J Cancer Res Clin Oncol (1998) 124: 374–382

ORIGINAL PAPER

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Renal cell cancer correlated with occupational exposure to trichloroethene

Received: 4 June 1997 / Accepted: 19 March 1998

Abstract A previous cohort-study in a cardboard factory demonstrated that high and prolonged occupational exposure to trichloroethene (C_2HCl_3) is associated with an increased incidence of renal cell cancer. The present hospital-based case/control study investigates occupational exposure in 58 patients with renal cell cancer with special emphasis on C_2HCl_3 and the structurally and toxicologically closely related compound tetrachloroethene (C_2Cl_4). A group of 84 patients from the accident wards of three general hospitals in the same area served as controls. Of the 58 cases, 19 had histories of occupational C_2HCl_3 exposure for at least 2 years and none had been exposed to C_2Cl_4; of the 84 controls, 5 had been occupationally exposed to C_2HCl_3 and 2 to C_2Cl_4. After adjustment for other risk factors, such as age, obesity, high blood pressure, smoking and chronic intake of diuretics, the study demonstrates an association of renal cell cancer with long-term exposure to C_2HCl_3 (odds ratio 10.80; 95% CI: 3.36–34.75).

Key words Trichloroethene · Occupational exposure · Renal cell tumors · Epidemiology

Abbreviations *CI* confidence interval · *NTP* National Toxicology Program · *OR* odds ratio · *PMR* proportional mortality ratio

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Introduction

Worldwide, trichloroethene (C_2HCl_3) has been used on a large scale as a degreasing solvent, predominantly in the metal and electronic industries, for more than eight decades (Bruckner et al. 1989; Torkelson and Rowe 1981). Many occupational hygiene studies have described C_2HCl_3 as a chemical of low acute and chronic toxicity at levels below the occupational exposure limits of 50–100 ppm (e.g. U.S. threshold limit value; German *maximale Arbeitsplatz-Konzentration*).

We recently found an increased incidence of renal cell carcinomas [standardized incidence ratio = 7.97, 95% confidence interval CI = 2.59–18.59, as compared to the Danish Cancer Registry] in a cohort of workers in a cardboard factory exposed for long periods of time to very high C_2HCl_3 concentrations (Henschler et al. 1995). This cohort study was initiated after a cluster of five renal cell cancer cases had been observed in that plant. In contrast, several cohort studies, some with large numbers of individuals occupationally exposed to C_2HCl_3, have failed to demonstrate increases in renal cell cancer morbidity or mortality rates (Anttila et al. 1995; Axelson et al. 1994; Spirtas et al. 1991). These contradictory results can, in part, be due to much lower exposure concentrations (Anttila et al. 1995; Axelson et al. 1994), or because renal cancer mortality instead of incidence was evaluated (Spirtas et al. 1991), which is expected to be a much less sensitive indicator since nephrectomy cures a high proportion of patients (Coleman et al. 1993; LaVecchia et al. 1992).

In parallel with these cohort studies, the identification of risk factors for renal cell cancer has been the objective of several case/control studies, especially in recent years. The results in relation to occupational factors can be summarized as follows: significant associations were found for employment in the blast-furnace or the coke-oven industries (odds ratio, OR 1.7; 95% CI: 1.1–2.7) or in the iron and steel industry (OR 1.6; 95% CI: 1.2–2.2), for exposures to asbestos (OR 1.4; 95% CI: 1.1–1.8),

cadmium (OR 2.0; 95% CI: 1.0–3.9), dry-cleaning solvents (OR 1.4; 95% CI: 1.1–1.7), gasoline (OR 1.6; 95% CI: 1.2–2.0), and other "petroleum products" (OR 1.6; 95% CI: 1.3–2.1) (Mandel et al. 1995). C_2HCl_3 and tetrachloroethene (C_2Cl_4) are extensively used in the iron and steel industry and dry-cleaning workplaces.

In two National Toxicology Program (NTP) gavage studies in rats, C_2HCl_3 was found to induce nephrotoxicity and renal tumors (NTP 1988; 1990), and, although the interpretation of the results was impaired because the maximum tolerated dose was exceeded (leading to insufficient survival rates in treated animals), dose-dependence was found for lesions considered to be pathogenic prestages of carcinomas: proximal tubular necrosis and proliferation, cystic degeneration, and cytokaryomegaly. Furthermore, a weak nephrocarcinogenic effect was obtained in an inhalation study in rats (Maltoni et al. 1988), and identical tumors were found in bioassays with the chemically closely related compounds tetrachloroethene (C_2Cl_4) [NCI (National Cancer Institute) 1986], hexachlorobutadiene (Kociba et al. 1977), and dichloroacetylene (Reichert et al. 1984).

The common molecular mechanism responsible for this nephrocarcinogenicity has been elucidated (Vamvakas et al. 1993): after enzymatic conjugation with glutathione, the glutathione adducts are processed to the respective cysteine adducts, which can be acetylated to mercapturic acids and excreted with the urine or cleaved, by an enzyme that is highly expressed in kidney tubule cells, to highly reactive chlorinated thioketenes. The cysteine adducts are genotoxic in a variety of bacterial and mammalian test systems in vivo and in vitro (Dekant et al. 1986b; Green and Odum 1985; Vamvakas et al. 1992, 1996, 1989, 1988). Mercapturic acids derived from C_2HCl_3 are formed and excreted dose-dependently in experimental animals and humans (Bernauer et al. 1996; Dekant et al. 1986a).

Although the presently available data cannot yet provide the final answer to whether high, prolonged exposure to C_2HCl_3 can cause renal cancer in humans, and three large cohort studies did not reveal any link, case/control studies, one cohort study and toxicity data lend credence to a possible etiological association. Therefore, the present hospital-based case/control study was designed to investigate further the role of occupational exposure to C_2HCl_3/C_2Cl_4 in the formation of renal cancer. Because of the high prevalence of C_2HCl_3 exposure, we decided to stay in the same area, excluding all cases and controls of the cohort study.

Subjects and methods

Definition of study groups

The study group (cases) consisted of all renal cell cancer patients who underwent nephrectomy between 1 December 1987 and 31 May 1992 in the Department of Urology of a country hospital in North Rhine Westphalia. A total of 73 patients with renal cell cancer were treated in this period and contacted by mail with 62 responses (= 85%). In 4 patients who died, no occupational exposure could be assessed. The remaining 58 patients with renal cell cancer were enrolled in this study. The hospital is located in a highly industrialized area with a large number of small plants manufacturing metal and electric devices. It should be mentioned that none of the renal cell cancer cases considered in a previous study by Henschler et al. (1995) in the same area is included in the present study, to avoid double reporting and because of great differences in the exposure conditions, in that the previous subjects were working in a large factory, whereas the present ones were employed exclusively in small premises.

Regarding the appropriate selection of controls, matching is one option. In a paper by Wacholder et al. (1992a) this topic is discussed in greater detail. Matching can improve efficiency; however, this improvement is regarded as small to negligible. Therefore, this option was disregarded. Our controls were patients from the accident wards of three hospitals, not including the study hospital but located within an area with a radius of approximately 20 km, during 1993. The reasons for choosing this type of control were to exclude any diagnosis possibly related to exposure, to achieve a high rate of agreement to participate in the study and especially to undergo medical examinations to exclude renal cell cancer, and to ensure that the controls came from the same geographical area. About 75% of all controls contacted agreed to participate. All controls ($n = 84$) underwent abdominal sonography in order to exclude kidney cancer.

Body mass index, blood pressure, smoking habits, alcohol consumption, intake of diuretics as well as renal diseases other than cancer and familial occurrence of renal diseases and cancer were also recorded in both groups.

Tumor diagnosis

All cancer cases evaluated were under strict hospital control and the tumors were investigated histologically by expert pathologists. Since many epidemiological studies in relation to cancer are jeopardized by doubts about the validity of tumor diagnoses, an expert in the field of kidney tumors reread all histological slides of the cases in a double-blind manner and fully confirmed the first diagnoses. There has been no case in which problems arose in distinguishing renal cell cancer from urothelial tumors originating from the renal pelvis, nor any uncertainty about differentiating the samples from other types of renal tissue tumor. Hence, all the cases evaluated constitute renal cell carcinomas originating from the tubule epithelia.

Exposure

The occupational history, including exposure to any type of hazardous chemicals, was evaluated by personal interview using a specially designed questionnaire. Blinded interviewing was not practically possible, because cases and controls were questioned by physicians of the area. In the case of patients who had already died, an indirect history could be obtained from former colleagues and relatives. In addition to C_2HCl_3 or C_2Cl_4, occupational exposure to the following substances were assessed by evaluation of the jobs and by questionnaire: cadmium, lead, nickel, chromium, gasoline and other petroleum products, benzene, asbestos, pesticides and polychlorinated biphenyls. Patients reporting an occupational exposure to C_2HCl_3 and C_2Cl_4 were also evaluated by a more specific questionnaire to assess the conditions of exposure to these solvents in greater detail.

Most probably the samples of C_2HCl_3 to which the patients in the case group had been exposed were of technical grade and contained genotoxic epoxide stabilizers (Henschler et al. 1977) which are not known to be nephrocarcinogenic. Hexachlorobenzene, a nephrocarcinogen, acts through a mechanism different from that of C_2HCl_3 (α_{2u}-globulin accumulation). Although there is no evidence, a contribution of these impurities cannot be completely ruled out.

Air or biological monitoring data were not available for any of the cases or controls, because exposure took place predominantly in small premises decades ago when monitoring was not mandatory. No conclusion can be drawn from present-day situations since

the use of C_2HCl_3 in open systems has been completely replaced in Germany by closed facilities and/or by the use of other solvents.

Since data on former C_2HCl_3 concentrations in the air at the workplace or equivalent biomonitoring data for workers' exposure were not available, detailed information of work history and conditions were obtained from investigations by occupational hygienists from the Employer's Liability Insurance Association (Berufsgenossenschaft) and from reports of pending legal compensation cases involving occupational diseases due to C_2HCl_3. According to this information, the individual exposures were ranked in a semiquantitative way.

The level of individual exposure to C_2HCl_3 was rated by applying a system that integrates total exposure time, as well as frequency and severity of acute prenarcotic symptoms. Details of the procedure used are outlined in Table 1, which presents all the descriptors for each case and control, resulting in three categories: high (+++, 8 cases, 2 controls), medium (++, 9 cases, 2 controls) and low (+, 2 cases, 1 control). By analogy, the two controls exposed to C_2Cl_4 were ranked in the high- and medium-exposure category respectively. Most subjects in the case group had been engaged in metal-degreasing processes, in which, according to walk-through surveys and interviews with employees, industrial hygienists and occupational physicians, the metal devices were dipped into open tubs filled with C_2HCl_3. The temperature of the solvent ranged from above 60°C to room temperature. The C_2HCl_3 tubs did not have adequate hoods, nor were efficient ventilation devices used. During the degreasing procedure, which was carried out manually without gloves, the employees' breathing zone was directly above the degreasing tub. After the cleaning operation, the metal parts, wetted with C_2HCl_3, were left in the room for air drying or were dried with compressed-air machines. Hence, besides exposure during the actual degreasing procedure, there was an additional permanent background exposure due to the open C_2HCl_3 tubs and evaporation from the C_2HCl_3-cleaned metal parts. It was reported repeatedly that, in the past, C_2HCl_3 was used for practically all cleaning purposes in the plants including cleaning floors, cloths, and also hands and arms.

Statistics

The analysis included descriptive statistics with numbers and percentages or means and standard deviations. Differences in these variables between cases and controls were tested by the χ^2-or the *t*-test. The association between the exposure to C_2HCl_3 and/or C_2Cl_4 and renal cell cancer was measured by estimating the odds ratios (OR) including 95% confidence intervals (95% CI). In order to adjust for the effect of other factors, multivariate logistic regression was used. In an additional step, the data were stratified with respect to age and analyzed by the method of Mantel-Haenszel (Breslow and Day 1980). The OR were calculated within age groups and then summarized. In order to avoid a division by zero, which happens if either the number of unexposed cases or the number of exposed controls is zero, 0.5 was added to each number within that stratum (Kleinbaum et al. 1982). The significance level (α) used in all analyses was 5%. The *P* values given are based on two-sided tests. Only in situations where only the exposure was considered are the *P* values the result of one-sided tests. The power calculation at the beginning of the study was based on detecting a relative risk of at least 4 with $\alpha = 5\%$ and $\beta = 20\%$, assuming an exposure rate among the controls of 10%. This involved about 60 cases and 60 controls.

Results

A description of the variables for cases and controls is given in Table 2. The majority of subjects in both groups were male (cases: 67%; controls: 65%), aged between 27 and 83 years with a mean of 62 years (cases) and 51 years (controls). The difference was statistically significant. The cases involved patients with a higher body weight than the controls (76 versus 71 kg) but both groups had a similar body mass index. The cases had a statistically significantly higher blood pressure (systolic and diastolic). The smoking behavior was also statistically different according to the proportion of smokers (cases: 14%; controls: 35%) and ex-smokers (cases: 35%; controls: 21%). However, when former and current smokers were taken together, there was no longer any difference between the two groups. In about 50% of the cases and 44% of the controls the subjects claimed to be non-smokers. The intake of diuretics with respect to frequency (cases: 19%, controls: 5%) and duration (mean 10 versus 4 years) differed between the two groups. There was no difference with respect to alcohol consumption. The majority of patients in both the case and control groups reported a moderate consumption of less than two drinks per week (cases: 64%; controls: 67%).

Up to three jobs were recorded for each of the 58 cases and 84 controls. One patient in the case group and three control subjects did not report any job; 46% of the cases and 26% of the controls reported only one job, 31% of the cases and 42% of the controls held two jobs and the remainder had three jobs. The majority of the patients with more than one job had simply changed company and not profession, or quit their job. Several study members had worked as locksmiths and as metal workers. Since there was special interest in occupations with a possible exposure to C_2HCl_3, all these are listed as metal workers (Table 3). In the two groups, 28% and 26% had worked at least once in a metal-related industry. In both groups, about 9% had worked as a locksmith, electrician or in the paper industry and 12% had been white-collar workers.

Nineteen of the case group (33%) had been exposed to C_2HCl_3; none had been exposed to C_2Cl_4 (Table 3). Five controls (6%) had been exposed to C_2HCl_3 and two to C_2Cl_4 (2%).

Information about the duration of exposure and time from onset of exposure to renal cancer diagnosis or interview (for controls) is given in Table 4. Among the cases, 19 patients had been regularly/repeatedly exposed to C_2HCl_3 for periods of at least 3 years up to a maximum of 40 years (see also Table 1) with a mean of 16 years. The latency time between the beginning of exposure and diagnosis of renal cell cancer was, on average, 33 years. The mean of the age at time of diagnosis within the group of exposed cases was 58 years.

The duration of exposure for the 7 controls was shorter (mean: 8 years). There is a statistically significant difference in the latency time between the beginning of exposure and tumor diagnosis (mean for cases: 33 years; for controls: 18 years). Among cases and controls, the subgroups of exposed individuals were younger than the unexposed individuals.

All C_2HCl_3-exposed subjects (cases) had been regularly dealing with C_2HCl_3 under conditions nowadays regarded as unacceptable. Room temperatures were often between 30°C and 50°C in poorly ventilated and small working areas. The concentrations of C_2HCl_3 in the atmosphere were, according to the symptoms de-

Table 1 Description of exposure conditions (duration, frequency, severity) and of acute symptoms following exposure to C_2HCl_3 in renal cell cancer patients ($n = 19$), and in controls exposed to C_2HCl_3 ($n = 5$) or C_2Cl_4 ($n = 2$). Symptoms were graded as 0: none, 1: light symptoms (light dizziness, modest headache), 2: moderate symptoms (light daze, clear dizziness, headache), and 3: severe symptoms (daze vertigo, severe headache, nausea, which did not permit the subject to remain exposed). A rating of exposure was established by integrating symptomatology and exposure duration and frequency, according to the following criteria: +++ prenarcotic symptoms grade 3, ≥ 3×/week, for ≥ 3 years or prenarcotic symptoms grade 3, ≥ 2×/week, for ≥ 15 years or prenarcotic syptoms grade 2, ≥ 2×/week, for ≥ 30 years; ++ prenarcotic symptoms grade 3, duration and frequency not sufficient for +++ or prenarcotic symptoms grade 2, ≥ 1×/week, for ≥ 3 years; + prenarcotic symptoms grade 2, duration and frequency not sufficient for ++ or any prenarcotic symptoms recorded, grades 0 or 1. * Higher values than average because of extra shifts

Case no.	Duration of work (years)	Total time of exposure (h)	Handling of C_2HCl_3	Prenarcotic symptoms			Other exposures	Rated exposure level
				Grade	Frequency	Duration (years)		
Renal cell cancer cases with TCE exposure								
1	21	29 500	Open tubs, 60°C, < 200 I	3	3×/week	21	Metal dust (copper, nickel)	+++
2	20	1100	Open tubs, < 200 I blow-drying with compressed air	2	2×/week	20	Cooling lubricants	++
3	5	9800*	Manually cleaning with buckets, rags	3	Daily	1	Metal dust (copper, nickel)	++
				2	2×/week	4		
4	15	6600	Open tubs, < 200 I	3	2×/week	14	Glue on acrylate base, wood preservative, cooling lubricants (without nitrite)	++
5	19	2300	Open tubs, 60°C, > 200 I	3	Daily	19	Aliphatic/naphthenic hydrocarbons, sulfuric, hydrochloric acid, sodium hydroxide	+++
6	38	68 400	Open tubs, 60°C, > 200 I	3	Daily	38	Asbestos (gloves), rubber, sulfur, vulcanization agent	+++
7	11	22 900*	Welding hot tubs containing C_2HCl_3 residues, hot C_2HCl_3 vapor (80°C), small welding box without ventilation	3	Daily	11	Metal dust (copper, nickel, brass)	+++
8	3	6300*	Open tubs, 60°C, > 200 I	3	Daily	3	Metal dust (copper, brass), asbestos	+++
9	4	1100	Polishing metal parts partly moistened	0				+
10	40	72 000	Open tubs, > 200 I	2	2×/week	40		+++
11	30	3900	Open tubs, < 200 I	2	2×/week	30		+++
12	25	13 000	Open tubs, < 200 I	2	1×/week	25	Metal dust (copper, aluminium, brass), different oils and fats, cellulose lacquer	++

Table 1 (*contd*)

Case no.	Duration of work (years)	Total time of exposure (h)	Handling of C_2HCl_3	Prenarcotic symptoms			Other exposures	Rated exposure level
				Grade	Frequency	Duration (years)		
13	8	2100	Open tubs, < 200 l	1	1×/week	8		+
14	19	13 000	Manually cleaning of hot sieves (90°C) with C_2HCl_3 (small working area, no ventilation)	3	1×/week	19	Sodium hydroxide, sodium peroxide, hydrochloric acid	++
			Manually cleaning of cold metal parts with C_2HCl_3 rags	1	Daily	19		
15	11	5700	Open tubs, < 200 l	2	1×/week	11	Cellulose thinner	++
16	20	2600	Open tubs, < 200 l	2	Daily	20	Melamine, cresole triethylamine, phenol, formaldehyde	++
17	24	10 000	Open tubs, < 2001 in small rooms	3	2×/week	24	Sulfuric acid, hydrochloric acid	+++
18	4	650	Open tubs, < 2001	3	2×/week	4	Clear varnish	++
19	3	1600	Open tubs, < 2001	2	Daily	3		++
Control cases with exposure								
1	24	18 700	Open tubs, 60°C, < 2001	3	Daily	24		+++
2	6	6200	Open tubs, < 2001	2	1×/week	6		++
3	6	2500	Placing baskets in hot C_2HCl_3 ovens, 60°C	2	1×/week	6	Handling of liquid aluminium	++
4	5	2100	Manually cleaning with buckets, rags	0				+
5	3	1800	Manually cleaning with buckets, rags	0				+
Control cases with C_2Cl_4 exposure								
1	26	54 000	Dry cleaning, boiling of residual C_2Cl_4 sludge, 60°C	3	2×/week	24		+++
			Operating dry-cleaning machines	2	Daily	24		
2	13	8800	Dry-cleaning, operating dry-cleaning machines	2	Daily	13		++

Table 2 Characteristics of selected variables for cases and controls separated by the exposure to C_2HCl_3 or C_2Cl_4

Variable	Cases (n = 58)	Controls (n = 84)	P
Quantitative ($\bar{x}$ ± *SD*)			
Age (years)	62 ± 9.7	51 ± 13.0	< 0.01
Weight (kg)	76 ± 11.5	71 ± 8.7	< 0.05
Body mass index	27 ± 3.1	27 ± 3.4	0.76
Blood pressure			
Systolic (kPa)	18.8 ± 2.4	17.8 ± 1.9	< 0.05
Diastolic	11.2 ± 1.3	10.7 ± 1.0	< 0.01
Qualitative (*percentages in parentheses*)			
Male sex	39 (67)	55 (65)	0.83
Smoker status			< 0.05
Nonsmoker	29 (50)	37 (44)	
Former smoker	20 (34)	18 (21)	
Current smoker	8 (14)	29 (35)	
Alcohol consumption			0.34
None	9 (16)	7 (8)	
Moderate (< twice per week)	37 (64)	56 (67)	
Frequent (< twice per week)	11 (19)	21 (25)	
Intake of diuretics	11 (19)	4 (5)	< 0.01
Duration of intake (years, $\bar{x}$ ± SD)	10 ± 8.2	4 ± 2.7	0.12

* Data on smoker status and alcohol consumption were available for 57/58 cases

Table 3 Number of individuals exposed to C_2HCl_3 or C_2Cl_4 and occupations

Parameter	Cases (n = 58)		Controls (n = 84)	
	(n)	(%)	(n)	(%)
Exposure patterns				
Exposure to C_2HCl_3	19	33	5	6
Exposure to C_2Cl_4	0	0	2	2
Exposure to neither	39	67	77	92
Occupations				
Worker in the metal industry	16	28	22	26
Locksmith	2	3	7	8
Electrician	2	3	1	1
Worker in the paper industry	2	3	0	0
Employee in dry-cleaning facilities	–	–	2	2
Other occupations	35	60	49	58
White-collar worker	7	12	10	12
Blue-collar worker	5	9	15	18
Salesman	11	19	8	10
Diverse	12	21	16	19
Missing	1	2	3	4

scribed by the subjects probably severalfold higher than the current occupational exposure limit of 50 ppm. One person reported that they often noticed a massive smell of C_2HCl_3 at work and eight described frequently recurring prenarcotic symptoms such as dizziness, headache, nausea and drowsiness, which forced them to leave their workplaces for a short period of time to get fresh air and recover from these acute complaints.

Additional exposure to other working materials with a proven or suspected carcinogenic potential, such as asbestos, was explicitly asked about. Two patients reported additional short exposures to asbestos (see Table 1). Since 1980, three patients had been exposed to additional cleaning solvents such as chlorine- and fluorine-free hydrocarbons, 1,1,1-trichloroethane, dichloromethane and "white spirit".

Among the 84 controls, 5 had been occupationally exposed to C_2HCl_3. Only 1 control subject, who had worked for 24 years at a heated C_2HCl_3 tub (60°C) for about 3 h/day, reported recurring dizziness, headache and nausea. Another control had worked for 5 years at a temporarily open metal-degreasing tub for 3–4 h daily. The other 3 exposed control subjects had had only oc-

Table 4 Mean values (±SD) for duration of exposure and time from onset of exposure to diagnosis or interview (= latency period), the year of first exposure to C_2HCl_3 or C_2Cl_4 and age at diagnosis or interview for exposed cases and controls

Parameter	Cases (n = 19)	Controls (n = 7)	P
Duration of exposure (years)	16 ± 11.3	8 ± 7.7	0.15
Latency period (years)	33 ± 10.4	18 ± 7.2	< 0.01
Year of first exposure	1957 ± 10.4	1975 ± 7	< 0.01
Age (years) at diagnosis or interview	58 ± 10.5	46 ± 7.6	< 0.05

casional contact with C_2HCl_3 and none of them recalled any C_2HCl_3-related symptoms during their exposure. Two additional controls were occupationally exposed to C_2Cl_4; both had worked as dry cleaners (8 h/day) in the years from 1948 to 1974 and from 1968 to 1980 respectively. One of them, who had also been exposed to high concentrations of C_2Cl_4 by recycling the solvent once a weak for about 3 h, reported moderate headache and dizziness.

To adjust for the influence of risk factors known from the literature, logistic regression was applied. Exposures to C_2HCl_3 and C_2Cl_4 were combined because of their identical toxicological mechanisms, as described above. The odds ratio for the exposure, adjusted for age, gender, smoking, body mass index, blood pressure and intake of diuretics, was statistically significantly increased at OR 10.80 (95% CI: 3.36–34.75). From all the factors considered, only the influence of age ($P < 0.01$) and diastolic blood presure ($P < 0.05$), besides the exposure, remained statistically significant.

The analysis of intensity of exposure compared with that of the unexposed individuals, adjusted for age and diastolic blood pressure (Table 5), showed an OR of 6.61 (95% CI: 0.50–87.76) in the low-level category, OR 11.92 (95% CI: 2.55–55.60) in the medium-level and OR 11.42 (95% CI: 1.96–66.79) in the high-level category (Table 5). There is a significant increase in the odds ratios with respect to the exposure levels ($P < 0.05$). Another approach to adjust for confounders is to stratify the data with respect to these factors. Since age was the factor with the greatest difference besides exposure to C_2HCl_3, the data were stratified into 10-year age groups (Table 6). The Mantel-Haenszel estimate of the odds ratio gave a value 8.96 (95% CI: 2.90–27.75). All analyses show a strong association between the exposure to C_2HCl_3 and renal cell cancer.

Table 5 Comparison between cases and controls for various levels of exposure to C_2HCl_3 (or C_2Cl_4) (for definition of the categories see table 1). Odds ratios including 95% confidence interval [OR (95% CI)] adjusted for age and diastolic blood pressure

Exposure category	Cases	Controls	OR (95% CI)
+++	8	2[a]	11.42 (1.96–66.79)
++	9	3[a]	11.92 (2.55–55.60)
+	2	2	6.61 (0.50–87.76)
no exposure	39	77	1.0

[a] One exposed to C_2Cl_4

Discussion

This case/control study shows a statistically significant association between an exposure to C_2HCl_3 and the risk of developing renal cell cancer. The study corroborates the findings of a previous investigation in cardboard-factory workers exposed for long periods to very high concentrations of C_2HCl_3 (Henschler et al. 1995), and validates the hypothesis formulated at the outset of this paper of an association between exposure to C_2HCl_3 and renal cell cancer.

The exposure data for both solvents (C_2HCl_3 and C_2Cl_4) were combined; however, since none of the nephrectomized patients was exposed to C_2Cl_4, the study actually demonstrates an association between C_2HCl_3 exposure and renal cell cancer. C_2HCl_3 is widely used for metal degreasing or cleaning. Therefore, studies analyzing jobs in the iron and steel industry and metalware work have to be considered when evaluating the epidemiological consistency. In the studies carried out so far, the odds ratios for renal cell cancer are about 1.0 or higher: 0.99 (Greenland et al. 1994), 1.18 (McCredie and Stewart 1993), 1.6 (Mandel et al. 1995) and 1.87 (Partanen et al. 1991). If the same procedure is used as described previously [comment by Ulm et al. (1996) and reply by N. Weiss] the odds ratio for all four studies combined is significantly increased (OR = 1.47, 95% CI: 1.17–1.86). A high proportion of jobs in the iron and steel industry is associated with exposure to C_2HCl_3 although other exposures cannot be ruled out in these studies. Therefore studies considering metal degreasing and cleaning, or focusing especially on exposures to C_2HCl_3, are of particular interest. Three case/control studies were found in the literature. Asal et al. (1988) reported on OR of 1.7 (95% CI: 0.7–3.8) for metal degreasing. An increased risk in relation to degreasing solvents (OR: 3.4; 95% CI: 0.92–12.66) was found by Sharpe et al. (1989). However, neither value reached statistical significance. No association between exposure to C_2HCl_3 and renal cell cancer could be demonstrated in another case/control study (Siemiatycki 1991) (OR: 0.8; 95% CI: 0.4–2.0). If all studies are taken together,

Table 6 Analysis of the association between exposure to C_2HCl_3 and renal cell cancer in different age groups, given in the form of odds ratios with continuity correction

	Cases		Controls		
Age (years)	*n*	Exposed	*n*	Exposed	OR (95% CI)
< 40	2	2	22	1	71.7[a] (2.3–227.1)
40–50	3	2	15	4	5.5 (0.4–71.33)
50–60	22	10	27	2	10.4 (2.0–55.2)
60–70	18	1	14	0	2.5[a] (0.1–65.8)
> 70	13	4	6	0	6.2[a] (0.3–135.0)
Σ	58	19	84	7	8.96 (2.90–27.75)

[a] OR was computed after adding 0.5 to each cell within the fourfold table in that stratum

the results are more in favor than against an etiological association between C_2HCl_3 exposure and renal cell cancer formation.

Closely related to C_2HCl_3 is C_2Cl_4, which is widely used in the dry-cleaning industry. In a meta-analysis, studies considering an association between occupation in the dry-cleaning industry and renal cell cancer were included. The odds ratios in the case/control studies considered were between 0.7 and 3.42, resulting in a statistically significant overall OR of 1.49 ($P < 0.05$) (Ulm et al. 1996 and reply by N. Weiss). Furthermore, two proportional mortality ratio studies have been published with OR of 2.57 and 3.8 (Duh and Asal 1984; Katz and Jowett 1981). Considering chlorinated solvents, a German case/control study (Schlehofer et al. 1995), which was part of the international study (Mandel et al. 1995), revealed an elevated risk associated with exposure especially to C_2Cl_4 and carbon tetrachloride (OR 2.52; 95% CI: 1.2–5.2). On the other hand, two cohort studies on dry-cleaning workers have been published. In the NIOSH study (Ruder et al. 1994) four kidney cancer deaths were observed, which leads to a standardized mortality ratio (SMR) of 1.46. In contrast, in the study from the NCI (Blair et al. 1990) two cases were observed compared with four expected leading to a SMR of 0.5. There was a discussion about the interpretation of all kidney-cancer-related epidemiological findings between our group and N. Weiss (Ulm et al. 1996 and reply by N. Weiss)[1].

If more weight is put on evidence from the cohort studies, the available data do not support a nephrocarcinogenic action of C_2Cl_4 as N. Weiss concludes. If, on the other hand, case/control and proportional mortality ratio studies are considered as equally informative, one ends up with a higher degree of probability for a causal relationship.

Increased risks of renal cell cancer have also been reported for exposures to asbestos, cadmium, gasoline and/or other petroleum products. Therefore, information about exposure to these compounds was sought in all cases and controls of the present study. In only two cases was an additional, short exposure to asbestos reported. Therefore, a confounding with asbestos can be excluded.

We have considered the possibility that the association between C_2HCl_3 and renal cell cancer could be due to bias and confounding. One source of bias could be the way the controls were selected. This issue is discussed in the literature e.g. (Lasky and Stolley 1994). To achieve a similar quality of information and a low rate of refusals, we preferred hospital controls (Wacholder et al. 1992b). Even in this situation there is a fair amount of debate about which diseases are suitable. All diagnoses likely to be related to exposure should be excluded. In order to reduce the chance of misclassification we checked that none of the controls could possibly be a case by sonography. To fulfill all these criteria best we selected controls from the accident wards out of three hospitals located in the same area as the hospital attended by the patients comprising the case group. Thus selection bias does not appear to be a plausible explanation for the results. Recall bias, an inherent problem in case/control studies, cannot be completely ruled-out in our evaluation. However, the response rates among the cases and controls were sufficiently high. Another source of bias could be related to the way the information about the exposure was obtained. In the first step a questionnaire was used to collect information about the possible risk factors as well as about the jobs. If one of the jobs reported was related to a possible exposure, a further investigation was started, independent of the status of the patient (case or control). The only obvious problem is the different age distribution between cases and controls, and we used several procedures to take account of this difference. Even when we restricted the criteria to ensure similar age distributions, by excluding some of the controls, similar results were obtained. Therefore, it seems unlikely that the observed OR of about 10 can be explained by any bias.

Although the increase of the overall OR from all studies is relatively small when our study is included [rising from 1.47 (95% CI: 1.17–1.86) to 1.59 (95% CI: 1.27–2.00)], the present study adds to the weight of evidence that C_2HCl_3 has a carcinogenic activity in long-term and severely exposed human beings. The evidence of this relationship is additionally corroborated by a variety of experimental findings:

– A mechanistic explanation for the induction of renal cell tumors by C_2HCl_3 and closely related α-chlorine-substituted vinyl compounds has been elaborated by several research groups, and this glutathione-dependent bioactivation mechanism of C_2HCl_3 and C_2Cl_4 is operative in both humans and rats (Bernauer et al. 1996; Birner et al. 1996; Commandeur and Vermeulen 1990; Dekant et al. 1986a; Green and Odum 1985; Vamvakas et al. 1993).

– An identical tumor type is found in experimental animals, predominantly rats, after exposure to C_2HCl_3, C_2Cl_4, and chemically related chloroorganic compounds (Kociba et al. 1977; Maltoni et al. 1988; NCI 1986; NTP 1988, 1990).

– According to recent epidemiological investigations, exposure to C_2Cl_4 also elevates the risk of renal cell cancer formation (Ulm et al. 1996).

In summary, the present case/control study demonstrates an association between high, prolonged C_2HCl_3 exposure and increased incidence of renal cancer, which was first demonstrated in a recent cluster investigation.

Acknowledgement We are most grateful to Professor S. Stoerkel (Pathologisches Institut der Kliniken der Stadt Wuppertal, Heusenstrasse 40, D-42283 Wuppertal) for carefully reviewing, in a double-blind operation, the histopathological slides of all tumors of the present study.

[1]A more detailed description and discussion of the data will be published elsewere

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