

## Acute Intoxication with Trichloroethene: Clinical Symptoms, Toxicokinetics, Metabolism, and Development of Biochemical Parameters for Renal Damage

Thomas Brüning,\* Spiros Vamvakas,† Vasilios Makropoulos,\* and Gerhard Birner†

\*Department of Work Physiology, University of Dortmund, Ardeystraße 67, D-44139 Dortmund, Federal Republic of Germany; and

†Department of Toxicology, University of Würzburg, Versbacherstraße 9, D-97078 Würzburg, Federal Republic of Germany

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The present study reports on a 17-year-old male who ingested approximately 70 ml trichloroethene (TRI) in a suicide attempt. The patient developed fever, tremor, general motor restlessness, and sinus tachycardia and lost consciousness 5 h after poisoning. After 5 days of intubation under narcosis with forced hyperventilation and diuresis he regained consciousness. During this period blood and urine were collected and TRI and its metabolites were quantified. The highest concentration of TRI in blood was detected 13 h after ingestion. Trichloroethanol and trichloroacetic acid, metabolites of the cytochrome P450-mediated pathway, and *N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine from the glutathione-dependent pathway of TRI were quantified in urine samples. Besides these known metabolites in humans, chloroacetic acid and dichloroacetic acid were identified for the first time in urine of a human exposed to TRI. Although the patient exhibited normal levels of glucose and total protein in urine, excretion of  $\alpha_1$ - and  $\beta_2$ -microglobulin as well as  $\beta$ -NAG was significantly increased. In addition to these typical markers of selective tubule damage, analysis of the urinary protein pattern by SDS-PAGE revealed increased excretion of several low-molecular-mass proteins between 10,000 and 50,000 Da, clearly indicating tubular damage. Based on the elucidated glutathione-dependent mechanism for the nephrotoxicity of TRI, activation of the formed *S*-conjugates by  $\beta$ -lyases to reactive intermediates may account for the observed renal effects after a single, high dose of TRI. © 1998 Society of Toxicology.

Trichloroethene (TRI) has been widely used in metal degreasing for decades. In a report on 288 industrial inhalatory poisonings due to TRI the most common symptoms were severe depression of the central nervous system and loss of consciousness. In addition, gastrointestinal (nausea or vomiting) and respiratory symptoms were frequently observed, while hepatic, cardiac, or renal effects were induced only in very few cases (McCarthy and Jones, 1983). Oral ingestions

of TRI have been reported to induce tachyarrhythmias and severe hypotension and in a very few cases also impairment of liver function (Meyer, 1966).

Several studies have been conducted to investigate the association between tumor formation and the exposure to TRI by contaminated drinking water (Isacson *et al.*, 1985; Lagakos *et al.*, 1986; Cohn *et al.*, 1994). In the United States, TRI concentrations in drinking water of 0.2–49  $\mu\text{g/liter}$  for 1976–77 have been reported (Thomas, 1989). An increased risk for childhood leukemia (Lagakos *et al.*, 1986), leukemia in women, and non-Hodgkin's lymphoma were described (Cohn *et al.*, 1994); however, methodological problems, particularly the fact that tumor incidence and TRI concentrations were evaluated at the same time point, complicate the interpretation of these epidemiological studies. The target organs of TRI in experimental animals are the mouse liver and lung and the rat kidney. In addition to acute and chronic organ toxicity, life-long application of high TRI doses (in corn oil by gavage) increased the rates of hepatocellular and lung carcinomas in B6C3F<sub>1</sub> mice and caused a small increase in the incidence of renal tumors in male rats (USNTP 1988, 1990). The carcinogenicity of TRI has been repeatedly debated in the last 2 decades (Henschler *et al.*, 1977; Kimbrough *et al.*, 1985; Candura and Faustman, 1991; ECETOC, 1994). Bioactivation reactions have been linked with TRI carcinogenicity. As shown in rats and mice, TRI is metabolized by two competing pathways (Fig. 1), i.e., oxidation by cytochromes P450 and conjugation with glutathione by glutathione *S*-transferases (Byington and Leibman, 1965; Leibman, 1965; Dekant *et al.*, 1986; Commandeur and Vermeulen, 1990; Dekant *et al.*, 1990; Goeptar *et al.*, 1995). Glutathione conjugation of TRI results in formation of *S*-(dichlorovinyl)glutathione (DCVG) and may be responsible for the nephrotoxic effects of TRI. *S*-(1,2-Dichlorovinyl)-glutathione is cleaved by the enzymes of mercapturic acid pathway to *S*-(1,2-dichlorovinyl)-*L*-cysteine (DCVC) which is a substrate for renal cysteine conjugate  $\beta$ -lyases leading to formation of a highly electrophilic chloroethioketene (Dekant *et al.*, 1991, 1993; Koob and Dekant, 1991). Evidence

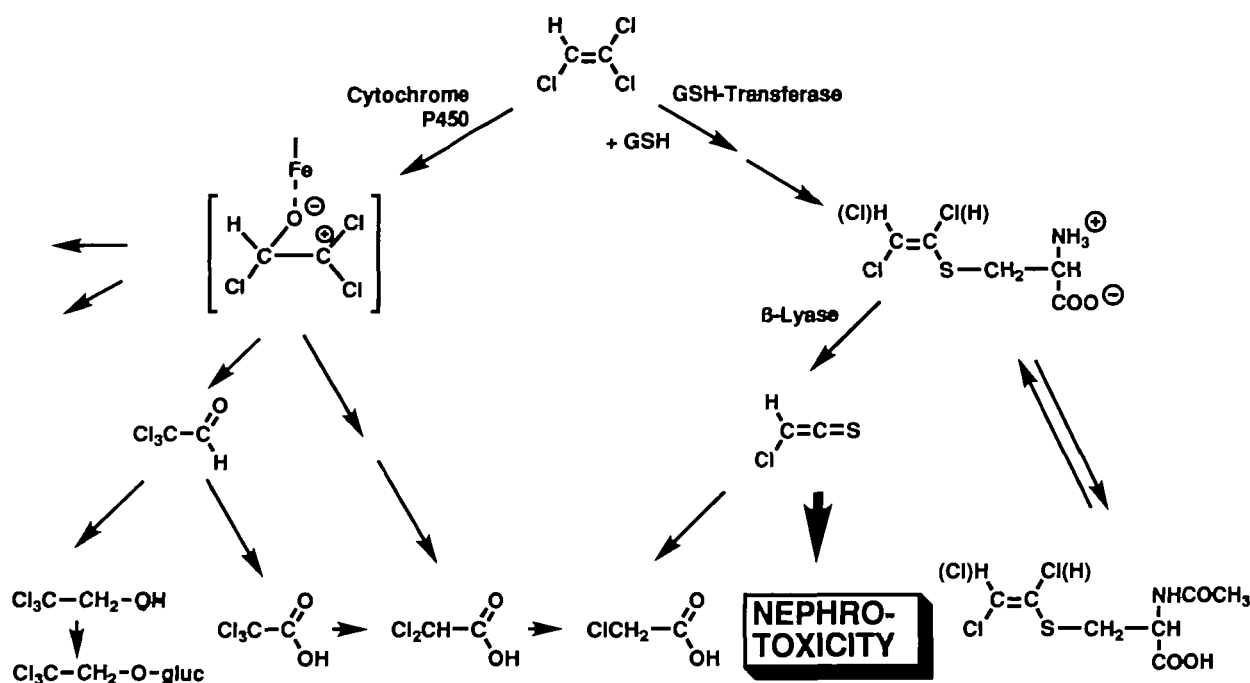


FIG. 1. Metabolism of TRI in humans.

for the bioactivation of TRI by this pathway in humans has been obtained by the identification of *N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine in the urine of humans exposed to TRI at the workplace or in controlled experiments (Birner *et al.*, 1993; Bernauer *et al.*, 1996). A decisive impact of TRI-derived *S*-conjugates and action of  $\beta$ -lyase in human nephrotoxicity of TRI have been proposed and linked with formation of renal cell cancer after long-term exposure to high concentrations of TRI at the workplace, as reported in a recent epidemiological study (Henschler *et al.*, 1995).

The present report presents clinical and biochemical data of a 17-year-old male, who had ingested about 70 ml TRI in a suicide attempt. Metabolic studies of acute human intoxications with TRI are not available. We now describe the identification of two novel metabolites of TRI in humans, confirm the formation of TRI-derived glutathione *S*-conjugates in man by quantification of the corresponding mercapturic acids, and demonstrate the time course of induction of renal toxicity in a human subject.

## METHODS

**SDS-polyacrylamide gel electrophoresis.** SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was used to differentiate urinary protein patterns. An optimized method of a SDS-PAGE was employed, using special "phast-gels" (Pharmacia, Freiburg, FRG) with very high resolution (Kierdorf *et al.*, 1993). By this method up to 20 different proteins can be discriminated without background staining.

After adjusting the urine protein concentration to 600 mg/liter, 40  $\mu$ l of

urine was mixed with 10  $\mu$ l of SDS buffer (5%) and incubated for 30 min at 37°C. One microliter of this mixture was transferred to a phast-gel and the electrophoresis was carried out at 10 mA. Phosphorylase b (MW 94,000), bovine albumin (MW 67,000), ovalbumin (MW 43,000), carbonic anhydrase (MW 30,000), soybean trypsin inhibitor (MW 20,100), and  $\alpha$ -lactalbumin (MW 14,400) were used as markers. The gels were silver-stained and analyzed by laser densitometry using an Ultrascan XL laser densitometer and a specific Gel Scan XL computer program (Pharmacia). According to the protein patterns, glomerular, tubular, or mixed early renal damage was diagnosed (Kierdorf *et al.*, 1993).

**Quantification of creatinine and urinary proteins.** Creatinine in the urine samples was determined colorimetrically (Heinegard and Tiderström, 1973). Urinary protein constituents were determined as following: total protein using the biuret method, albumin and  $\alpha_1$ -microglobulin by a nephelometric assay (Beckman Instruments, Galway, Ireland),  $\beta_2$ -microglobulin by a radioimmunoassay (Pharmacia),  $\beta$ -*N*-acetylglucosaminidase by an enzymatic assay using 3-cresolsulfonphthaleinyl-*N*-acetyl- $\beta$ -*D*-glucosaminide as substrate (Boehringer, Mannheim, FRG).

**Determination of TRI concentrations in serum.** TRI was quantified by gas chromatography using the head-space technique evaluated by the DFG (1994).

**Quantification of trichloroacetic acid and 2,2,2-trichloroethanol in urine.** Trichloroacetic acid and 2,2,2-trichloroethanol were determined according to the modified method of Fujiwara (Tanaka and Ikeda, 1968). This involved the addition of 2.5 ml of pyridine, 1.25 ml of a sodium hydroxide solution (5 N) to 0.5 ml of urine and shaking at 70°C for 15 min. After cooling, 1.25 ml of the pyridine solution was diluted with 0.5 ml water and the UV absorption at 530 nm was determined with a Uvikon 860 photometer (Kontron, Konstanz, FRG). The concentration of trichloroacetic acid was quantified based on a standard curve. For the quantification of 2,2,2-trichloroethanol, 350  $\mu$ l of chromic acid and 150  $\mu$ l of urine were heated at 70°C for 5 min. After cooling, 3 ml of a sodium hydroxide solution (5 N) and 3.5 ml pyridine were added and the mixture was incubated for 15 min at 70°C. Further processing and quantitation of the trichloroacetate

formed was performed as described above. The difference of both absorbances indicated the concentration of 2,2,2-trichloroethanol.

**Quantification of *N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine in urine.** Reference compounds were synthesized according to Dekant *et al.* (1986). A total of 0.63 nmol  $d_3$ -*N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine in methanol (50  $\mu$ l) as internal standard was added to 3 ml of urine. The solution was then adjusted to pH 1 with hydrochloric acid (2 N) and extracted four times with 2 ml of diethylether. After removing the solvent *in vacuo*, the obtained residue was dissolved in 1 ml of water. The obtained solution was added to a preconditioned  $C_{18}$  column (200 mg/3 ml; adsorbex, ICT, Bad Homburg, FRG). To remove contaminants the column was washed with water/trifluoroacetic acid (pH 2) and water/methanol (8:2, v/v). The mercapturic acids were eluted finally with 2 ml of methanol. The solvent was removed by evaporation, and the dry residue was redissolved in 0.5 ml of methanol and derivatized by adding 3 ml of an ethereal solution of diazomethane. After evaporation of the ether, the residue was dissolved in 1.0 ml of chloroform. One microliter of the derivatized sample was analyzed by GC/MS, using splitless injection (valve time 0.5 min).

**Identification of urinary metabolites by gas chromatography/mass spectrometry.** Urine (5–10 ml) was acidified to pH 2 with concentrated sulfuric acid and extracted three times with 20 ml of diethylether each. The ether extracts were evaporated under reduced pressure, and the residue was derivatized by adding 3 ml of an ethereal solution of diazomethane to form the methyl ester of the acetic acids. After removing the solvent and dissolving the residue in 1 ml chloroform, 1  $\mu$ l of the solution was analyzed by GC/MS, using splitless injection (valve time 0.5 min).

**Gas chromatography/mass spectrometry.** GC/MS analyses were performed on a Fisons Trio 2000 mass spectrometer coupled to a Carlo Erba 8000 series GC and equipped with an AS 800 autosampler (Fisons Instruments, Mainz, FRG). For quantification of the mercapturic acids, a DB1 (J&W Scientific, Folsom, CA) fused-silica capillary column (30 m, 0.25 mm i.d., 0.1- $\mu$ m film thickness) with helium as carrier gas (2 ml/min) was used. Quantitation of *N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine was performed with chemical ionization and negative ion detection in the single ion monitoring mode. Characteristic fragments with  $m/z$  178,  $m/z$  180,  $m/z$  182,  $m/z$  184,  $m/z$  235, and  $m/z$  237 were monitored for quantification of *N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine, *N*-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine, and  $d_3$ -*N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine. Quantification of the mercapturic acids was carried out relative to the content of the internal standard and referenced to calibration curves with authentic material. For gas chromatographic separation, a linear temperature program (10°C/min) from 40 to 300°C was applied; injector temperature was 200°C and transfer line temperature was 250°C. The ion source temperature was adjusted to 150°C and methane (1 Torr) was used as reactant gas.

The identification of chloroacetic acids and 2,2,2-trichloroethanol in the urine was performed using chemical ionization with positive ion detection and methane as reactant gas. The temperature of the ion source was 180°C. Full-scan spectra were recorded from  $m/z$  50 to 650. Gas chromatographical conditions were a DB 1701 (J&W Scientific) fused-silica column (15 m, 0.32 mm i.d., 1- $\mu$ m film thickness), He carrier gas, linear temperature program from 40°C/2 min to 100°C with 10°C/min, injector temperature 200°C, and transfer line 250°C.

## RESULTS

### Case Description and Clinical Symptoms

A 17-year-old male was admitted to an emergency hospital 1 h after having ingested about 70 ml TRI in a suicide attempt. On admission the patient was lethargic, but did not

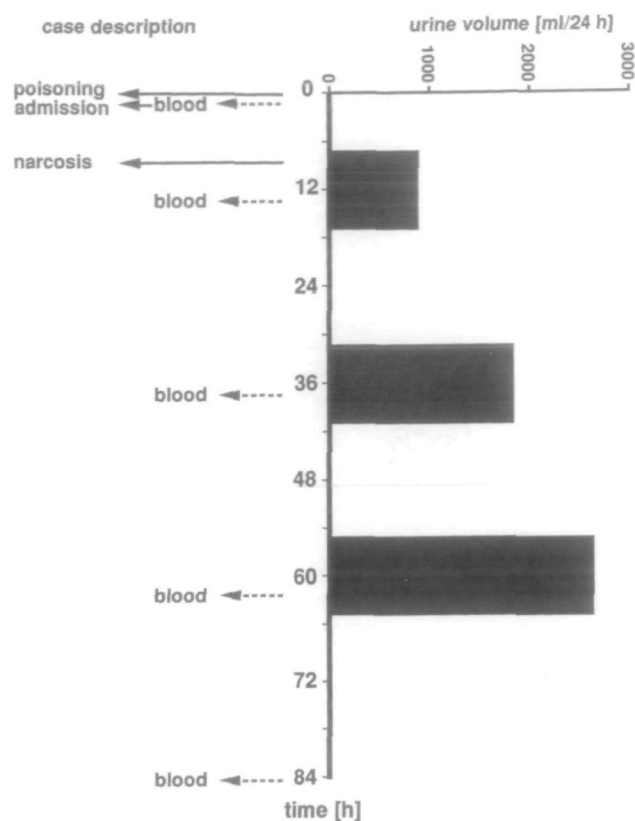


FIG. 2. Schematic description of the case of poisoning with TRI and urinary excretion volumes.

present with cardiac arrhythmia or other cardiac or respiratory problems. Although a stomach lavage was immediately carried out, he gradually became semicomatous in the following hours. After developing tremor and general motor restlessness, he finally lost consciousness 4.5 h after admission. The patient was intubated and forced hyperventilation was initiated under propofol narcosis 9 h after admission. Diuretic medication consisted of furosemid (20 mg iv) every 3 h, starting from 10 until 40 h after poisoning. The hyperventilation under narcosis was carried out for 55 h and the patient received volume substitution of 9900 ml over 62 h; the urinary excretion volume was 5300 ml (Fig. 2).

The muscle tremor persisted for approximately 18 h after poisoning. A sinus tachycardia developed after 20 h and endured for the first 5 days; intermittently the patient had ectopic beats and atrial flutter. Twenty-eight hours after admission, the patient developed fever that reached 39.1°C in the following 3 h. After administration of a cephalosporin antibiotic the fever gradually subsided in the course of the next day. The patient was extubated 93 h after poisoning and transferred to a psychiatric department.

### Clinical Chemistry

On admission and on the following days of hospitalization the patient did not reveal hematological disorders; serum

TABLE 1  
Excretion of Proteins in the Urine after Ingestion of Trichloroethene (70 ml)

	$\alpha_1$ -Microglobulin (mg/liter urine)	$\beta_2$ -Microglobulin ( $\mu$ g/liter urine)	$\beta$ -NAG (units/liter urine)	Albumin (mg/liter urine)
Sample A (0–7 h after poisoning)	3.4	160	3.4	8.6
Sample B (36–40 h after poisoning)	23.5	670	18.2	24
Sample C (46–63 h after poisoning)	20.5	480	18.0	10.9
Sample D (5 months after poisoning)	14.1	450	9.8	13.2
Normal level	<12	<350	<7	<16

electrolytes, bicarbonate, creatinine, urea, total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatin kinase, hemoglobin, hematocrit, prothrombin and partial thromboplastin time, as well as creatinine clearance and urinary output were within the normal range, respectively, as expected according to the medication. In addition, there were no hematological indices of shock, such as fibrinogen-monomers or -dimers.

#### Urinary Parameters of Nephrotoxicity

The urinary excretion of protein and glucose was within the normal range at all time points. For additional assessment of renal function several urinary marker proteins were determined by specific assays: albumin as indicator for changes of glomerular function and  $\alpha_1$ -,  $\beta_2$ -microglobulin, and  $\beta$ -NAG activity indicative for tubular alterations (Table 1). Urine collected 36–40 h (sample B) after ingestion of TRI showed a clear increase in excretion of albumin,  $\alpha_1$ -,  $\beta_2$ -microglobulin, and  $\beta$ -NAG with respect to sample A (0–7 h after poisoning). The urinary excretion of these proteins was found to be in the normal range 5 months later, although still several fold higher compared to the patient's initial values.

#### SDS–Polyacrylamide Gel Electrophoresis

An optimized SDS–PAGE offering the advantage of a high-resolution separation of approximately 20 different urinary proteins according to their molecular size was used (Fig. 3). It was possible to differentiate between pathological protein patterns in the excreted urine which indicate tubular, glomerular, and mixed renal damage (Servais *et al.*, 1995).

The urinary protein pattern was assessed by SDS–PAGE in samples collected 7 h, 36–40 h, 46–63 h, and 5 months after poisoning (Fig. 3). The first sample contained less than 50 mg/liter protein, preferentially albumin at normal concentration, and did not reveal any indication of tubular or glomerular damage (Fig. 3, lane 1). In contrast, 36–40 h after TRI ingestion, analysis of the urine by SDS–PAGE revealed the excretion of several low-molecular-mass proteins between 10,000 and 55,000 Da (Fig. 3, lane 2) indicative of tubular damage. An increase in urinary excretion of  $\alpha_1$ -mi-

croglobulin,  $\beta_2$ -microglobulin, and several other low-molecular-mass proteins (40–60 kDa) was found. An additional urine sample of the patient, 5 months after poisoning was analyzed to check the persistence of the changes in the urinary protein pattern (Fig. 3, lane 4). A clear decrease in excretion of  $\alpha_1$ -microglobulin and  $\beta_2$ -microglobulin was detected; however, both proteins were still present. Additionally excreted low-molecular-weight proteins could no more be found; however, a new, not further identified protein band (7000 Da) was observed.

#### Quantification of TRI in Blood

Trichloroethene in blood was quantified by gas chromatography according to a validated standard method (DFG,

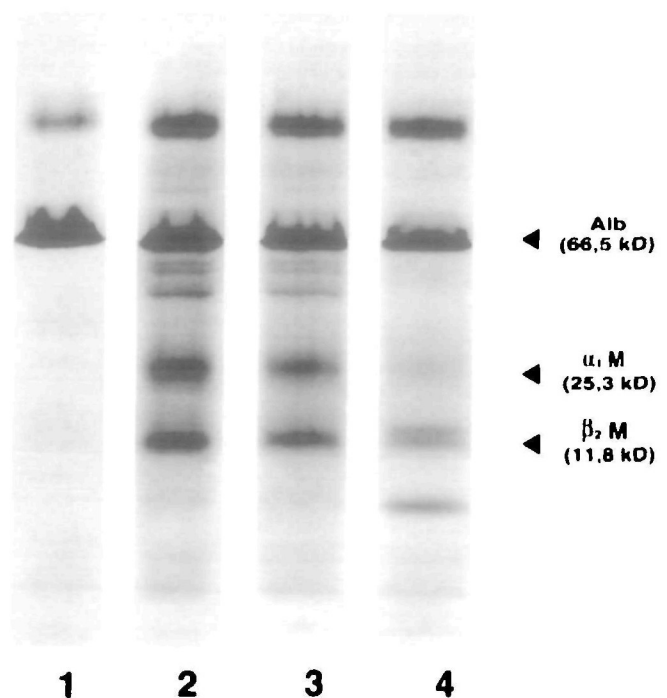


FIG. 3. SDS–PAGE of proteins excreted in urine 0–7 h (lane 1), 36–40 h (lane 2), 46–63 h (lane 3), and 5 months (lane 4) after poisoning with TRI.

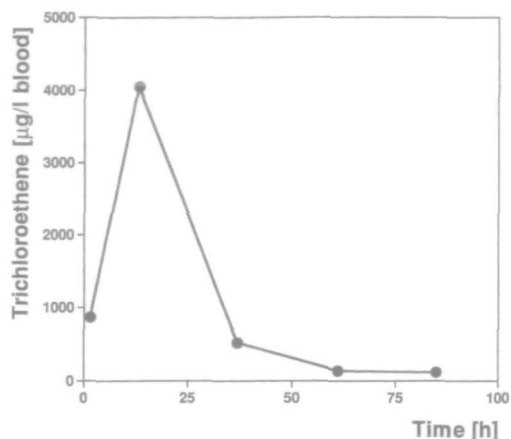


FIG. 4. Concentration of TRI in blood over 63 h after ingestion of 70 ml.

1994). Blood samples (10 ml) were obtained from the cephalica 1.5, 13, 37, 61, and 85 h after poisoning. The highest concentration of 4050 µg/liter blood of TRI was detected 13 h after oral ingestion (Fig. 4).

#### Identification of Cytochrome P450-Mediated TRI Metabolites

Highest concentrations of metabolites of the oxidative pathway of TRI in urine were expected in the sample collected between 36 and 40 h after poisoning. Aliquots of this sample were acidified and extracted with diethylether. After derivatization with diazomethane to form methyl esters, the samples were analyzed by GC/MS using chemical ionization in the positive ion mode (Fig. 5). Full scan spectra of 2,2,2-trichloroethanol and of the methyl esters of chloroacetic acid, dichloroacetic acid, and trichloroacetic acid (Figs. 5A–5D) were obtained. The spectrum of the peak with RT 3.67 showed the presence of two chlorine atoms in the molecule:  $m/z$  143 ( $^{35}\text{Cl}$ ) represents the molecular ion of dichloroacetic acid methyl ester and  $m/z$  107 ( $^{35}\text{Cl}$ ) the loss of  $-\text{HCl}$ . The molecular ion  $m/z$  177 ( $^{35}\text{Cl}$ ) in the spectrum of the peak with RT 4.53 containing three chlorines was characteristic for the trichloroacetic acid methyl ester. The fragment with  $m/z$  141 ( $^{35}\text{Cl}$ ) was formed by the elimination of  $-\text{HCl}$ . The metabolite with RT 4.81 represented the spectrum of chloroacetic acid methyl ester with  $m/z$  108 ( $^{35}\text{Cl}$ ) containing one chlorine atom. The spectrum of the peak with RT 5.81 showed a molecular ion with  $m/z$  149 ( $^{35}\text{Cl}$ ) and three chlorines and the fragments  $m/z$  131 [ $\text{M}^+ - \text{CH}_4$ ],  $m/z$  113 [ $\text{M}^+ - \text{HCl}$ ], and  $m/z$  79 [ $-2\text{Cl}$ ] characteristic for 2,2,2-trichloroethanol.

The retention times of all peaks and their fragmentations were identical with those obtained with authentic reference compounds. These compounds also were not detected in urine samples from control persons (not exposed to TRI) treated in the same way. All mass spectrometry data conclu-

sively led to the identification of chloroacetic and dichloroacetic acid as TRI metabolites and confirmed the known formation of trichloroacetic acid and trichloroethanol in humans after exposure to TRI.

#### Quantification of Urinary Metabolites

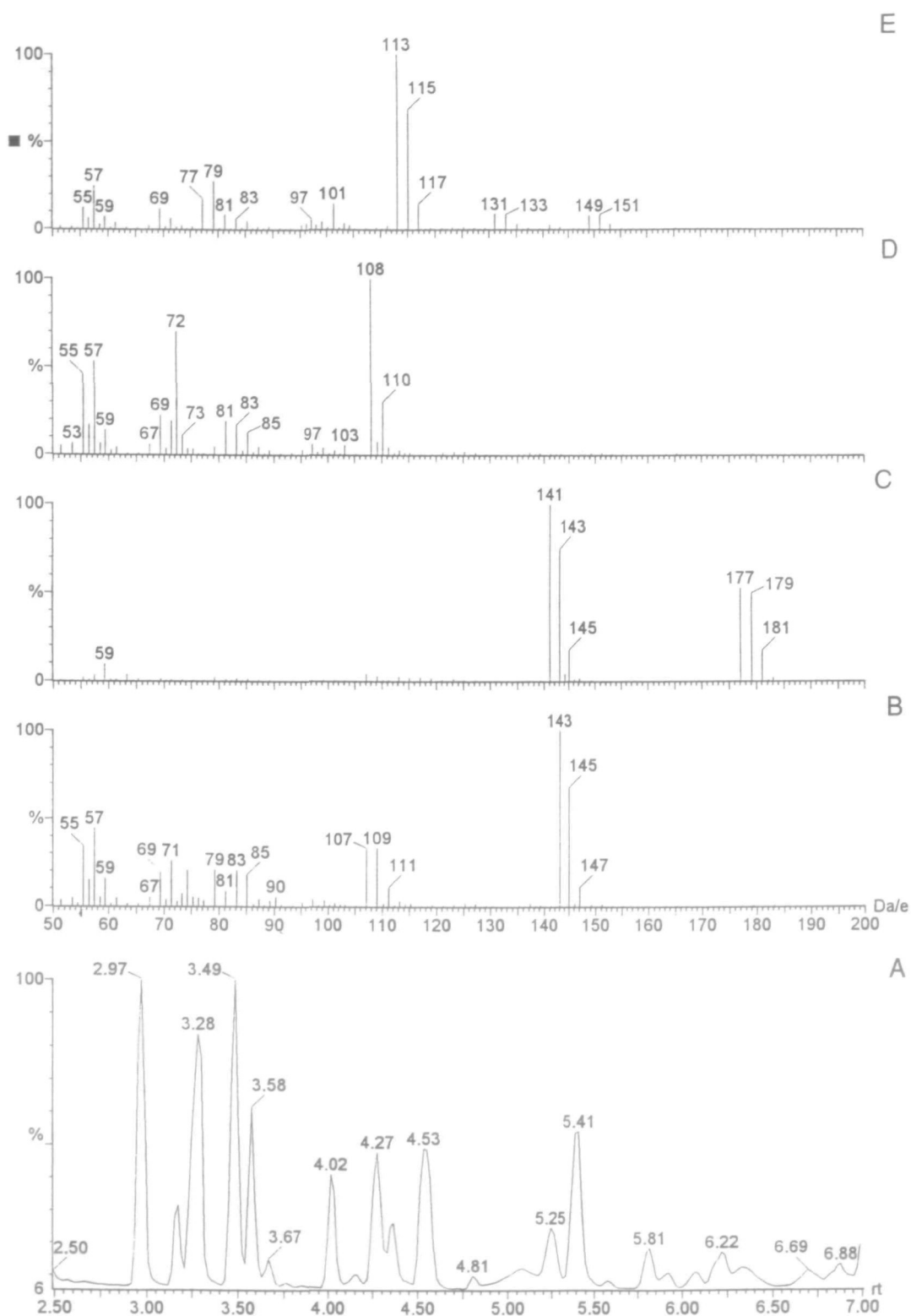
The following samples for quantification of urinary metabolites were obtained: urine A (7 h after poisoning), urine B (36 to 40 h after poisoning), and urine C (46 to 63 h after poisoning). The amounts of excreted metabolites relative to the amount of creatinine are presented in Table 2. The largest amount of trichloroacetic acid was found in sample B, while the excretion of trichloroethanol showed a maximum in sample C. This excretion pattern is not in agreement with the known kinetics of the TRI metabolites with expected half-lives of about 10 and 100 h for trichloroethanol and trichloroacetic acid, respectively. *N*-Acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine were excreted over the entire period of 63 h; the highest concentration was determined in sample C.

Since not all urine samples were available for analysis, we had to extrapolate from the aliquots obtained to the entire urine volume excreted during the 63-h treatment to calculate the total excretion of metabolites. During this period 7.2% of dose was found to be excreted as trichloroacetic acid and 1.3% as trichloroethanol. The amount of both mercapturic acids was 0.001% of dose. However, trichloroethanol and mercapturic acid excretions showed a continuing increase with time, indicating that maximum concentrations were probably achieved after the 63-h observation period.

## DISCUSSION

In contrast to the numerous cases of acute poisoning with TRI after pulmonary exposure, there are hardly any reports on oral intoxication. In the case described here the patient showed the well-known symptoms of an acute intoxication with solvents. He became semicomatous and developed symptoms of CNS depression including tremor, general motor restlessness, and finally loss of consciousness. A suicide attempt of a 29-year-old female ingesting 80–90 ml TRI also resulted in coma for 5 days (Kuntz and Neumann-Mangoldt, 1965). These symptoms were in accordance with the known narcotic effects of high TRI doses. Because of its depressing effects on the central nervous system TRI was used in medical narcosis for a short time in the past (Smith, 1966). Halogenated hydrocarbons are known to sensitize the myocardium to adrenergic transmitters, thus causing cardiac arrhythmias. Signs for cardiotoxicity like sinus tachycardia and ectopias were observed 20 h after poisoning in the present case.

In a study with 288 cases of acute intoxication with TRI beside the neurotoxic effects, liver toxicity was found in 5



**FIG. 5.** Total ion current (A) of the urine 36–40 h after poisoning with TRI. Mass spectra of dichloroacetic acid (RT 3.67) (B), trichloroacetic acid (RT 4.53) (C), chloroacetic acid (RT 4.81) (D), and of 2,2,2-trichloroethanol (RT 5.81) (E) obtained from the urine sample after work-up and derivatization of the chloroacetic acids with diazomethane.

TABLE 2  
Excretion of Urinary Metabolites over 63 h after Ingestion of 70 ml Trichloroethene

	Trichloroacetic acid (nmol/mg creatinine)	2,2,2-Trichloroethanol (nmol/mg creatinine)	<i>N</i> -Acetyl- <i>S</i> - (dichlorovinyl)-L-cysteine <sup>a</sup> (nmol/mg creatinine)
Sample A (0–7 h after poisoning)	16	4,340	0.26
Sample B (36–40 h after poisoning)	3400	2,640	0.42
Sample C (46–63 h after poisoning)	410	12,590	1.25

<sup>a</sup> Represents the sum of both isomers, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine.

cases, but no renal damage was observed (McCarthy and Jones, 1983). Hepatotoxicity, but no evidence for renal toxicity, was also reported after chronic exposure to low levels of TRI (Selden *et al.*, 1993). Nephrotoxicity associated with occupational exposure to TRI has been reported only in a few cases and always in mixed exposures with other industrial solvents (Brogren *et al.*, 1986). Hence, clear evidence for nephrotoxic effects of TRI in humans has been lacking to date (Goepfert *et al.*, 1995). In the case of acute intoxication described here, the typical standard clinical parameters for nephrotoxicity, i.e., glucose and total protein excretion as well as serum creatinine and BUN concentration, were also negative. However, the patient exhibited significantly increased excretion of  $\alpha_1$ - and  $\beta_2$ -microglobulin as well as  $\beta$ -NAG, typical markers of tubular damage. In addition, increased albumin excretion was found in the 36- to 40-h sample. Increased albumin excretion usually indicates glomerular damage, but a slightly elevated urinary excretion of this large protein may also result from impaired tubular reabsorption of albumin traces escaping the normal glomerular barrier. The tubule damage indicated by the above findings was confirmed by a very sensitive method, SDS-PAGE, which can accurately discriminate between tubular and glomerular damage. SDS-PAGE in combination with laser densitometry has already been used for the analysis of urinary proteins in several studies in humans (Mann *et al.*, 1995; Oser and Boesken, 1995; Bazzi *et al.*, 1997). In experimental animals, the renal toxicity of the established nephrotoxin *S*-(1,2-dichlorovinyl)-L-cysteine has been assessed with SDS-PAGE using this method (Davis *et al.*, 1995). We applied this method for the first time to look for renal damage in humans after TRI exposure. In this case of poisoning with TRI, we found a clear change in the pattern of urinary proteins being indicative of damage to the renal tubule.

In rats, after chronic inhalation of high TRI doses an increase of renal tubular adenocarcinomas was reported (USNTP, 1990). The organ-selective toxicity is due to a bioactivation of TRI by glutathione *S*-transferases and a further processing of the formed GSH conjugate to a reactive metabolite, a chlorothioketene in the kidney. The occurrence

of the mercapturic acid/ $\beta$ -lyase pathway in rats has been established in recent years (Dekant *et al.*, 1986, 1990; Commandeur and Vermeulen, 1990). The excretion of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine in urine of trichloroethene-exposed persons provided evidence that this pathway is also operative in humans (Birner *et al.*, 1993; Bernauer *et al.*, 1996). The proposed mechanism based on metabolic activation to chlorothioketenes has also been discussed to be responsible for the increased renal cancer incidence found in a cohort of cardboard workers exposed to TRI (Henschler *et al.*, 1995).

The biotransformation of TRI in rodents has been well investigated (for reviews see Dekant, 1986; Bruckner *et al.*, 1989; Davidson and Beliles, 1991; Goepfert *et al.*, 1995). In humans the excretion of trichloroethanol, its glucuronide, trichloroacetic acid (Monster *et al.*, 1976, Monster, 1979), and of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine (Birner *et al.*, 1993; Bernauer *et al.*, 1996) in urine has been described and confirmed in the present case. Oxalic acid and *N*-(hydroxyacetyl)aminoethanol have been described as minor TRI metabolites (Dekant *et al.*, 1984). In the present study, we have been able to identify chloroacetic acid and dichloroacetic acid as urinary TRI metabolites for the first time in humans (Fig. 1). Chloroacetic acid has been described as trace metabolite in rats (Green and Prout, 1985) and its formation may be explained by dehalogenation of dichloroacetate as well as by hydrolysis of the intermediate chlorothioketene. Dichloroacetic acid in blood and urine of mice and rats after exposure to TRI has been found in several studies at low concentrations (Green and Prout, 1985; Dekant *et al.*, 1986; Hathaway, 1980; Larson and Bull, 1992).

Excretion kinetics of trichloroacetic acid and trichloroethanol determined after oral TRI ingestion are not in line with the known half-lives of these metabolites, with trichloroethanol being eliminated faster than trichloroacetic acid. This discrepancy may be due, at least in part, to the fact that our patient received furosemid for 30 h in the intensive care unit. Furosemid has a plasma protein binding of approximately 98% and may thus interfere with the protein binding of TRI metabolites.

In summary, this is the first demonstration that a single oral dose of TRI can induce nephrotoxic effects in humans, probably by the formation of glutathione-mediated toxication products. Taking into account (i) the nephrotoxicity of this solvent in experimental animals, (ii) the identical toxication pathway in rodents and humans, and (iii) the recently demonstrated association between human kidney cancer and occupational exposure to TRI, the present study provides an important endorsement of a possible nephrotoxic potential of TRI in humans.

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