The Measurement of 2-Thiothiazolidine-4-carboxylic Acid as an Index of the *in Vivo* Release of CS_2 by Dithiocarbamates

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Dithiocarbamates and their disulfides are used extensively as agricultural fungicides, as accelerators of the vulcanization process of rubber in industry, and as therapeutic agents in medicine. The widespread uses of these compounds in agriculture, industry, and medicine provide many avenues of exposure to the human population. Subchronic to chronic exposures to some dithiocarbamates have resulted in the development of neuropathy in humans and experimental animals. Decomposition to CS_2 presents a potential mechanism through which the toxicity of dithiocarbamates may be mediated. The purpose of this study was to determine the potential of dithiocarbamates to release CS_2 *in vivo*. The ability to release CS_2 was assessed by measuring urinary 2-thiothiazolidine-4-carboxylic acid (TTCA), which is used in industry to measure the exposure of workers to CS_2. In this study, rats were housed individually in metabolic cages and given daily equimolar ip or po doses (1.5 mmol/kg) of *N,N*-diethyldithiocarbamate (DEDC), disulfiram (DS), *N*-methyldithiocarbamate (NMDC), or CS_2 for 5 days, and TTCA was measured in urine collected at 24 h intervals. For each compound administered, TTCA was produced in all of the treated animals and the amount of TTCA eliminated in urine from po administration was significantly greater than that from ip administration. The relative rates of TTCA elimination in urine were DS > DEDC ≅ CS_2 > NMDC for both routes of administration. Following administration of *N,N*-diethyl[^{13}C=S]dithiocarbamate, carbon-13 enrichment at the thiocarbonyl carbon of TTCA was demonstrated using ^{13}C NMR. Analysis of urinary TTCA proved to be useful both for establishing the *in vivo* release of CS_2 by dithiocarbamate containing compounds and for evaluating the bioavailability of CS_2. The results appear especially relevant to disulfiram, which is given orally for sustained periods in the treatment of alcoholism and has resulted in the development of neuropathy in susceptible individuals.

Introduction

Dithiocarbamates are used extensively in agriculture against a broad spectrum of plant diseases caused by fungi. In industry, dithiocarbamates and their disulfides are used as accelerators of the vulcanization process of rubber, as antioxidants in the preparation of plastics, and as slimicides in water-cooling systems (*1*). Because of their strong chelation properties, dithiocarbamates are used as scavengers in waste-water treatment (*1*) and in medicine for the treatment of certain metal poisonings (*2*) and Wilson's disease (*3, 4*). Potential adjunctive oncologic therapies employing dithiocarbamates include diminishing the toxicity of cisplatin and enhancing the efficacy of the chemotherapeutic agents, cyclophosphamide and bleomycin (*3*−*6*). In the area of AIDS research, dithiocarbamates are being considered because of their ability to bind the Zn^{2+}-dependent TAT gene product of HIV, a metal regulatory protein essential for viral replication (*7*) and as an immunomodulator (*8, 9*). Disulfiram (DS),[1] the bis(thiocarbamoyl) disulfide of diethyldithiocarbamate (DEDC), is used as an aversion therapy for the treatment of chronic alcoholism (*10*). According to WHO (*1*), the worldwide consumption of dithiocarbamates in agriculture and industry is between 25 000 and 35 000 metric tons per year. The numerous and widespread applications in agriculture and industry, along with the medical uses of these compounds, provide many avenues through which the human population is exposed.

Human symptoms of acute intoxication from exposure to dithiocarbamates and their disulfides range from nausea and ataxia to death, including behavioral symptoms such as depression, lethargy, loss of libido, delirium, and psychoses (*11, 12*). Dithiocarbamates and their disulfides have been shown to be neurotoxic (*3, 13*), and clinical use of DS has produced a distinct neuropathy described as a sensorimotor central−peripheral distal axonopathy with axonal degeneration (*12, 14*). The initial symptom of DS neuropathy is usually distal sensory impairment which is followed by weakness. These sensory and motor deficits progress proximally upon continued use of the drug (*15, 16*).

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[1] Abbreviations: DS, disulfiram; DEDC, *N,N*-diethyldithiocarbamate; NMDC, *N*-methyldithiocarbamate; TTCA, 2-thiothiazolidine-4-carboxylic acid; T_3CA, tetrahydro-2-thioxo-2*H*-1,3-thiazine-4-carboxylic acid.

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Previous investigations have presented several potential mechanisms to account for the toxicity of dithiocarbamates. The majority of these have been based on their metal-chelating properties. Some studies have attributed the neurotoxicity of dithiocarbamates to interference with catecholamine synthesis, resulting from the metal-chelating abilities of dithiocarbamates (*4*, *10*, *17*). By effectively chelating copper, dithiocarbamates can potentially inhibit dopamine β-hydroxylase (DBH), a copper-containing enzyme necessary in the biosynthesis of norepinephrine (*11*, *17*). Similarly, dithiocarbamates may also inhibit metalloenzymes involved with mitochondrial respiration, *e.g.*, cytochrome *c* oxidase and monoamine oxidase B, thereby decreasing oxidative metabolism (*7*). In addition, the ability of dithiocarbamates to bind divalent metal ions, including Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}, to form a more lipophilic complex which is readily distributed to the brain, facilitates the accumulation of these metals in the brain (*11*). Alternatively, dithiocarbamates may bind essential thiol groups to form mixed disulfides causing changes in enzyme conformation, regardless of the metal cofactor (*7*, *10*).

A principal decomposition product of dithiocarbamates in aqueous solution is CS_2 (*18*−*20*). Considering the recognized toxicity of CS_2, the ability of dithiocarbamates to form CS_2 rapidly in aqueous solution suggests that CS_2 may contribute to the toxic effects of dithiocarbamates (*14*, *15*). A previous study has shown that liberation of CS_2 from DEDC *in vitro* leads to covalent cross-linking of proteins (*21*). Initially, CS_2 reacts with ϵ-amino groups on lysyl residues of proteins to form ϵ-monoalkyldithiocarbamates followed by decomposition to isothiocyanate; a second nucleophilic addition by either amino or sulfhydryl moieties can occur to form thiourea or dithiocarbamate ester cross-links, respectively. CS_2-mediated covalent cross-linking *in vivo* has also been demonstrated using erythrocyte spectrin (*22*), and covalent cross-linking of neurofilament proteins has been proposed as a common mechanism to account for the identical neuropathies produced by 2,5-hexanedione and CS_2 (*23*). Additionally, the CS_2 produced by dithiocarbamates can react with α-amino groups of amino acids to form a dithiocarbamate adduct which may give rise to a cyclic structure of the thiazolidinium type (*24*). TTCA, which is a thio derivative of thiazolidine, has been postulated to be formed by the reaction of CS_2 with the sulfhydryl group of the cysteinyl moiety of GSH or cysteine (Figure 1) (*25*, *26*). TTCA has been shown to be a sensitive indicator of internal exposure to CS_2 and has become the preferred measurement over the less sensitive and nonspecific iodine−azide test (*27*−*29*).

In the present study, the ability of dithiocarbamates to generate CS_2 *in vivo* was evaluated using urinary TTCA measurements in rats given equimolar doses of representative dithiocarbamates or CS_2 over a period of 5 days. The relative bioavailability of CS_2 generated from a dithiocarbamate disulfide, monoalkyldithiocarbamate, and dialkyldithiocarbamate was compared to the administration of CS_2 by parenteral and oral routes. In addition, the origin of the thiocarbonyl carbon in TTCA was established using *N*,*N*-diethyl[^{13}C=S]dithiocarbamate and ^{13}C NMR.

Materials and Methods

Chemicals. Caution: *Carbon disulfide is volatile, flammable, toxic, and a skin irritant; therefore, gloves and a fume hood should be used when handling this compound. Diazomethane (CH_2N_2) is poisonous and explosive. It is handled in polished glassware under a fume hood.* Carbon disulfide was obtained from EM Science, Gibbstown, NJ. Labeled carbon disulfide, $^{13}CS_2$, was purchased from Cambridge Isotope Laboratories, Andover, MA. Disulfiram [DS; tetraethylthiuram disulfide; bis(diethylthiocarbamoyl) disulfide] and corn oil were obtained from Sigma Chemical Co., St. Louis, MO. *N*,*N*-Diethyldithiocarbamic acid, sodium salt, trihydrate (DEDC), was obtained from Acros Organics, Pittsburgh, PA. DL-Homocystine was obtained from Fluka Chemical Corp., Ronkonkoma, NY. Methylamine, diethylamine, and Dizald were purchased from Aldrich Chemicals, Milwaukee, WI. Sodium *N*-methyldithiocarbamate (NMDC), sodium *N*,*N*-diethyl[^{13}C=S]-dithiocarbamate, and unlabeled TTCA were prepared according to published procedures (*21*, *30*, *31*).

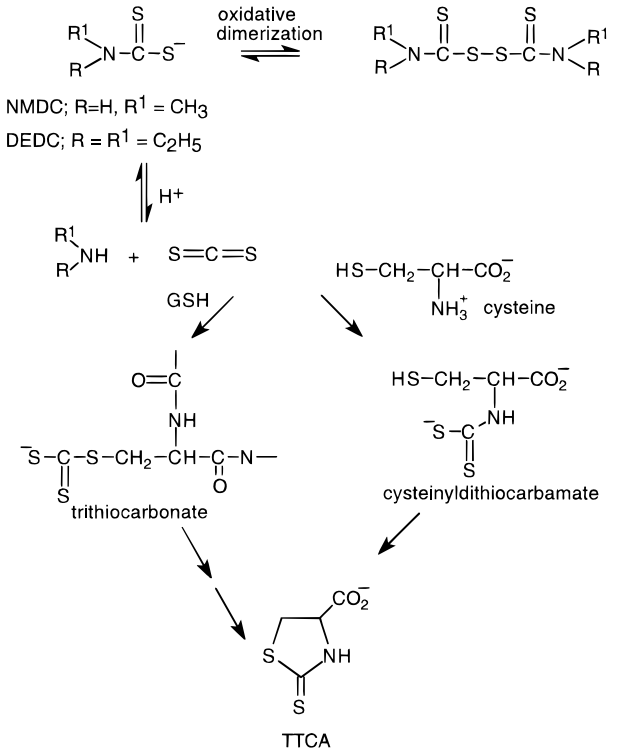

Figure 1. The decomposition of dithiocarbamate showing the generation of CS_2 and the subsequent reaction with the sulfhydryl group on the cysteinyl moiety of GSH to form a trithiocarbonate that cyclizes to form TTCA. In concept, CS_2 also may react with the α-NH_2 of free cysteine to form a dithiocarbamate which cyclizes to TTCA.

GC/MS Measurements. A Hewlett-Packard 5890 Series II gas chromatograph connected to a 5971A mass selective detector was used. An HP-5 column (30 m × 0.25 mm × 0.25 μm) was used with temperature programming (70 °C for 2 min and 20 °C/min to 210 °C and hold).

NMR Measurements. A Varian Unity 500 spectrometer was used. Spectra were acquired at 125 MHz with a sweep width of 29 000 Hz and a pulse repetition of 1.8 s. Deuterium oxide was used as an internal lock signal. Chemical shifts were referenced to external aqueous 3-(trimethylsilyl)-1-propanesulfonic acid and adjusted to TMS scale by subtraction of 1.7 ppm.

[^{13}C=S]-2-Thiothiazolidine-4-carboxylic Acid ([^{13}C=S]-TTCA). L-Cystine (240 mg, 1 mmol) and K_2CO_3 (280 mg, 2 mmol) were taken in water (2 mL), and $^{13}CS_2$ (60 μL, 1 mmol) was added with stirring. The stirring was continued for 18 h at room temperature. The reaction mixture was then acidified to pH 1 with concentrated HCl and extracted with ethyl acetate (3 × 5 mL). The extracts were combined, dried over Na_2SO_4, and evaporated. The crude product was crystallized from 1 N HCl; 80 mg (50%).

Tetrahydro-2-thioxo-2*H*-1,3-thiazine-4-carboxylic Acid (T_3CA). Homocystine (6.7 g, 25 mmol) and K_2CO_3 (7 g, 50 mmol) were mixed together in 40 mL of water, to which CS_2 (6 mL, 100 mmol) was added, and the solution was stirred for 18 h at room temperature. The reaction mixture was acidified to pH 1 with 6 N HCl and extracted with ethyl acetate (3 × 20 mL). The extracts were combined, dried, and filtered through Celite and evaporated. The residue was dissolved in 40 mL of hot 0.5 N HCl, filtered, and cooled to obtain 1 g of white crystals: mp 168−169 °C; UV λ_{max} 283 nm (ϵ 14 300) and 246 (8050); ^{13}C NMR (water) δ 23.2 (C-5), 28.5 (C-6), 56.7 (C-4), 174.3 (CO_2H), and 196.6 (C═S). On methylation with excess diazomethane, the methyl ester of the 3-methyl derivative was obtained quantitatively: ^{13}C NMR ($CDCl_3$) δ 13.3 (N-CH_3), 22.7 (C-5), 25.72 (C-6), 52.18 (-OCH_3), 59.9 (C-4), 159.0 (C═S), and 172.1 (C═O); MS *m*/*z* 205 (M^+) and 146 ($M - CO_2CH_3$).

Animals and Exposures. Male Sprague-Dawley rats, 200−250 g, obtained from Charles River Breeding Laboratories (Raleigh, NC), were housed individually in metabolic cages, given finely-ground Purina rodent chow and water *ad libitum*, and placed in a room on a diurnal light cycle. Control urine samples were collected at 24 h intervals for a period of 2−3 days prior to dithiocarbamate or CS_2 administration so that each animal could serve as its own control. Body weights were determined prior to dosing. The rats were given 1.5 mmol/kg body wt doses of DEDC, DS, NMDC, or CS_2, either ip or po (gavage) once a day for 5 days ($n = 3$). This dose was chosen based on preliminary experiments which had shown this dose to produce detectable levels of TTCA without signs of overt toxicity. Urine was collected at room temperature from the urine collection tubes of the metabolic cages 24 h after each dose and just prior to administering the next dose. Urine was also collected at 24, 48, and 72 h after the final dose. The urine samples were kept at −20 °C until ready for use. Carbon disulfide was administered in corn oil such that 0.1 mL was given for each 100 g of body wt. NMDC and DEDC were given in 0.1 M phosphate buffer, pH 7.5, such that 1 mL of solution was given for every 100 g of body wt. DS was prepared in 1,2-propanediol/water (1:2). The suspension (solubility of DS is 0.2 mg/dL of H_2O) (*32*) was sonicated in order to more finely break up particles to enable passage through a 19-gauge needle or a gavage tube. Purity of the test compounds was determined spectrophotometrically to be approximately 99%. The animals were dosed within 1 h of preparing test solutions. Release of CS_2 from the dose preparations prior to administration was ≤4% over a 2 h period.

TTCA Determination. Prior to analysis of the test samples, a standard curve was generated. Control urine was centrifuged 15 min at 7500 rpm, and then filtered via suction through a 0.45 μm filter. Ten milliliter aliquots were spiked with varying concentrations of TTCA and a constant concentration of T_3CA. The spiked urines were taken through the entire procedure for the analysis of TTCA, which was modified from van Doorn *et al.* (*26*). Filtered urine was spiked with a constant concentration of T_3CA and then acidified with concentrated HCl to pH 2. The urine was extracted with ethyl acetate (3 × 2 volumes), and the extracts were combined and dried over Na_2SO_4. The pooled extracts were taken to dryness by evaporation and then reconstituted with 2 × 500 μL of methanol; 500 μL of the methanolic solution was methylated with excess diazomethane, taken to dryness, and reconstituted in 100 μL of ethyl acetate, of which 0.5 μL was injected onto the GC/MS and detected, using multiple ion monitoring, as the dimethyl derivative of TTCA. The molecular ion (M^+) identified as the dimethyl derivative was detected at a mass/charge (*m*/*z*) ratio of 191, and the fragment at *m*/*z* 132 corresponds to the loss of the carbomethoxy side chain. The concentration of TTCA in the urine was determined by comparing the ratio of the peak area for TTCA/T_3CA to a standard curve generated using peak area ratios for varying concentrations of TTCA and a constant concentration of T_3CA. The lower limit of detection for TTCA by this method was 10 pmol.

Incorporation of [^{13}C]DEDC into TTCA. [^{13}C═S]DEDC (2 mmol/kg/day, ip) was given to 3 rats housed in metabolic cages for 3 days and daily urines were collected. The urine samples were taken through the assay for TTCA determination as stated above. Labeled TTCA was determined from the multiple ion mass spectrum obtained from synthetic dimethyl [^{13}C═S]TTCA. The pooled methylated extracted samples were purified by flash chromatography (3:1 *n*-hexane/ethyl acetate) and subjected to ^{13}C NMR as previously stated.

Statistical Comparisons. Statistical analysis for differences over time were determined by ANOVA. The two-sample *t*-test for means was used to determine differences between treatment groups. A *p* value of <0.05 was taken as the level of significance. All analyses were performed using Microsoft Excel software.

Results

Elimination of TTCA in Urine. TTCA was present in the urine of all treated animals for both routes of administration. TTCA was detected by GC/MS as the dimethyl derivative of TTCA (M^+; *m*/*z* 191) and the fragment at *m*/*z* 132 representing the loss of the carbomethoxy side chain. No TTCA was detected in control urine. The animals showed no visible symptoms of toxicity or clinical evidence of neuropathy, *e.g.*, hind limb paralysis, during the course of treatment.

Incorporation of [^{13}C═S]DEDC into TTCA. ^{13}C-Enriched TTCA was detected by GC/MS as the dimethyl derivative of TTCA (M^+; *m*/*z* 192) and the fragment resulting from loss of the carbomethoxy side chain (*m*/*z* 133) (Figure 2). Figure 3 shows the downfield region of proton-decoupled ^{13}C NMR spectra corresponding to the methylated urine extracts. The ^{13}C NMR spectrum obtained from the methylated urine extracts of [^{13}C═S]-DEDC-treated rats showed the presence of three new resonances. Following the addition of synthetic [^{13}C═S]-TTCA, there was an increase in the intensity of the resonance at 170.3 ppm, indicating that this signal corresponded to the thiocarbonyl carbon of TTCA. The other two peaks appearing in the spectra remain unidentified. No signal was detected at 170.3 ppm in extracts of urine obtained from rats administered non-enriched DEDC.

Intraperitoneal Administration of Dithiocarbamates and CS_2. Data obtained show that the mean 24 h TTCA elimination over the time course of treatment for NMDC, DEDC, and CS_2 remained relatively unchanged, whereas the mean 24 h excretion of TTCA from DS treatment increased (Figure 4). There were significant differences ($p < 0.05$) in mean 24 h TTCA elimination over the course of 5 days when comparing DS with CS_2, NMDC with CS_2, DEDC with NMDC, and DS with NMDC. Exceptions in statistical significance were found when comparing the mean 24 h TTCA elimination of DEDC with CS_2 ($p = 0.05$) and DEDC with DS ($p = 0.14$). The relative rate of elimination of TTCA in urine was DS > DEDC ≅ CS_2 > NMDC. Detectable levels of TTCA remained in urine for at least 48 h after cessation of exposure for all compounds.

Oral Administration of Dithiocarbamates and CS_2. In treatment groups where dithiocarbamates and CS_2 were administered po, the mean 24 h TTCA elimination from NMDC was relatively constant over the 5-day treatment period (Figure 5). Among treatment groups, there were significant differences when comparing the mean 24 h TTCA elimination of DS with CS_2, NMDC with CS_2, DEDC with DS, DEDC with NMDC, and DS

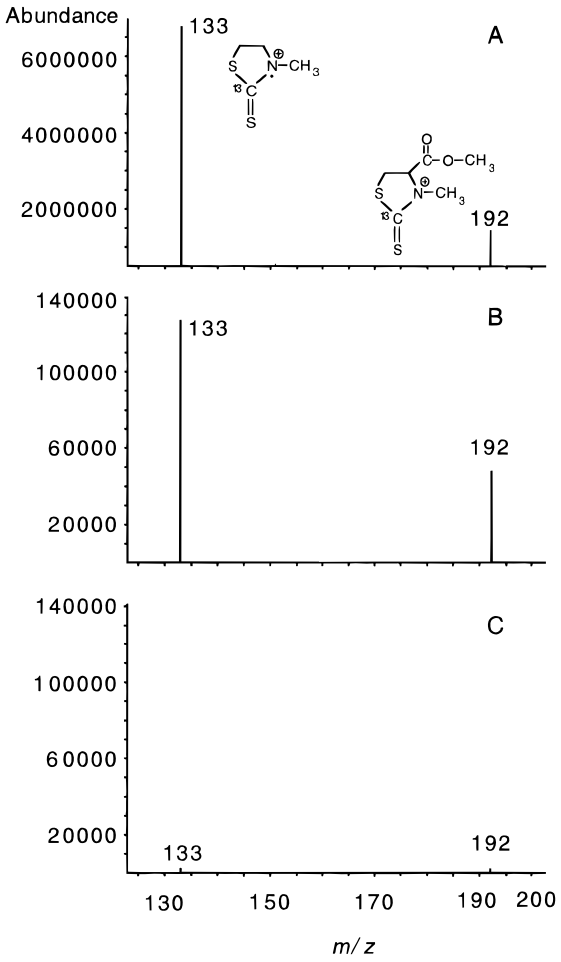

Figure 2. Identification of derivatized [^{13}C]TTCA by GC/MS. Following derivatization with diazomethane, the urine extracts were analyzed by GC/MS. The multiple ion mass spectrum of derivatized synthetic [^{13}C═S]TTCA (A) shows the molecular ion (M^+; *m/z* 192) to be the dimethyl derivative. The fragment at *m/z* 133 represents the loss of the carbomethoxy side chain. The multiple ion mass spectrum of derivatized extracted urine from [^{13}C═S]DEDC-treated rats (B) shows the presence of derivatized [^{13}C]TTCA, as indicated by the presence of ions at *m/z* 192 and 133, a difference of one atomic mass unit from unlabeled derivatized TTCA. The multiple ion mass spectrum of extracted urine from control rats (C) shows the absence of TTCA.

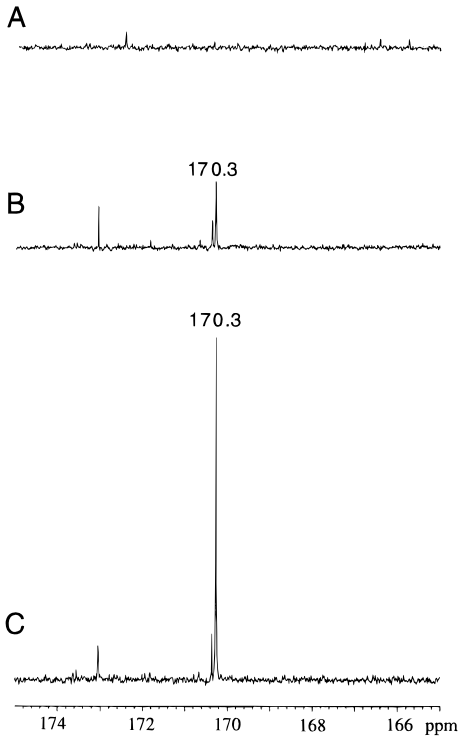

Figure 3. ^{13}C NMR spectra demonstrating the incorporation of $^{13}CS_2$ into the [^{13}C═S]TTCA structure. (A) The downfield region of the proton-decoupled ^{13}C NMR spectrum obtained from extracted control urine. (B) The ^{13}C NMR spectrum obtained from extracted urine of [^{13}C═S]DEDC-treated rats showing the presence of three new resonances. Synthetic [^{13}C═S]TTCA was added to the sample and reanalyzed (C). The intensification of the resonance at 170.3 ppm following addition of [^{13}C═S]-2-thiothiazolidine-4-carboxylic acid identifies this signal as the thiocarbonyl carbon of TTCA. The other two peaks remain unidentified.

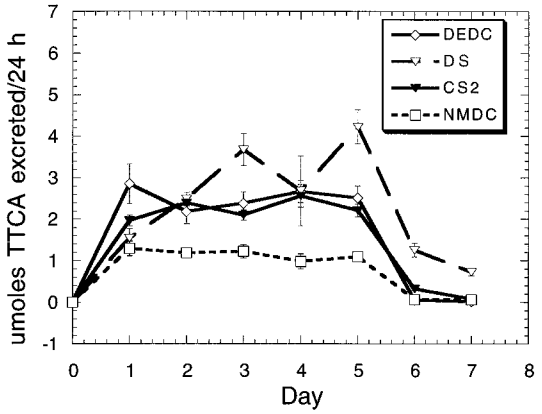

Figure 4. Urinary TTCA elimination rates obtained from ip administration of DEDC, DS, NMDC, or CS_2. The first treatment corresponds to day zero, and each urine sample was collected for 24 h after dosing (n = 3, mean ± SEM). Days 6 and 7 represent 48 and 72 h after the 5th and final dose.

with NMDC. Only the comparison of mean 24 h TTCA elimination between DEDC and CS_2 over the 5-day treatment period was not statistically different. The relative rates of TTCA elimination in urine were DS > DEDC ≅ CS_2 > NMDC. The average 24 h urinary TTCA elimination was significantly greater from po administration for all compounds tested than from ip administration (Table 1).

Discussion

TTCA has been used as a biological monitor of internal exposure for CS_2 in humans (*28, 33*) and in rats (*34, 35*) with the advantage over analysis of free CS_2 in blood of being both nonvolatile and detectable in urine. A positive correlation between exposure level and urinary excretion of TTCA exists (*29, 36, 37*), and Kivistö *et al.* (*35*) showed linearity between oral exposures of up to 30 mg/kg CS_2 and the elimination of TTCA in the urine of rats. Although only a small percentage of the absorbed CS_2 is excreted as TTCA, it is readily detectable in urine (*35, 38*), and TTCA demonstrates greater specificity over previously used metabolites. Simon *et al.* (*39*) determined that diet could be considered a source of TTCA, especially a diet rich in brassica vegetables such as cabbage, from which an extract could yield up to 3 mg of TTCA/kg. TTCA has also been identified in kidney bean seedlings treated with a dithiocarbamate fungicide and in the dwarf pea and in Sakurajima radish as an endogenous growth inhibitor (*39*). Dietary sources of TTCA were not a factor in the present study in that each animal served as its own control and diet was strictly regulated. None of the control urines contained TTCA when analyzed by GC/MS.

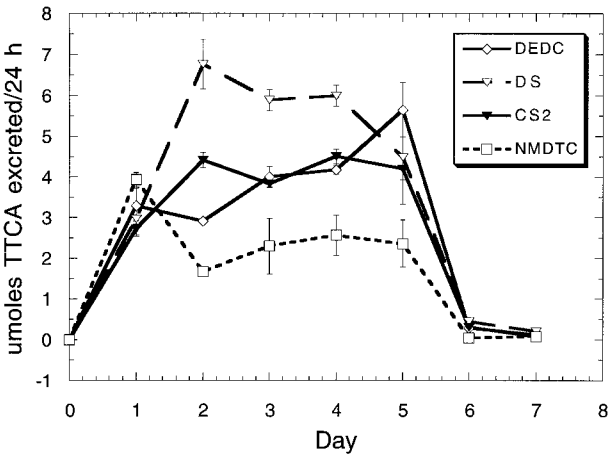

Figure 5. Urinary TTCA concentrations from rats administered DEDC, DS, NMDC, or CS_2, po. Treatment was started on day zero, and urine was collected 24 h after administration of compound just prior to the subsequent dose. Days 6 and 7 represent 48 and 72 h after the 5th and final dose ($n = 3$, mean ± SEM).

Table 1. Mean 24 h Urinary Excretion of TTCA[a]

	μmol of TTCA/24 h			
	CS_2	DEDC	DS	NMDC
ip	2.24 ± 0.08	2.52 ± 0.14	2.93 ± 0.31	1.16 ± 0.06
po	3.93 ± 0.19	4.00 ± 0.29	5.22 ± 0.44	2.56 ± 0.27

[a] $n = 15$; values are expressed as mean ± SE.

The metabolic pathway responsible for the generation of TTCA has not been established. It has been proposed that TTCA is formed through the addition of CS_2 to the sulfhydryl group of the cysteinyl moiety of glutathione (*25, 26*). Alternatively, addition of CS_2 to the α-amino group of cysteine followed by cyclization could also result in production of TTCA (Figure 1). Support for a role of glutathione in TTCA generation has been provided in a previous study that demonstrated urinary excretion rates of TTCA to parallel levels of hepatic glutathione following exposure to CS_2 (*35*). The incorporation of the CS_2 carbon into the thiocarbonyl carbon of TTCA observed in the present investigation is also consistent with the biotransformation of CS_2 being initiated through the addition of CS_2 to cysteine or glutathione.

A common decomposition product of monoalkyldithiocarbamates, dialkyldithiocarbamates, and dithiocarbamate disulfides is CS_2 (Figure 1), and it has been previously proposed that CS_2 may be the proximate or ultimate toxicant of dithiocarbamates (*16, 21, 40, 41*). Under neutral and acidic conditions, monoalkyl- and dialkyldithiocarbamates may undergo acid-promoted decomposition to CS_2 and the parent amine. Steric and electronic effects both contribute to increase the rate of decomposition to CS_2 and parent amine by un-ionized dialkyldithiocarbamates relative to monoalkyldithiocarbamates (*18, 19*). Once produced, CS_2 may then react with an ϵ-amino group of lysine to form a monoalkyldithiocarbamate which can lead either to the formation of isothiocyanate and ^{-}SH or to the regeneration of CS_2 and amine (*20*). Both monoalkyl- and dialkyldithiocarbamates may also undergo oxidative dimerization to form a bis(thiocarbamoyl) disulfide which in the case of monoalkyl bis(thiocarbamoyl) disulfides can oxidize further to isothiocyanate and sulfur under appropriate reaction conditions (*18*). Under physiological conditions, the disulfide is also readily reduced to yield two dithiocarbamates which may decompose to regenerate CS_2 and the parent amine (*10, 23*).

Production of TTCA from administration of dithiocarbamates is consistent with previous studies which have demonstrated the presence of free CS_2 in blood obtained from patients receiving DS (*40*) and in exhaled air of rats administered DEDC iv (*42*). The urinary excretion of 3.9 μmol/24 h of TTCA following administration of 1.5 mmol/kg po CS_2 measured here is in good agreement with the 3.6 μmol/24 h reported for a 1.3 mmol/kg dose of po CS_2 reported previously (*35*). The excretion of TTCA following oral administration of equimolar doses of CS_2 and DEDC were similar, consonant with a previous *in vitro* investigation showing DEDC to be an efficient vehicle for introducing CS_2 into aqueous systems (*21*). The higher levels of TTCA produced by DS relative to an equimolar dose of CS_2 were most likely due to the ability of DS to be reduced rapidly *in vivo* to two molecules of DEDC (*43*). The urinary excretion rate of 2.6 μmol of TTCA/24 h obtained for po administration of 1.5 mmol/kg NMDC is close to that of 2.3 μmol/24 h reported for 0.4 mmol/kg po CS_2 (*35*). The reduced production of TTCA for NMDC relative to DEDC may result from several potential contributing mechanisms. For one, the observed rate constants for decomposition to parent amine and CS_2 of dialkyldithiocarbamates are approximately 2 orders of magnitude greater than those corresponding to analogous monoalkyldithiocarbamates (*19*). Additionally, hepatotoxicity may contribute to diminished TTCA excretion (*35*) and may be relevant here considering the much greater cytotoxicity reported for NMDC relative to DEDC (*4*). Evidence has also been presented to indicate that TTCA excretion parallels hepatic glutathione levels (*35*) and NMDC has the potential to reduce free glutathione levels and consequently TTCA excretion either through direct enzymatic conjugation (*44*) or via generation of a methyl isothiocyanate electrophile (*31*).

Differences were observed in both the day to day variability and total amount of TTCA eliminated in urine between the two routes of administration. When dithiocarbamates and CS_2 were given ip, the 24 h excretion of TTCA into the urine was relatively constant, with the exception of DS, which appeared to increase with subsequent doses. This apparent increase may have resulted from accumulation of solid DS in the abdomen due to a diminished rate of absorption of this insoluble compound relative to the other test compounds. In contrast, the urinary TTCA obtained from po administration showed only NMDC to maintain a relatively constant rate of TTCA in urine. The other compounds showed significant variability over the 5-day treatment period and may reflect a greater variation in absorption by the oral route of exposure mediated by factors such as the presence of ingesta. There were significantly greater amounts of TTCA excreted in urine following po administration compared to ip injection for each compound. Although the greater quantities of TTCA obtained for po administration of dithiocarbamates may have resulted from an increased contribution of acid-promoted hydrolysis in the stomach, the difference observed for CS_2 appears to require further interpretation. Alternatively, quantitative differences in the biotransformation of CS_2 to TTCA due to route of exposure may be dependent upon effective dose rates and the resulting levels attained in the liver. A previous comparison of the uptake rate of CS_2 to excretion of TTCA in urine for gavage and inhalation exposure showed that the proportion of CS_2 transformed

to TTCA decreased as the rate of CS_2 uptake increased (*35*). Consequently, when a considerably smaller absorbed dose of CS_2 was administered via inhalation over a 6 h period, greater quantities of TTCA were excreted than when a bolus dose of CS_2 was administered via gavage. Similarly, decreased formation of TTCA may have resulted in the present investigation from a more rapid uptake of CS_2 following ip injection as compared to po administration.

There has been previous documentation for the presence of TTCA in the urine of humans and rats exposed to CS_2. In the present study, we have used TTCA to further establish *in vivo* release of CS_2 from dithiocarbamates as a potential mechanism of toxicity. We have demonstrated TTCA in the urine of rats challenged with DEDC, DS, and NMDC and have also shown that the CS_2 released *in vivo* by DEDC is incorporated into TTCA at the thiocarbonyl carbon. Levels of TTCA produced by these compounds followed the pattern predicted from the molar content of CS_2 and the relative rates of acid-promoted decomposition to parent amine and CS_2 observed *in vitro*. It is interesting to note that oral exposure to dithiocarbamates resulted in greater levels of TTCA relative to parenteral administration and that DS and DEDC produced levels of TTCA that were greater than and equivalent to CS_2, respectively. Although somewhat useful for evaluating acute exposures, the rapid elimination of TTCA following cessation of exposure suggests that a biomarker demonstrating a cumulative dose response, as observed for spectrin cross-linking, may be more suitable for monitoring chronic exposures as occur in DS therapy. The differences observed here in TTCA levels resulting from route of exposure may reflect differences in the bioavailability of CS_2 that are relevant when considering routes of administration for dithiocarbamate-based medical agents.

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Meeting Calendar

August 25−29, 1996	American Chemical Society Division of Chemical Toxicology (probationary) First Symposium Program [*Chem. Res. Toxicol.* **9** (2), 562, 1996].
October 20−24, 1996	2nd World Congress on Alternatives and Animal Use in the Life Sciences [*Chem. Res. Toxicol.* **8** (4), 624, 1995].
October 20−24, 1996	Seventh North American ISSX Meeting [*Chem. Res. Toxicol.* **9** (1), 349, 1996].
June 30−July 3, 1997	Sixth European ISSX Meeting [*Chem. Res. Toxicol.* **9** (1), 350, 1996].

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