c) or GS ($r = 0.437$, $p < 0.01$, figure 2(d)), and between TCA and TUS ($r = 0.502$, $p < 0.001$, figure 2(e)) or GS ($r = 0.306$, $p < 0.05$, figure 2(f)).

Finally, when all subjects were considered, a slight, but statistically significant

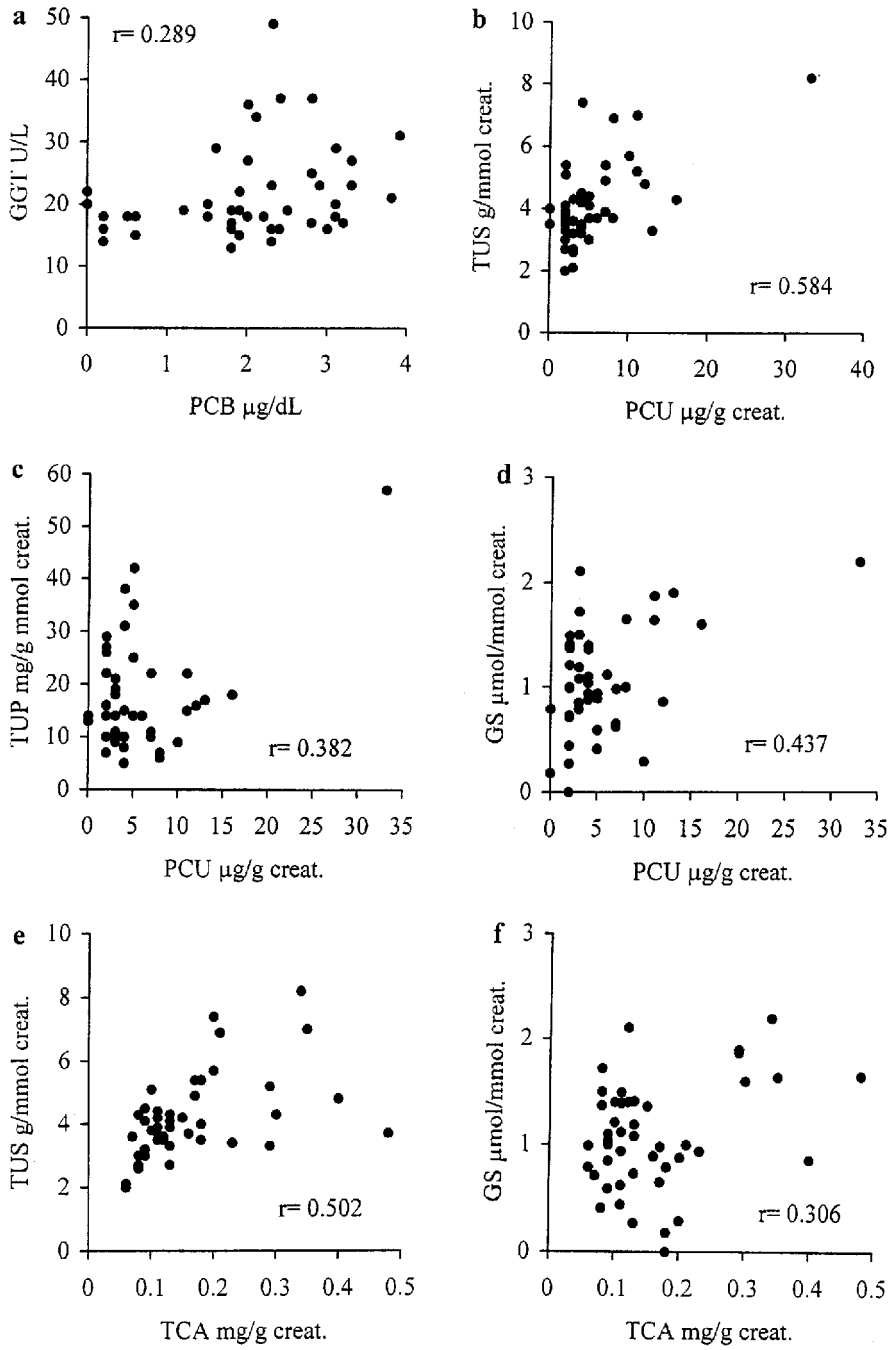

Figure 2. Relationship between PCB and GGT (**a**, $y = 2.145x + 17.366$, $p < 0.05$), PCU and TUS (**b**, $y = 0.140x + 3.405$, $p < 0.001$), TUP (**c** $y = 0.728x + 14.354$, $p < 0.01$) or GS (**d**, $y = 0.040x + 0.862$, $p < 0.01$), TCA and TUS (**e**, $y = 6.908x + 3.052$, $p < 0.001$) or GS (**f**, $y = 1.621x + 0.820$, $p < 0.05$) determined in pre-shift urine of ironing-shop workers (45 subjects).

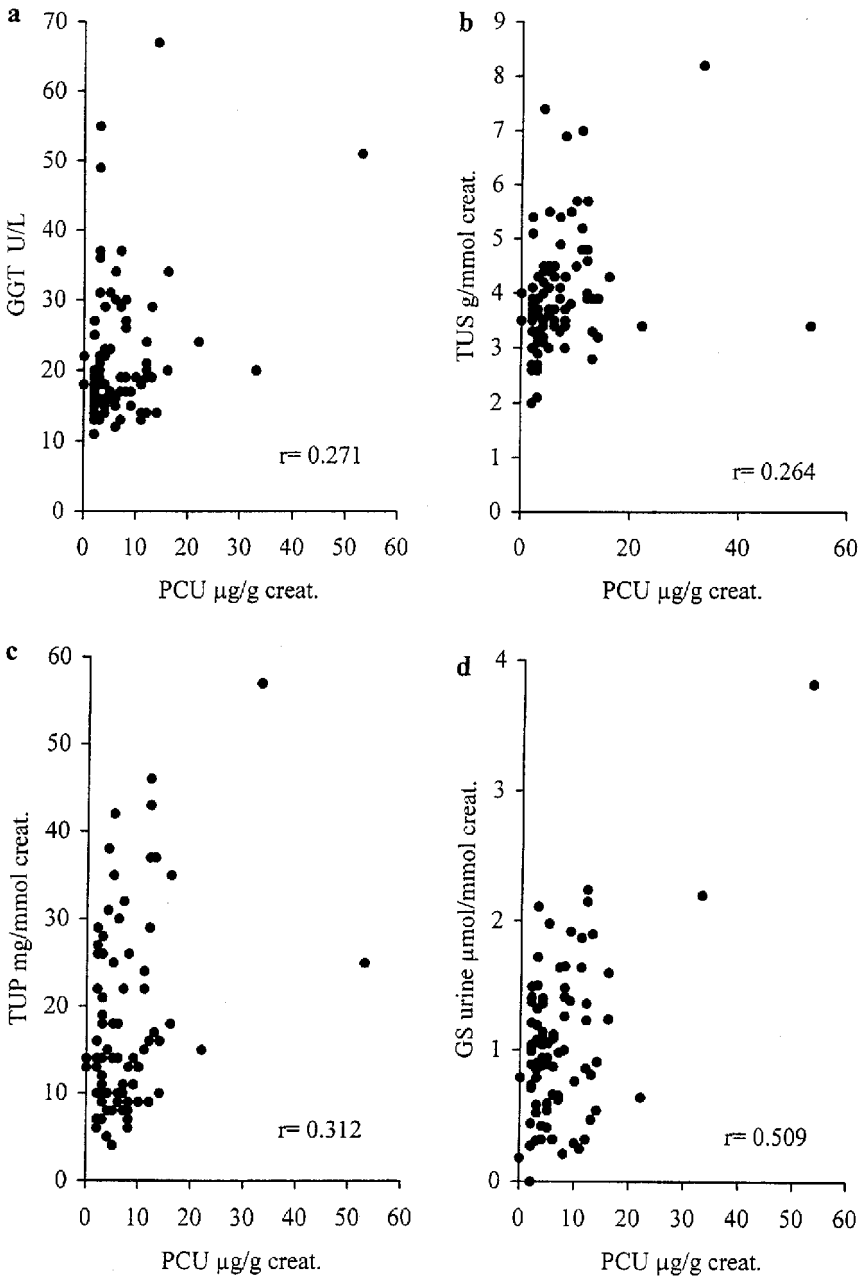

Figure 3. Relationship between PCU in pre-shift and GGT (**a**, $y = 0.363x + 19.593$, $p < 0.01$), TUS (**b**, $y = 0.040x + 3.721$, $p < 0.05$), TUP (**c**, $y = 0.465x + 14.741$, $p < 0.01$) and GS (**d**, $y = 0.042x + 0.766$, $p < 0.001$) in all workers (85 subjects).

correlation was observed between pre-shift PCU, the only urine test available for both groups, and GGT ($r = 0.271$, $p < 0.01$, figure 3(a)), TUS ($r = 0.264$, $p < 0.05$, figure 3(b)) and TUP ($r = 0.312$, $p < 0.01$, figure 3(c)). In addition, a highly statistically significant correlation was also observed between PCU and GS ($r = 0.509$, $p < 0.001$, figure 3(d)). However, a multivariate analysis performed in all

subjects showed a significant influence of age on AST ($p < 0.05$) and ALT ($p < 0.025$), of age (prevalently) and alcohol consumption (< 0.005) on GGT and alcohol consumption (< 0.025) on TUP (data not shown).

Discussion

The environmental and biological monitoring of dry-cleaning workers showed, in the present study, air PER values about three-fold lower, on the average, than the current A.C.G.I.H. TLV-TWA. Interestingly, ironing workers also showed measurable, although much lower (one to two orders of magnitude), levels of solvent in their blood. A possible explanation for this finding is the exposure of ironing workers to residual PER present in the clothes and released during ironing. In fact, in the non-exposed general population detectable, although very low, PCB levels (ng l^{-1}) are usually found (Ashley *et al*. 1994).

Dry-cleaning workers showed a good correlation between environmental PER concentrations and exposure markers such as PCB measured in post- and pre-shift and PCU in post-shift. A significant but weaker correlation was also found between PCU in pre-shift and TCA in post-shift. When we calculated the PCB value determined before the last shift of the work-week corresponding, on the regression equation between PCB and PER in air, to an air PER concentration equal to the A.C.G.I.H. TLV-TWA value, a value of 67.7 μg dl^{-1} was found, a figure which is about 1.34-fold higher than the current A.C.G.I.H. BEI. Moreover, although no BEI is suggested by A.C.G.I.H. for PCB and PCU determined in post-shift in the day before the end of the work-week and corresponding to the A.C.G.I.H. TLV-TWA, we calculated these values and found them to be 134 μg dl^{-1} and 94 μg g^{-1} creat., respectively.

Interestingly, the urinary TCA level measured post-shift in our study gave higher figures than the A.C.G.I.H. BEI (about three-fold higher). This may be explained by the observation of the very low, although measurable, air concentrations of TRI found in some dry-cleaning shops (results not shown), and may also explain the low correlation found between urinary TCA and environmental PER levels. Given the much higher (about two orders of magnitude) rate of TCA formation from TRI than from PER, even trace amounts of TRI would significantly contribute to TCA excretion in PER-exposed subjects.

Health surveillance of the dry-cleaning workers showed no effect on liver or kidney function tests attributable to PER. The statistically significant increase of AST levels observed in the present study in dry-cleaners as compared with ironing workers, cannot be confidently attributed to PER, due to the age difference between the two groups and the positive correlation known to exist in the non-exposed general population between age and transaminases (Trevisan *et al*. 1999b). Moreover, no correlation was found between either of the transaminases (AST or ALT) and any of the biological indices of exposure, except for GGT, which showed a slightly statistically significant correlation with some of these indices. The toxicological significance of this observation, however, is difficult to assess and may be due to the well known influence of age and alcohol consumption on GGT values.

TUS and TUP are currently used as non-specific indices of kidney disturbance, whereas urinary enzymes are used as markers of tubular damage caused by various xenobiotics and to detect specific subcellular targets (Price 1982) and/or

segmentary damage (Trevisan *et al.* 1999a). In addition, serum creatinine is used as a non-specific marker of glomerular function as well as a monitor of impairment of the nephron in various diseases.

In the present study, ACE, NAG, and GS activities were measured in urine to monitor possible damage of the proximal tubule caused by PER. ACE, an enzyme distributed in the brush border of all the segments of the proximal tubule though prevalently in the *pars recta* (Sudo 1981), is highly sensitive to acute and chronic tubulotoxicity caused by various chemicals, such as mercuric chloride (Trevisan *et al.* 1996) and cadmium (Chiesura *et al.* 1984). NAG, a lysosomal enzyme prevalently distributed along the *pars convoluta* but also present in the *pars recta* in rats (Le Hir *et al.* 1979) and rabbits (Bourbouze *et al.* 1984), but prevalently in the *pars recta* in humans (Schmid *et al.* 1986), is largely used to detect damage of the proximal tubule caused by xenobiotics such as solvents (Brogren *et al.* 1986) and metals (Meyer *et al.* 1984, Chia *et al.* 1994, Usuda *et al.* 1999), and by silica (Ng *et al.* 1992). An age-related increase of this enzyme activity in urine was also observed (Hultberg *et al.* 1988). In addition, a good correlation ($r = 0.48$, $p < 0.01$) was found between NAG and TCA in urine of workers exposed to TRI (Selden *et al.* 1993). Finally, the detection in urine of GS, a mitochondrial enzyme localized in the early and late portion of the S_3 segment (Burch *et al.* 1978), was recently suggested (Trevisan *et al.* 1999a) as a marker of S_3 segment-specific damage in rats treated with hexachloro-1:3-butadiene.

In this study, only GS showed a good correlation with environmental PER and the biological indices of exposure in dry-cleaners, in ironing-shop workers, and in all subjects. TUS and TUP also showed a good correlation with PCU measured in pre-shift but only in ironing-shop workers and in all subjects. ACE and NAG did not show any statistically significant correlation with any of the exposure markers studied. No urinary marker showed an increase with age (data not shown), whereas TUP showed a alcohol consumption-related increase. No difference in serum creatinine between the two groups nor any correlation between serum creatinine and any of the exposure indices was observed.

The results suggest that exposure to PER may have dose-related effects on the kidney of dry-cleaners exposed to air concentrations below the A.C.G.I.H. TLV-TWA, in agreement with the findings of other authors (Vyskocil *et al.* 1990, Mutti *et al.* 1992, Verplanke *et al.* 1999). These effects were very small and pre clinical in nature, but appear to be well correlated, on a group basis, with various exposure markers. An increased excretion of GS in the urine appears to be the most reliable index of effect in humans exposed to low PER level and could indicate an S_3 segment-specific effect of the solvent on the proximal tubule, in contrast with effect observed in animal, where an accumulation of α_{2u}-globulin in the S_2 segment was reported (Bergamaschi *et al.* 1992). The observed effects of PER on the kidney are likely to be related to the activation of the PER-derived trichlorovinylcysteine conjugate to the nephrotoxic trichlorovinyltioketene via ß-lyase, a common mechanism of bioactivation and toxicity of many halogenated alkenes.

In conclusion, the present environmental and biological monitoring study on 40 female dry-cleaning workers showed several statistically significant correlations between exposure and effect biomarkers. This suggests a weak but dose-dependent effect on the kidney of PER air levels three-fold lower, on the average, than the current A.C.G.I.H. TLV-TWA. An increase of GS excretion in urine appears to be the most specific and sensitive biomarker of kidney change. Moreover, AST in

serum was found to be significantly higher in dry-cleaners, but this finding is probably due to the age difference with respect to ironing group and, furthermore, was not correlated with any exposure biomarker. PER in blood and in urine appeared to be better indices of exposure than TCA in urine, due to the presence in several dry-cleaning shops of low, but metabolically significant levels of trichloroethylene. Interestingly, the data indicate that ironing also may result in the absorption of low, but detectable amounts of PER, probably due to direct exposure of the workers to clothes which had been previously dry-cleaned.

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