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Pathological excretion patterns of urinary proteins in renal cell cancer patients exposed to trichloroethylene

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A study was carried out to investigate urinary protein excretion patterns by means of SDS-polyacrylamide-gel-electrophoresis (SDS-PAGE) in renal cell cancer patients who had previously been exposed to high levels of trichloroethylene. Thirty-eight out of 41 (93%) renal cell cancer patients investigated had former extensive trichloroethylene exposure, but only 23 out of 50 (46%) renal cell cancer patients without a history of occupational exposure to trichloroethylene revealed urinary protein patterns indicative of toxic effects on the tubular system. One hundred controls without histories of overt renal disease and not occupationally exposed to trichloroethylene were examined in the same way; only 11 (11%) of them displayed protein excretion patterns indicative of damage to the renal tubule. These results are supported by α_1-microglobulin excretion data. The following conclusions are drawn: (1) Substantially more cases of tubular damage are found amongst renal cell carcinoma patients having been exposed to substantial levels of trichloroethylene over many years as compared with renal cell carcinoma patients not exposed to trichloroethylene. (2) The results support the view that chronic tubular damage is a precondition for the nephrocarcinogenic effect of trichloroethylene. (3) The findings indicate that urine protein patterns, on the basis of the SDS-PAGE methodology, represent a 'biological effect parameter' for the medical surveillance of persons occupationally exposed to trichloroethylene.

Key words: α_1-microglobulin; nephrotoxicity; renal cell cancer; SDS-PAGE; trichloroethylene.

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INTRODUCTION

Trichloroethylene (TRI) is a solvent predominantly used in the metal industry as a degreaser. Recently, its use in Germany has declined, primarily due to regulatory activity associated with permissible emissions and waste disposal. Apart from its toxic effects on the central nervous system and the liver, toxicity to the kidney has also been observed, particularly after high occupational exposures.[1] On the basis of animal studies in which liver tumours have been observed in mice and kidney tumours in rats,[2,3] trichloroethylene has been classified by the IARC as probably carcinogenic to humans (Group 2A) based on *limited evidence* in humans for the carcinogenicity of trichloroethylene and *sufficient evidence* in experimental animals.[4] In addition, epidemiological studies point to nephrocarcinogenicity of trichloroethylene to humans. In a cohort study, Henschler *et al.*[5] have described increased incidences of renal cell cancers in industrial workers who had been exposed to high levels of trichloroethylene over many years; this was corroborated by a case–control study.[6]

On the basis of animal experiments the argument has been put forward that chronic kidney toxicity of trichloroethylene is a precondition for the development of renal cell cancer after high exposures to trichloroethylene,[7] *i.e.*, at concentrations consistently higher than the former Occupational Exposure Limits (v.i.).

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Trichloroethylene is metabolized in humans by two competing metabolic pathways. At low levels of exposure to trichloroethylene, the major oxidative metabolic pathway is highly predominant. At concentrations higher than ~100 ppm this becomes saturated,[8] with the consequence that more trichloroethylene is metabolized *via* the alternative glutathione-dependent metabolic pathway which leads to formation of highly reactive and nephrotoxic metabolites, *e.g.*, S-dichlorovinyl-cysteine.[9–11] The site of generation of these metabolites within the nephron and, in consequence, of nephrotoxicity is the proximal tubule. The locally generated reactive metabolites are also genotoxic.[12] The process of renal cell cancer formation in persons having been extensively exposed to trichloroethylene is regarded to be based on both the local genotoxicity and cytotoxicity of the metabolites generated.[13,14]

The present study compares one group of persons not suffering from overt renal diseases and without a history of exposure to trichloroethylene with two groups of patients who had been nephrectomized because of renal cell cancer, one group without and the other with histories of extensive occupational exposure to trichloroethylene. The groups were examined with regard to early parameters of nephrotoxicity, namely urinary excretion of low-molecular proteins in general (separated by the SDS-PAGE method) and excretion of α_1-microglobulin. The study aims at developing more sensitive and targeted methods for the medical surveillance of persons occupationally exposed to trichloroethylene.

METHODS

Study groups

In connection with pending cases involving compensation for an occupational disease, 41 patients having been exposed to high levels of trichloroethylene over many years and suffering from renal cell cancer were assessed at the Institute of Occupational Physiology at the University of Dortmund (IfADo).

Prenarcotic symptoms like feeling of drunkenness, dizziness, headache and drowsiness had occurred frequently in all these patients when they were exposed to trichloroethylene. Also, the workers often had to leave the work area for some time to recover in fresh air. Typical occupational activities were degreasing of metal wares, production of rubber boxes for storage batteries, welding metal surfaces wet with trichloroethylene, cleaning and degreasing activities in the textile industry and cleaning of felts and sieves in cardboard machines. In a number of cases the workers had been exposed to hot trichloroethylene vapours. For the most part, hoods, ventilating systems and suitable gloves were not available.

Because of the historic nature of the exposure conditions, a semi-quantitative exposure evaluation system was used based on subjects' memory recall of occurrence of acute prenarcotic symptoms when using trichloroethylene. Such symptoms are known to occur at exposure peaks well above current Occupational Exposure Limits (see DFG[13]). In Germany, the MAK for trichloroethylene was set to 50 ppm in 1976.[13] From the data it can be anticipated that 'light' (light dizziness, moderate headache) or 'moderate' (light daze, clear dizziness, headache) symptoms may occur at peak concentrations up to about 500 ppm and 'severe' symptoms (daze, severe dizziness, vertigo, severe headache, nausea which did not allow a person to remain in exposure conditions) at concentrations consistently higher than 500 ppm. The severity ('light', 'moderate', 'severe') and frequency (episodes per week) of these peak exposure symptoms as well as the duration (years) of work under these conditions were entered into a semi-quantitative (+, ++, +++) rating system which was developed by Vamvakas *et al.*[6] Details of this exposure classification and the questionnaire used in this investigation have been described previously.[6]

To compare the frequency of pathologic protein excretions in renal cell cancer patients not exposed to trichloroethylene, we also examined 50 patients of a urological hospital department located in an area characterized by a long tradition with the metal industry where the use of trichloroethylene was very common. The postoperative period after nephrectomy, because of renal cell cancer (2.4 years), was similar to that of the first group of trichloroethylene-exposed tumour patients (2.6 years).

Both groups with kidney tumours were compared with a group of 100 patients of the surgical hospital department who were not suffering from a renal disease and who also had not been occupationally exposed to trichloroethylene.

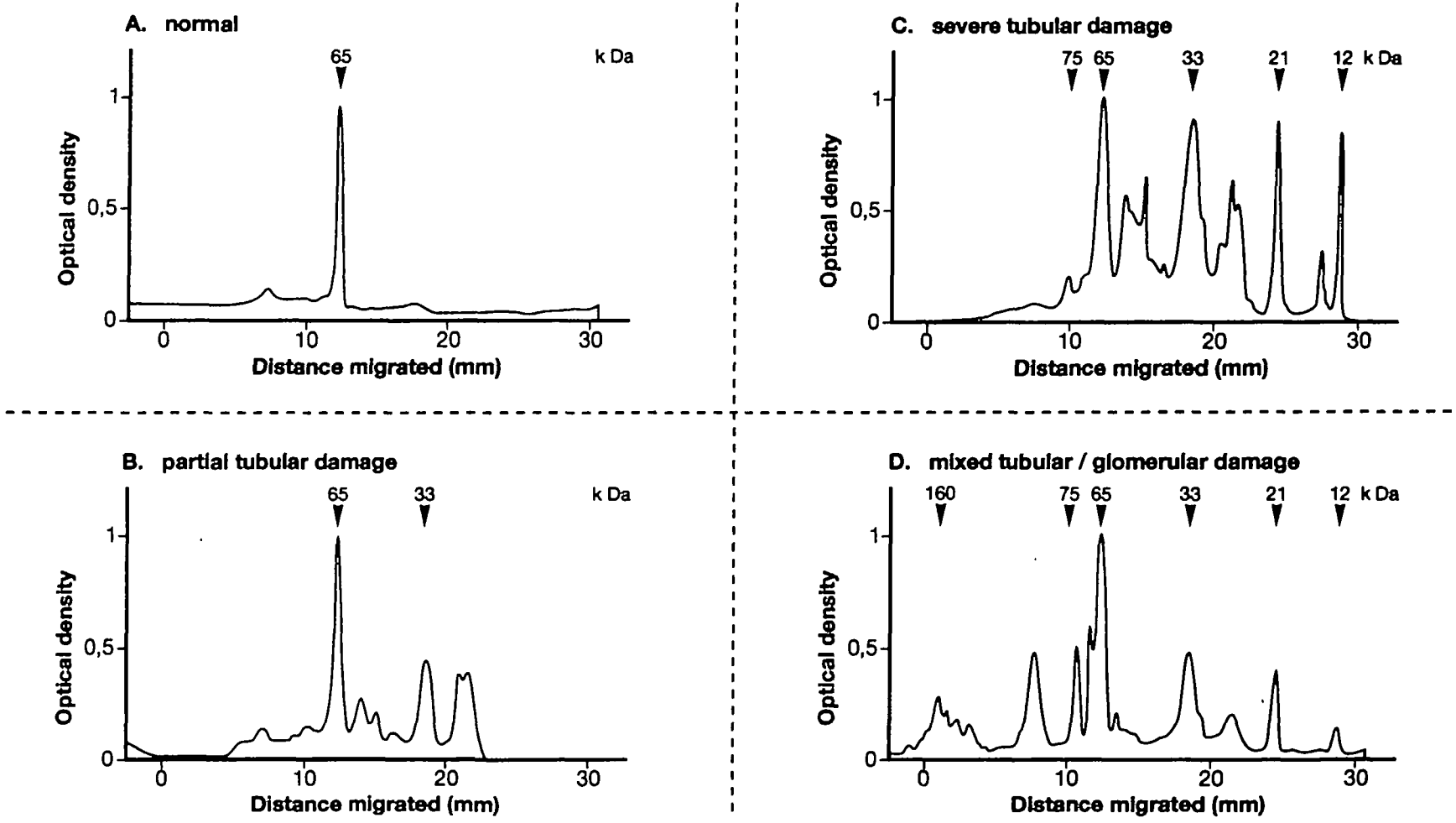
The questionnaire used to assess occupational exposures to trichloroethylene[6] was used in all three groups (including controls) of the present study.

Analytical methods

SDS-polyacrylamide gel electrophoresis. An analytical screening procedure[16,17] was employed to differentiate the urinary protein patterns of the subjects within the study groups. This procedure is an optimized method of SDS-polyacrylamide-gel-electrophoresis (SDS-PAGE) using the Phast System (Pharmacia, Freiburg), silver-staining and consecutive laser densitometry. The densitometry data are stored in an electronic databank and can therefore be used to assess individual clinical developments of protein excretion behaviours. In contrast to the quantitative determination of specific single serum proteins and tubular enzymes excreted in the urine, this semi-quantitative method offers the advantage of high-resolution separation between about 20 different urinary proteins along with their molecular size.[18] Different pathological protein excretion patterns are indicative of 'tubular', 'glomerular' or 'mixed' renal damage[16,17] (see Figure 1). The evaluation was always carried out by the same clinical nephrologist in a blind manner, without any knowledge of names, exposure or disease status of the patients.

Quantification of creatinine and urinary proteins. Creatinine in the urinary samples was determined colorimetric-

Figure 1. Typical SDS-PAGE profiles of urinary proteins.[15,17] **(A)** normal condition, showing the albumin peak at 65 kDa only; **(B)** 'partial' tubular damage; **(C)** 'severe' tubular damage; **(D)** 'mixed' tubular and glomerular damage (excretion also of proteins with molecular weights higher than albumin).



ally.[19] Total protein using the biuret method and α_1-microglobulin determination were based on a commercial nephelometric assay (Beckman Instruments, Galway, Ireland).

RESULTS

The characteristics of the three study groups are given in Table 1. In group 1 (41 renal cell cancer patients previously exposed to trichloroethylene) the average duration of occupational exposure was 18.1 years. For this group, Table 2 gives a compilation of the semi-quantitative exposure assessment (+, ++, +++), the semi-quantitative results of SDS-PAGE analysis, as well as the individual α_1-microglobulin excretions. In all patients of this group, trichloroethylene exposures, assessed retrospectively on the basis of the reported pre-narcotic symptoms, were rated high (++) or very high (+++). All the workers had been exposed to trichloroethylene under working conditions which are now considered unacceptable and are no longer permitted. There is a clear tendency towards more severe tubular damage among those in which exposure has been ranked 'very high' (+++) as opposed to 'high' (++) exposures. Table 3 compares the results of SDS-PAGE determinations in the three study groups. Protein excretion patterns pointing to tubular damage in the remaining kidney were identified in 38 out of 41 renal cell cancer patients who had been exposed to 'high' or 'very high' levels of trichloroethylene (group 1). In one case a protein excretion indicative of glomerular damage was diagnosed, and only two cases ($\sim$ 5%) showed virtually normal protein excretion patterns.

This is much in contrast to the patterns observed in group 2. Out of 50 patients without trichloroethylene exposure who had received nephrectomy because of renal cell cancer, 27 showed normal protein excretion patterns (corresponding to 54%). This means that a substantially lower percentage of tubular damage occurred among the group of nephrectomized hospital patients with renal cell cancer but not previously exposed to trichloroethylene than in renal cell cancer patients who had been exposed. No occupational exposure to nephrotoxic substances could be traced back in the patients of group 2 as the potential cause of tubular damage, except for one case. This patient had been exposed to perchloroethylene from 1960–75 as a dry-cleaner.

In the group of 100 surgical patients without overt signs of renal disease and no exposure to trichloroethylene (group 3) 86% displayed normal urinary protein excretion patterns based on the SDS-PAGE method. The spectrum of pathological results in the remaining 15 patients represents the background of subclinical changes found in the general population in Germany.[16]

Table 4 presents the results of the α_1-microglobulin determinations which are generally supportive of the SDS-PAGE data. Very clearly, excretion of this indicator protein is highest in group 1, renal cell cancer patients exposed to trichloroethylene, followed by group 2, renal cell cancer patients without a history of such exposure.

The SDS-PAGE method used is a semi-quantitative assessment of urinary proteins with molecular weights between 10–160 kDa. By this method up to 20 different proteins are discriminated, and changes in the excretion patterns provide information about tubular and/or glomerular dysfunctions. Figure 1 shows urinary protein patterns which characterize typical features of kidney damage. Physiologically, the only significant protein peak is that of albumin (panel A). Under conditions classified as 'partial tubular damage' (panel B), some additional bands are observed in the molecular weight region below albumin. In cases of 'severe tubular damage' (panel C), the whole spectrum of proteins between 10 and 65 kDa appears. Finally, under conditions of 'mixed tubular and glomerular damage' (panel D), not only low molecular weight proteins are excreted but in addition, several protein bands in the 65–160 kDa region which are indicative of structural damage to the glomerular basal membrane are also excreted.

DISCUSSION

A comprehensive concept of the human carcinogenicity of trichloroethylene is based on the general 'initiation–promotion model' of chemical carcinogenesis. The glutathione-S-transferase/β-lyase metabolic pathway leads to local formation of genotoxic metabolites of trichloroethylene in the kidney.[20] In consequence, specific mutations in the VHL tumour suppressor gene have been detected in renal cell cancers of persons with histories of high occupational exposures to trichloroethylene.[14] This demonstrates that trichloroethylene, by local action of metabolites, exerts a tumour 'initiating' effect.

Table 1. Characteristics of the study groups 1–3

	Renal cell cancer cases formerly exposed to TRI	*Renal cell cancer cases without TRI exposure*	*Healthy controls without TRI exposure*
Number of subjects (*n*)	41	50	100
Sex (m = male, f = female)	37 m, 4 f	42 m, 8 f	76 m, 24 f
Body weight (median)	74 kg	72 kg	72 kg
Date of birth (median)	1932	1934	1935
Smoking habits (S = smoker, NS = non-smoker)	27 S, 14 NS	33 S, 17 NS	68 S, 32 NS
Blood pressure (median)	140/80 mm Hg	135/75 mm Hg	130/75 mm Hg
Average duration of exposure to TRI	18.1 years	—	—

Table 2. Evaluation of the histories of trichloroethylene exposure and protein marker excretion in renal cell cancer patients (group 1)

No.	Gender	Smoking habit*	Year of birth	TRI exposure	SDS-PAGE	α_1M(mg/l)	Urinary creatinine (g/l)	Total protein in urine (g/l)
1	Male	S	1941	++	Part tub	16.3	0.96	0.28
2	Male	N	1931	+++	Part tub/gl.	14.2	1.18	0.12
3	Male	N	1934	+++	Sev tub	45.8	1.02	0.17
4	Male	S	1934	++	Part tub	18.1	1.05	0.08
5	Male	S	1917	+++	Sev tub	23.2	0.99	0.32
6	Male	N	1953	+++	Sev tub	29.3	1.21	0.13
7	Male	S	1931	+++	Part tub	19.1	0.92	0.10
8	Male	S	1944	++	Part tub	12.9	0.89	0.06
9	Male	S	1931	+++	Sev tub	21.5	1.08	0.09
10	Female	N	1936	++	Sev tub	14.7	0.97	0.08
11	Male	S	1952	++	Part tub	18.9	1.12	0.16
12	Female	N	1939	++	Normal	5.3	0.93	0.05
13	Male	S	1921	++	Sev tub	17.8	0.99	0.12
14	Male	S	1929	+++	Sev tub/gl	18.2	1.29	0.76
15	Male	N	1931	+++	Sev tub	36.1	1.02	0.15
16	Male	S	1951	++	Normal	6.1	0.95	0.05
17	Male	N	1923	++	Sev tub	32.9	1.12	0.13
18	Male	S	1920	+++	Sev tub	62.8	1.05	0.24
19	Male	S	1935	++	Sev tub/gl	20.2	0.97	0.19
20	Male	S	1936	+++	Sev tub/gl	19.1	1.10	0.08
21	Male	N	1931	+++	Sev tub	17.8	1.01	0.09
22	Male	S	1908	+++	Sev tub	18.9	1.15	0.10
23	Female	S	1928	++	Part tub	16.7	0.93	0.07
24	Male	S	1938	++	Sev tub	19.8	0.98	0.09
25	Male	S	1931	++	Sev tub	22.3	1.03	0.09
26	Male	S	1926	+++	Sev tub	28.9	0.94	0.11
27	Male	S	1919	++	Sev tub	19.2	1.18	0.07
28	Male	N	1936	++	Part tub	15.2	0.99	0.07
29	Male	S	1936	++	Sev tub	17.3	1.04	0.08
30	Male	N	1936	+++	Sev tub	27.6	1.16	0.11
31	Male	S	1934	+++	Sev tub	32.1	0.97	0.12
32	Male	S	1934	+++	Sev tub	68.7	1.11	0.23
33	Male	N	1930	++	Sev tub	23.4	1.24	0.12
34	Male	S	1907	++	Sev tub	47.8	0.92	0.17
35	Male	S	1932	++	Part tub	16.5	0.95	0.09
36	Male	S	1945	++	Part tub	14.2	1.03	0.07
37	Male	S	1938	+++	Sev tub	39.1	1.17	0.13
38	Male	N	1939	++	Part tub	34.8	1.10	0.11
39	Male	N	1940	++	Sev tub	47.9	1.01	0.13
40	Male	N	1930	++	Gl.	12.1	1.26	0.93
41	Male	S	1937	++	Part tub	14.2	0.94	0.08

* S: smoker; N: non-smoker;

** SDS-PAGE results pointing to renal damage: part tub = partial tubular; sev tub = severe tubular; gl = glomerular; normal = physiological.

Table 3. Results of SDS-PAGE determinations in the three study groups: number of persons with different protein excretion patterns

Renal damage, assessed according to protein excretion patterns	Renal cell cancer cases formerly exposed to TRI (n = 41)	Renal cell cancer cases without TRI exposure (n = 50)	Healthy controls without TRI exposure (n = 100)
Severe tubular	24	14	2
Severe tubular and glomerular	2	1	1
Slight tubular	11	8	5
Slight tubular and glomerular	1	—	3
Glomerular	1	—	3
Normal	2	27	86

In addition, the rate of formation of malignant tumours is, to a large extent, influenced by local cytotoxic effects of the metabolites of trichloroethylene which superimpose a 'promotional' stimulus on the already 'initiated' cells.[7] At present, there are two biochemical concepts to explain the selective nephrotoxicity of trichloroethylene on the proximal tubule. On one hand, some reactive metabolites generated, *e.g.*, S-(1,2-dichlorovinyl)-L-cysteine, are cytotoxic.[11] Such cytotoxicity might well be induced by high peak concentrations of trichloroethylene. On the other hand, Green *et al.*[21] have advanced the concept that high doses of

Table 4. α_1-microglobulin excretions in the three study groups

α_1-microglobulin	*Renal cell cancer cases formerly exposed to TRI (group 1)*	*Renal cell cancer cases without TRI exposure (group 2)*	*Healthy controls without TRI exposure (group 3)*
Mean	24.6	11.3	5.5
Standard deviation	13.9	9.8	6.8
Median	19.1	8.9	<4.0
Range	5.3–68.7	<4.0–39.2	<4.0–41.2

trichloroethylene enhance the formation of formic acid, a known nephrotoxin, from pathways of intermediary metabolism. Both concepts imply a classical dose-dependence between trichloroethylene exposure and nephrotoxic effect. Hence, it is possible to explain why the human nephrocarcinogenicity of trichloroethylene is obviously primarily restricted to persons with histories of unusually high trichloroethylene exposures.[5,6]

The present data are consistent with this general concept as ~95% of the persons examined (group 1) with renal cell cancer after high exposures to trichloroethylene show signs of tubular toxicity in the remaining contralateral kidney.

The prevalence of signs of renal tubular damage (46%) in group 2 (patients nephrectomized for renal cancer, but without a history of trichloroethylene exposure), is in accordance with previous data[22] and is likely to be due to multiple causes. One explanation is so-called 'hyperfiltration' after nephrectomy, in response to loss of renal mass.[23] However, this effect is mostly regarded as reversible; the mean time periods elapsed between nephrectomy and the time of examination were 2.6 years in the trichloroethylene-exposed group, and 2.4 years in the non-exposed group. A more likely reason for the prevalence of nephrotoxicity, based on causes other than trichloroethylene, in renal cell cancer patients could be some exposure to nephrotoxins of the general population. Known examples of lifestyle exposure are analgesics[24] and food constituents which are produced by plants or by microorganisms.[25–29] A possible conclusion after looking at the elevated patterns of damage, as shown by SDS-PAGE (Table 3) and α_1-microglobulin (Table 4) in group 2 (non-trichloroethylene exposed renal cancer patients compared to normal controls), is that the non-exposed patients may have been previously exposed to nephrotoxic agents (possibly including other halogenated solvents). This would mean that tubulotoxicity in general could be regarded as a predisposing factor for renal cell cancer. In general, more research in this field is required.

The findings of the human carcinogenicity of trichloroethylene[5,6] have given rise to considerations of how to regulate occupational exposures to this solvent.[15] In this context, the issue of medical surveillance programmes for persons occupationally exposed to trichloroethylene has been raised. The present data are supportive of a concept that is being debated in Germany by the Committee of Dangerous Substances (Ausschuß für Gefahrstoffe, AGS). This recommends that trichloroethylene-exposed persons undergo a mandatory examination of urinary α_1-microglobulin which is supplemented by further parameters of early nephrotoxicity in cases where α_1-microglobulin is elevated. According to our results, one of these parameters could be the urinary protein excretion profile based on SDS-PAGE analysis.

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