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Urinary Thioether Biological Monitoring in the Interaction Between 1,2-Dichloroethane and Disulfiram in Sprague-Dawley Rats*

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The interaction between inhaled 1,2-dichloroethane (ethylene dichloride; EDC) and dietary Disulfiram (tetraethylthiuram disulfide; Antabuse; DSF) was investigated for male Sprague-Dawley rats in terms of urinary levels of thio-compounds extractable in ethyl acetate and then hydrolyzed in alkali (the classic urinary thioether assay). The assay was found to be an inadequate biological monitoring indicator for EDC or DSF exposure during the DSF/EDC interaction at exposures of 0, 153, 304 and 455 ppm EDC (7 hr/day, 5 days/week, 30 exposure days) for rats fed with AIN-76 diet fortified with 0.15% DSF. EDC inhibited the excretion of DSF-derived thio-compounds with increasing EDC concentration; the thioether content was dose-related in the absence of DSF. In situations where confounding agents generate neutral S-containing urinary metabolites without involvement of endogenous glutathione, the classic thioether assay requires supplementation by other biochemical monitoring strategies.

Introduction

The present paper reports part of an animal study[1-3] investigating the toxic interaction between 1,2-dichloroethane (ethylene dichloride; EDC) and an agent used in the management of chronic alcoholism, Antabuse (Disulfiram; tetraethylthiuram disulfide; DSF). This aspect of the investigation concerns the interaction in terms of urinary thio-compounds quantitated using the classic thioether assay[4] and compares the results with those elicited by EDC or DSF alone.

The monitoring of urinary thioethers (the major neutral thio-compounds present in urine) in humans has been widely used to assess if the glutathione conjugation/elimination pathway is coping with the internal exposure of many xenobiotics[4] and whether this exposure is by inhalation, ingestion, or through the skin.[5,6]

The rationale for the use of urinary thioethers as a screening index of chemical exposure is based on the fact that reactive electrophiles (*e.g.*, activated epoxides, carbonium ions, activated protonated intermediates, functional groups bearing a net positive charge) generated during the metabolism of many chemicals may react with the nucleophilic -SH group of reduced glutathione (GSH) in cells. The extent to which a generated electrophile conjugates with GSH depends[7] upon its electrophilic reactivity (non-enzymic process) and the activity of the cytosolic glutathione-S-transferases (GSTs). GSH conjugates are then converted to cysteine conjugates in two enzymatic reactions: cleavage of the glutamyl group by gamma-glutamyl transferases and the glycyl moiety by cysteinylglycinase.[8] The cysteine conjugate can be N-acetylated by N-acetyl transferase isoenzymes in the microsomes, and subsequently, the thioethers are excreted in the urine and isolated on acidification as mercapturic acids. Glutathione conjugation thus results in the formation of thioethers which are excreted as glutathione conjugates in the bile and as cysteine conjugates, premercapturic acids, mercapturic acids and other thioethers in the bile and urine. The enterohepatic circulation reabsorbs many of the biliary thioethers, which are subsequently excreted in the urine. Most mercapturic acids that have been identified as urinary metabolites originate from xenobiotic compounds, but some endogenous compounds are sources.[7,9] Thus, the sulfur atom of a "true urinary thioether" is endogenously derived. The reaction of electrophilic intermediates with GSH is generally a protective function, preventing damaging covalent binding of electrophiles to nucleophilic receptor sites and macromolecules, which may initiate toxic responses. In the case of EDC, however, bioactivation is thought to occur via an electrophilic "sulfur half-mustard."[3]

EDC is extensively (48% to 85% over 24 hr) metabolized to urinary thioethers in rats[10] and mice.[11] The principal metabolites identified are S-carboxymethyl-cysteine; thiodiacetic acid (33% to 58%, and the sole urinary metabolite found in Sprague-Dawley rats);[12] chloroacetic acid; and thiodiacetic acid sulfoxide. Minor metabolites are 2-S-hydroxyethylcysteine and N-acetyl-S-(2-hydroxyethyl)cysteine and its sulfoxide.[13] The ratio among excretion products varies with EDC dose.[11] Except for the chloroacetic acid identified in mouse urine[11] and the sulfoxide, all the metabolites identified for EDC are thioethers. Thus, GSH conjugation/elimination plays a major role in EDC metabolism.

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Orally administered DSF is almost completely absorbed (70%-90%) in the rat[14] and man.[15] The major urinary metabolites of DSF in the rat are diethyldithiocarbamate-glucuronide (DDC-glucuronide); DDC-methyl ester; diethylamine; inorganic sulfate; and possibly, thiothiazolidine carboxylic acids derived from carbon dislufide to which DSF also is known to be metabolized in the liver.[4,16] DDC-glucuronide constitutes over 50% of DSF urinary metabolites.[17] DDC-glucuronide and the DDC-methyl ester are thiol esters which are neutral compounds chemically similar to thioethers.

In previously reported studies on the EDC/DSF interaction, biological residence half-times of 11.9 hr (for both acute and subchronic EDC exposure) and 20.8 hr (for acute and subchronic EDC exposure in the presence of DSF) were determined for ^{14}C-EDC administered i.p. assuming first-order ^{14}C-label excretion.[1] This showed that DSF decreased the urinary excretion of EDC-derived material. The excretion of EDC-derived thioethers also may be similarly affected, and determining this was one of the aims of the present study.

The urinary thioether assay, in principle, should be capable of integrating exposure to mixtures of chemicals which generate electrophiles *in situ* which conjugate GSH. Workers are exposed to many chemicals generally in an intermittent manner so that such an integrative test would be an invaluable screening index of exposure to a chemical mixture. The urinary excretion rate of endogenous thioethers is similar in chimpanzees and man, whereas great species differences have been found between the primates and rats[18] because of the differential specific activities of the GST isoenymes in the two species. Using 1-chloro-2,4-dinitrobenzene as a secondary substrate, however, Baars, *et al.*,[19] also have shown that most tissues of the same type from rats and humans have comparable GST activities except that rat liver and testes have 7- and 3-fold higher activity, respectively, and rat muscle had 10-fold lower activity than human tissues. Therefore, results from the rat animal model will still be directly relevant to man in terms of dose response and interaction with DSF. DSF might be expected to affect the thioether assay because DSF produces a neutral thio-compound (DDC-glucuronide) as its major metabolite. Whether the excretion is additive or non-additive is important for biological monitoring purposes when workers are exposed to mixtures of chemicals.

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, Mich.) weighing 130 to 145 g were quarantined on receipt for ten days while they were screened for viruses and mycoplasmas, housed in groups of five in stainless-steel cages and fed Purina rat chow. At the end of the quarantine period, animals were weighed, placed in individual cages according to a table of random numbers and reassigned to a test group.

Immediately following test group assignments, all animals were placed on powdered AIN-76 semisynthetic diet (Zeigler Brothers, Gardner, Pa.), including those with 0.15% DSF incorporated into their feed. EDC inhalation exposures were initiated after 10 days of feeding. Animal body weights were recorded twice per week during the first 2 weeks of exposure and once weekly thereafter. Food and DSF consumption per rat were recorded weekly. The EDC-exposed and control animals were permitted water and diet *ad libitum* except during the inhalation exposure periods. The light cycle in the animal holding area was 12 hr fluorescent white light-dark cycle. Temperature and relative humidity (RH) were recorded twice daily in the animal holding area and ranged from 20-24°C and 40-65%, respectively. Circulating room air changed 12-15 times per hour. Animal cages were changed once every 2 weeks; soiled cages were sanitized in an industrial cage washer at approximately 100°C. During the course of the study, all animals were observed twice daily (at the beginning and end of the exposure period) for signs of toxicity, morbidity and mortality. The rats (12 per group) were placed on AIN-76 diets with and without 0.15% DSF and exposed to EDC vapor [0; 153 ± 3; 304 ± 5; and 455 ± 7 ppm (mean ± S.D.)] for 7 hr/day, 5 days/week for the following 30 exposure days (Table I). The exposure conditions are described in detail elsewhere.[2] Because of lack of inhalation chambers, only one EDC concentration during a 30-day exposure period could be generated. Therefore, each period had its own control, DSF only, and EDC only exposed group, in addition to the interaction group.

Urine samples were collected at the end of EDC exposure days 1, 7, 12, 17, 22 and 30, each over a 17-hour period. The EDC exposure days also corresponded to the number of times the animals had been exposed to EDC (or air for EDC and DSF controls) at the stipulated target concentration. At the end of each exposure day, the chambers were purged with clean air for 1 hr to remove residual EDC. Immediately following removal from the exposure chambers on each sampling day, four rats from each group were placed in individual stainless-steel metabolism cages to facilitate individual collection of urine samples in 118-mL graduated screw-cap containers (Fisher Scientific Company, Pittsburgh, Pa), the same animals being sampled at each ensuing collection period. The initial animal selection was done randomly. This was necessary because of limited numbers of metabolic cages at the authors' disposal. The rest of the animals were placed in their animal holding area under the same conditions as specified after quarantine and as for the animals being monitored in the metabolic cages. A stainless-steel flap fitted into the funnel-shaped bottom end of each metabolic cage plus a plug of glass wool, prevented contamination of the urine samples with feces and feed droppings. The animals were supplied with water in individual water bottles, and feeding cups were transferred from the rats' regular cages to the metabolism cages. At the end of each collection period, individual cages and water bottles were washed with an industrial type disinfectant, rinsed in copious amounts of hot water and air dried.

TABLE I
Concentrations of Urinary Thio-Compounds Extractable in Ethyl Acetate and Then Hydrolysed in Alkali (Urinary Thio-Compounds) on Indicated Days After Exposure to EDC (7 Hrs/Day, 5 Days/Week) Over a 30-Day Period and Combined with Prior and Concurrent Dietary 0.15% DSF

Treatment Group	Urinary Thio-Compounds Concentration[A,B,C] Time, Days					
	1	7	12	17	22	30[D]
I. Air Control	5.1 ± 1.4	2.5 ± 1.5	2.4 ± 1.5	6.6 ± 1.2	6.3 ± 1.6	2.2 ± 2.1
II. EDC [153 ppm]	11.9 ± 1.2	11.7 ± 1.3	12.6 ± 1.4	18.7 ± 1.2	21.9 ± 1.1	18.7 ± 1.1
III. EDC [304 ppm]	12.3 ± 1.2	15.3 ± 1.2[E]	24.0 ± 1.1[E]	22.4 ± 1.2[E]	33.8 ± 1.2[E]	19.1 ± 1.2
IV. EDC [455 ppm]	20.4 ± 1.3[E]	20.7 ± 1.3[E]	23.6 ± 1.4[E]	31.8 ± 1.5[E]	27.6 ± 1.4[E]	24.3 ± 1.2[E]
V. DSF Control [0.15%]	222 ± 1	233 ± 1	220 ± 1	277 ± 1	278 ± 1	286 ± 1
VI. DSF + EDC [153 ppm]	180 ± 1[F]	217 ± 1[F]	218 ± 1	211 ± 1[F]	232 ± 1[F]	251 ± 1[F]
VII. DSF + EDC [304 ppm]	80 ± 1[F]	90 ± 1[F]	120 ± 1[F]	149 ± 1[F]	143 ± 1[F]	140 ± 1[F]
VIII. DSF + EDC [455 ppm]	61 ± 1[F]	68 ± 1[F]	95 ± 1[F]	100 ± 1[F]	109 ± 1[F]	111 ± 1[F]

[A]Values are geometric mean ± GSD of the urine collected from nine rats for Groups I and V, and four rats for all the other groups over a 17-hr period following the termination of EDC exposure for the stipulated day.

[B]In units of μmol SH equivalents/mmol creatinine.

[C]The values for EDC- or DSF-treated animals are all statistically significant relative to the appropriate air control at $p \leq 0.05$ using either Scheffe's test or one-way ANOVA.[22]

[D]Indicates days on which urine samples were collected from individual rats at the end of EDC exposure.

[E]Indicates $p \leq 0.05$ relative to the lowest EDC dose (153 ppm) on each exposure day (for EDC and DSF/EDC exposures) by Scheffe's Test or one-way ANOVA.[22]

[F]Indicates $p \leq 0.05$ relative to DSF alone on each exposure day by Scheffe's Test or one-way ANOVA.[22]

After recording the individual rat urine volumes, colors and specific gravities — measured with a hand-held Clinical Refractometer (National Instrument Co., Inc., Baltimore, Md.) — collected urine samples were stored at -40°C until required for analysis. Aliquots also were taken on the day of urine collection for the determination of creatinine concentration using the alkaline picrate method[20] and external standards. In a preliminary study, it was shown that urine storage for up to 9 months at -20°C did not affect the thioether concentration.

Chemicals

From Sigma Chemical Co., St. Louis, Mo., 5,5'-dithiobis-(2-nitrobenzoic acid) [DTNB, Ellman's Reagent]; thiodiacetic acid [thioglycolic acid]; and GSH were purchased. Creatinine and picric acid for the creatinine assay, Pesticide Grade EDC and ethyl acetate were obtained from Fisher Scientific Co., Pittsburgh, Pa. DSF originated from the Aldrich Chemical Co., Milwaukee, Wis. and was purified.[2] The purity of EDC and DSF used has been described.[2]

Thio-Compound Assay

The assay is the thioether assay as described by Van Doorn, *et al.*[4] It consists of the extraction of neutral and protonatable organics by ethyl acetate from an acidified saturated salt solution of urine, thus eliminating interference by such amino acids as cysteine, cystine, methionine and peptides. Free thiols, thiocarboxylic acids, thiol esters and thioethers are all extracted. The difference in -SH content before and after alkaline hydrolysis is related to hydrolysable thioethers and thiol esters. The units are expressed in -SH equivalents/mmol creatinine.

When frozen urine samples were required for analyses, they were thawed; 4-mL aliquots were transferred into Kimax® borosilicate culture tubes, stoppered, and centrifuged for 10 min at 2000 g (Sorvall Superspeed RC2-B centrifuge). Two mL samples of the supernatant then were transferred to Teflon® screw-capped glass tubes (2 per sample) and the pH adjusted to 1.0-1.5 with 0.2 mL 4N HCl; these samples then were left at room temperature for 15 min. Sodium chloride (0.5-1.0 g) was added, then 6 mL of ethyl acetate, followed by extraction for 30 min on a rotorack (Fisher Hematology/Chemistry Mixer, Model 346). The organic and the aqueous layers were centrifuged at 5000 g for 5 min before the organic layer was carefully separated (Pasteur pipet). The aqueous layer was again extracted for 20 min and the two organic extracts combined; anhydrous sodium sulfate (1 g) was added, and the solution evaporated to dryness at 30°C under vacuum using a rotary evaporator.

TABLE II
Correlations (as r, the Correlation Coefficient) Between Ethyl Acetate Extractable Alkali Hydrolyzable Thio-Compounds (y), in μmol SH/mmol Creatinine, in the Presence and Absence of 0.15% Disulfiram (DSF) in the AIN-76 Diet when Doses of 1,2-Dichloroethane (x in ppm)[A] Are Increased

Exposure Day	No DSF		0.15% DSF	
	Regression Equation[A]	r	Regression Equation[A]	r
1	y = 0.031x + 5.48	0.96	y = -0.39x + 223	-0.97
7	y = 0.039x + 3.82	0.98	y = -0.41x + 245	-0.94
12	y = 0.050x + 441	0.94	y = -0.32x + 234	-0.94
17	y = 0.053x + 7.98	0.98	y = -0.40x + 273	-1.00
22	y = 0.044x + 10.03	0.91	y = -0.40x + 280	-0.99
30	y = 0.045x + 6.00	0.90	y = -0.42x + 293	-0.97

[A]x is 0, 153, 304 and 455 ppm (v/v).

The residue was taken-up in four 0.25 mL aliquots of distilled water. Alkaline hydrolysis was performed on 0.5 mL samples in borosilicate glass tubes with Teflon-lined screw caps by the addition of 0.25 mL of 4N NaOH, heating the closed tubes in a boiling water bath for 50 min, and then cooling for 10 min in a slush of sodium chloride and ice.

Then 4N HCl (0.25 mL) was added to each tube and thoroughly mixed to neutralize the 4N NaOH; 5 min later, 0.2 mL aliquots in duplicate were transferred into clean glass tubes and sulfhydryl concentrations determined according to the method of Ellman,[21] with a slight modification. A 2.8 mL solution of Ellman's reagent (DTNB), 0.33 mM in 0.1 M sodium dihydrogen phosphate and 0.001 M disodium EDTA, pH 8.0, were added to each tube to give a total volume of 3.0 mL. Absorbances were read at 412 nm in a 1 cm cell. The concentration of SH-groups was calculated from the corrected absorbances using a molar absorptivity of 13.98 mM/cm previously verified from a calibration curve using GSH. The SH content also was assayed without NaOH hydrolysis, and the difference before and after alkaline hydrolysis represented the ethyl acetate extractable alkali hydrolyzable thio-compound concentration. Corrections were made for the contribution of the sample and DTNB to the absorbance. The concentration was expressed in μmol SH/mmol creatinine. The detection limit was approximately 4 μM.

The recovery of thiodiacetic acid, a major metabolite of EDC, from a buffer system was also studied. Six concentrations (0.01 mM - 1.0 mM) in 0.1 M phosphate buffer, pH 7.5, were assayed and the thioether content calculated. The comparative efficiencies of thioether extraction using methyl and ethyl acetate and dichloromethane also were evaluated using five extractions.

Statistical Methods

One-way analysis of variance (ANOVA) as contained by the Statistical Analysis System (SAS) program was employed. Means were compared by Scheffe's test of multiple comparison,[22] with a $p \leq 0.05$ as the criterion of significance.

Results

Thio-Compounds Extraction Efficiency

The first two extractions of acidified urine samples with ethyl acetate recovered 95%-100% of the ethyl acetate extractables from EDC- and EDC/DSF-treated animals. Ethyl acetate also was shown to be 5 to 7 times more effective than dichloromethane or methyl acetate; five to six extraction steps were necessary to equal the efficiency of two extractions with ethyl acetate. The recovery of thiodiacetic acid was 95 ± 8% (mean ± SD, n = 6). This showed that negligible oxidation of thiols occurred with this method since GSH at room temperature was the reference thiol employed in this estimation. The same results were obtained for thiodiacetic acid subjected to just the alkaline hydrolysis step. A time study revealed an optimum reaction time of 1 hr to allow complete alkaline hydrolysis with negligible oxida-

TABLE III
Correlations Between Urinary Thio-Compounds Extractable by Ethyl Acetate and Then Alkali Hydrolyzable, and Other Indicators of EDC/DSF and EDC Exposure at Day 30 of EDC Exposure

Parameter[A]	Correlation Coefficient	
	EDC/DSF[B]	EDC Alone[B]
EDC-exposure concentration	-0.94	+0.81
Liver weight	-0.91	+0.95
Liver GSH	-0.97	+0.61
Liver cytochrome P_{450}	+0.85	-0.96
Serum SDH[C]	-0.97	+0.38
Serum APT[D]	-0.87	+0.29
Serum 5'-NT[E]	-0.72	+0.28

[A]The methods for determination of all parameters can be found in reference 3, except for EDC exposure concentration and liver weight (reference 2).

[B]Correlation coefficients are either positive or negative.

[C]Sorbitol dehydrogenase.

[D]Alkaline phosphatase.

[E]5'-Nucleotidase.

tion of the thiols. Lower control values (approximately half) were found than those in previous studies in rats,[18,23] possibly because of the use of AIN-76 diet instead of Chow diets. There were no significant differences in urine specific gravity or color between exposed and non-exposed animals.

Kinetics of Urinary Excretion of Ethyl Acetate Extractable Alkali Hydrolyzable Thio-Compounds

Table I shows the concentrations of urinary ethyl acetate extractable alkali hydrolyzable thio-compounds ("urinary thio-compounds") uncorrected for control values and expressed as μmol-SH/mmol creatinine over the collection time period. The day designations for groups II, III, IV, VI, VII and VIII also correspond to the number of times the animals were exposed to EDC. For the groups with DSF in their diets, the first urine samples were collected after 10 days of dietary DSF exposure, *i.e.,* on the first day of EDC exposure.

The creatinine corrected urinary thio-compound values are reported as geometric mean (GM) ± geometric standard deviation (GSD) because the data followed a log normal distribution as shown by Chi-square test.[22] Relative GSDs generally were less than 2% of the GMs (Table I), except for the air-control (group I) where levels were near the detection limit.

The groups treated with EDC alone, at different concentrations, generally showed parallel excretion patterns throughout the exposure period. The thio-compound content and EDC exposure level were not as highly correlated on days 1, 12, 22 and 30 [r = 0.90 to 0.96 for these days (Table II)] as on days 7 and 17 (r = 0.98). Steady state urinary thio-compound excretion generally occurred for most EDC exposure levels by day 22. For the EDC/DSF combination groups, in contrast, the levels were inversely related to EDC concentration (r = −0.94 to −1.0). For groups VII and VIII, the excretion per unit time (in days) was small compared to group VI as EDC exposure continued, while for group VI the excretion rate approached that of DSF alone (group V). This indicates that EDC inhibits the excretion of "thio-compounds" derived from DSF. The thio-compounds from DSF under these conditions far outweighed the contribution from EDC.

Discussion

Not only does DSF affect urinary excretion of EDC-derived compounds, as shown by ^{14}C-radiolabel studies,[1] EDC also affects the elimination of urinary thio-compounds derived from DSF as revealed by the decreased thio-compounds excreted by the EDC/DSF groups with increasing EDC concentration. Urinary biological monitoring is feasible for EDC alone. Since the EDC levels used[2] in this study were equivalent to dosages of 98, 194 and 291 mg/kg/day for exposures at 153, 304 and 455 ppm, respectively, the highest two EDC doses may cause saturation kinetics, as noted before for female mice.[11] This sometimes occurred (days 12 and 22), and there appears to be a complex time-dependence not describable by any published toxicodynamic model. More research is required in this area.

The urinary thio-compounds derived from DSF do not arise from endogenous GSH, unlike those from EDC alone. In fact, the thiolesters from DSF, which are extractable in ethyl acetate, are hydrolyzed at the alkaline hydrolysis stage to thiols which are then detected by the Ellman reaction. This confounds the classic test for thioethers[4] but does enable quantitation of all the major metabolites of both EDC and DSF.

The decreased urinary thio-compounds observed for the EDC/DSF groups might be caused by a number of time-dependent factors. One factor is compromised hepatobiliary and/or kidney excretory functions; bile dust proliferation was noted in these animals at day 30;[2] and a marker of hepatobiliary system damage, serum 5′-nucleotidase, was increased.[3] There was no kidney pathology[2] or change in urinary creatinine concentrations in this study.

Another factor could be DSF inhibition or saturation of a microsome-dependent reaction of the glutathione pathway. Both EDC and DSF decrease cytochrome P-450 at day 30.[3] The cysteine S-conjugate N-acetyltransferase[24] or the enzymes of the thiomethyl shunt pathway[24] — particularly cysteine conjugate beta-lyase (S-1,2-dichlorovinyl-L-cysteine is a known substrate) and the thiol, S-methyltransferase (for which DDC is a known substrate) — may be affected by DSF, leading to the accumulation of bioactive glutathione conjugates or reactive intermediates derived from EDC and/or DSF. All the GSTs apart from GST-E (GST-5-5) were not affected by EDC in the presence of DSF.[3]

Increased shunting of EDC-derived thio-compounds to the feces or fat was not enough to account for the lengthened ^{14}C-EDC half-time.[1] The appearance of enhanced EDC in rat breath in the presence of DSF,[25] however, indicates an inhibition of the early steps of EDC detoxification.

Table III shows the correlations between the geometric means of the urinary thio-compound values and the mean values of invasive biological monitoring parameters related to adverse effects at day 30 of exposure. The adverse effects have been discussed already elsewhere.[2,3] For EDC/DSF, the exposure parameters most highly correlated with urinary thio-compound level were, in order, serum sorbitol dehydrogenase (SDH) = liver GSH, EDC concentration, liver weight, alkaline phosphatase (APT), cytochrome P_{450} and 5′-nucleotidase (5′-NT) — all except 5′-NT exceeding an r value of 0.85. Recent work implies that the accumulation of 5′-NT and APT (as well as acetylcholinesterase) in blood could be related to the attack on liver cell membrane surface proteins anchored by phosphatidylinositol sensitive to phospholipase C.[26] In contrast, the only parameters correlating above a value of 0.80 for EDC alone were cytochrome P_{450}, liver weight and EDC concentration, in descending order. The high correlations with both treatments for these parameters indicate that effects on liver function may account for the urinary effects and that an early microsomal dependent step in the glutathione elimination pathway is definitely involved in the interaction. More research is required to determine which specific enzyme systems are implicated.

Selective monitoring of the urinary metabolites of EDC (namely thiodiacetic acid, thiodiacetic acid sulfoxide, or carboxymethylcysteine) during administration of DSF also might provide further information on how EDC and DSF interact, at least in this 2-component system. DSF has been shown[27] to increase the toxicity and carcinogenicity of the bromine analog (1,2-dibromoethane; EDB), and the major metabolite of EDB, N-acetyl-S-2-hydroxyethyl-L-cysteine, is greatly decreased in the urine of EDB/DSF-treated SPF Wistar rats relative to that of rats treated with EDB alone.[28] DSF also reduced the urinary excretion of ^{14}C-label from ^{14}C-EDC compared with EDC alone.[1] If this also is observed for specific EDC metabolites, as seems likely, biological monitoring for specific EDC urinary metabolites also would be as qualitative as monitoring total urinary thiocompounds and would not be related to the exposure or absorbed EDC doses.

For the workplace, the specific metabolite approach for EDC is complicated by other organics whose major metabolites are the same: namely, vinyl chloride;[29-31] *bis*-(2-chloroethyl)ether;[32] and 1,1,2-trichloroethane.[33] Moreover, S-carboxymethyl-L-cysteine used as a mucolytic agent in Europe as supportive therapy in respiratory disorders is metabolized to thiodiacetic acid[34] and also may interfere. Alcohol intake has been found to increase the urinary excretion of thiodiacetic acid.[31] A minor metabolite of EDC, S-carboxymethyl-L-cysteine has been demonstrated to accumulate in animals exposed to over 500 ppm vinyl chloride.[30] Thus, a thorough knowledge of the chemicals used in a particular industrial setting is necessary to be able to interpret results. Knowledge of diets and drug intake is required also and knowledge of interactions, *e.g.*, drugs such as barbital,[35] warfarin,[36] phenytoin,[37] metronidazole,[38] morphine [where biliary excretion was inhibited also[39]], nitrosodiethylamine,[40] azomethane,[41] and antipyrine[42] whose urinary excretion is inhibited by DSF.

Thus, measurement of specific urinary metabolites or the thio-compound content may need to be supplemented by other biochemical monitoring, for example, serum activities of 5'-NT, SDH, APT or any other parameter, like specific DNA adducts, that indicates organ damage. This implies that monitoring for adverse effects or for parameters predictive of these events should be a major research goal. Nevertheless, the monitoring of urinary thioethers still appears to be useful for screening purposes in situations where there are no simultaneous exposures to compounds capable of confounding the assay.

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