The Effects of Diethyldithiocarbamate and Carbon Disulfide on Acute Nephrotoxicity Induced by Furan, Bromobenzene and Cephaloridine in Mice

Yasusuke MASUDA, Nobue NAKAYAMA, Atsuko YAMAGUCHI and Masatomo MUROHASHI
Department of Toxicology, Niigata College of Pharmacy, Niigata 950-21, Japan
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Abstract—In previous studies (Masuda and Nakayama, 1983), diethyldithiocarbamate (DTC) and carbon disulfide (CS₂) have been found to be protective against acute nephrotoxicity induced by CHCl₃ and 1,1-dichloroethylene in normal and CCl₄-poisoned mice, and it has been suggested that the protective action of DTC and CS₂ might be mediated through inhibition of bioactivation of these nephrotoxicants in the kidney. As an extension of these studies, similar experiments were undertaken with furan, bromobenzene and cephaloridine, other nephrotoxic agents that are also thought to require metabolic activation. DTC or CS₂ prevented mice from suffering renal injury induced by furan and bromobenzene, as evidenced by suppression of elevations in plasma urea nitrogen concentration and kidney calcium content and of morphologic alterations. Cephaloridine nephrotoxicity, however, was not prevented. In CCl₄-poisoned mice, furan nephrotoxicity was augmented, whereas bromobenzene and cephaloridine nephrotoxicity was suppressed. The augmented furan nephrotoxicity was also prevented by DTC or CS₂. These observations suggest that furan, like CHCl₃ and 1,1-dichloroethylene, may exert nephrotoxicity through active metabolites formed in the kidney. For bromobenzene and cephaloridine nephrotoxicity, a renal bioactivation mechanism is suspected. DTC and CS₂ also protected against hepatotoxicity induced by furan and thiophene.

As we have previously reported, diethyldithiocarbamate (DTC) and carbon disulfide (CS₂) protected mice against liver injury induced by a variety of hepatotoxicants that require metabolic activation by the liver microsomal monooxygenase system (1). Both agents also prevented renal injury induced by CHCl₃ and 1,1-dichloroethylene (1,1-DCE) (2, 3). Since both agents inhibited microsomal drug metabolizing enzyme activities in the liver and kidney in these studies, we suggested that the protective action may be due to inhibition of the metabolic activation of these hepato- and nephrotoxicants.

As an extension of these studies, we examined whether DTC and CS₂ could suppress renal injury induced by furan, bromobenzene and cephaloridine.

In general, the nephrotoxicity of these nephrotoxicants including CHCl₃ and 1,1-DCE is considered to be mediated through active metabolites formed by the microsomal monooxygenase system (4–9). However, it is not always clear whether active metabolites are formed in the kidney per se or if they are formed in the liver and transported to the kidney. Recently, Breen et al. (10) showed that metabolites of acetaminophen covalently bound to kidney proteins in totally hepatectomized rats, although the relationship between the covalent binding and renal injury has yet to be determined.

In an attempt to check the involvement of the liver in the development of nephrotoxicity, we used CCl₄-poisoned mice (0.2 ml/kg, i.p., pretreated 24 hr earlier), in which severe hepatic cell necrosis accompanied by a marked loss of microsomal drug metabolizing enzymes is induced. The results showed that furan nephrotoxicity was not prevented by DTC or CS₂, whereas bromobenzene nephrotoxicity was suppressed. Cephaloridine nephrotoxicity, however, was not prevented. This suggests that bromobenzene nephrotoxicity is mediated through active metabolites formed in the kidney.

In conclusion, DTC and CS₂ are protective against acute renal injury induced by furan and bromobenzene, and they also protect against hepatotoxicity induced by furan and thiophene. Further studies are needed to clarify the mechanism of protection and the role of the liver in the development of nephrotoxicity.
enzyme activities was produced, whereas the kidney was normal as judged by biochemical and histopathologic assays, although some drug metabolizing enzyme activities of the kidney microsomal fraction were increased (2). In such CCl₄-poisoned mice, the nephrotoxicity of CHCl₃ and 1,1-DCE was markedly potentiated. This action was also blocked by DTC and CS₂, as reported previously (2, 3). These observations suggest that nephrotoxicity of CHCl₃ and 1,1-DCE may be attributed to their metabolic activation by the kidney microsomal monoxygenase system.

In the present study, by determining the possible protective action of DTC and CS₂ against other nephrotoxicants such as furan, bromobenzene and cephaloridine in normal and CCl₄-poisoned mice, we wished to deduce indirectly if a renal bioactivation mechanism could be applied to these nephrotoxicants.

The degree of renal injury was assessed by plasma urea concentration and histologic examination. Kidney calcium content was also measured since an increase in tissue calcium content is postulated to be associated with cell necrosis, especially in the liver injury induced by various hepatotoxicants (1, 11-13) and under an ischemic condition (14), and in addition, CHCl₃ and 1,1-DCE have been shown to increase calcium content in the kidney (2, 3). Hepatic damage was also examined in parallel.

Materials and Methods

Chemicals: The purity and sources of the tested compounds were as follows: furan (>95%, GC), bromobenzene (>98%, GC), thiophene (>98%, GC), sodium diethylthiocarbamate trihydrate (DTC) (99.5%, nonaqueous titration) and CS₂ (>99%, GC) (from Wako Pure Chemicals), and cephaloridine (Ceporan, from Japan Glaxo). Other chemicals were of reagent grade.

Animals and treatments: For experiments with furan and thiophene, SPF-grade male ddY mice, 7 weeks of age, purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals and housed in an air-conditioned animal room for at least 1 week, were used. Similar male C57BL/6 mice were used for the experiments with bromobenzene and cephaloridine since this strain was more sensitive to the nephrotoxic action of these agents. Food (MF cubic diet, Oriental Yeast) and water were given ad libitum throughout the experiment period.

The animals were sacrificed 24 hr after i.p. administration of furan (0.2 ml/kg), bromobenzene (1.0 ml/kg) and thiophene (0.5 ml/kg) dissolved in olive oil, and cephaloridine (2.0 g/kg, in distilled water). DTC (30 to 500 mg/kg, in distilled water) and nearly equimolar doses of CS₂ (10 to 170 mg/kg, in olive oil) were given p.o. 1 hr before the administration of nephrotoxicants. CCl₄-poisoned mice had received CCl₄ (0.2 ml/kg, i.p., in olive oil) 24 hr prior to the nephrotoxicant administration. All solutions were freshly prepared and given at a volume of 0.05 ml/10 g of body weight. Any changes of the treatment are described in the legend.

Biochemical assays: The mice were decapitated following cervical dislocation, the blood was rapidly collected in a heparinized syringe and the plasma was separated by centrifugation. Plasma urea nitrogen concentration was determined by the clinical urease-indophenol method originally developed by Searcy and Cox (15). Plasma glutamate pyruvate transaminase (GPT) activity was assayed according to the method of Reitman and Frankel (16). For determination of tissue calcium content, the left whole kidney or a portion of the liver was completely sonicated in 8% TCA, and the TCA extract was titrated with EDTA using calcein as an indicator (17).

Statistical analysis of the data was undertaken by Student's t-test for significance of the differences, and P<0.05 was considered significant.

Histopathologic examination: The same animals that were used for biochemical assays were examined histopathologically. The whole right kidney and a portion of the median lobe of the liver were fixed in 10% neutralized formalin, embedded in paraffin, cut in 4-μm slices and stained with hematoxylin and eosin.

Results

Furan: As shown in Table 1 and Fig. 1,
furan (0.2 ml/kg) markedly increased plasma urea nitrogen concentration and kidney calcium content; and histopathologically, severe coagulation necrosis of tubular cells of the kidney cortex, tubular precipitate and slight atrophy of glomeruli were observed, while medullary cells appeared normal. Plasma GPT activity and liver calcium content

Table 1. Protective effects of DTC and CS₂ against renal and hepatic injury induced by furan in normal mice

| Treatment (n) | Kidney | Liver |
|--------------|--------|-------|
|              | Plasma urea (mg N/dl) | Kidney calcium (µmol/g) | Plasma GPT (Karmen unit) | Liver calcium (µmol/g) |
| Control (6)  | 21.8±2.8* | 1.35±0.10 | 20±9 | 0.89±0.07 |
| Furan (0.2 ml/kg) (8) | 107.0±26.5*# | 4.91±1.70*# | 2410±1030*# | 11.02±5.78*# |
| DTC (30 mg/kg)+Furan (6) | 36.3±38.3* | 2.81±1.71** | 3920±3040 | 15.17±11.35 |
| DTC (100 mg/kg)+Furan (6) | 18.6±3.5* | 1.52±0.20* | 49±56* | 0.97±0.13* |
| CS₂ (10 mg/kg)+Furan (6) | 48.0±32.8* | 4.22±2.44* | 714±1460** | 1.44±0.77* |
| CS₂ (30 mg/kg)+Furan (6) | 19.0±3.1* | 1.60±0.59* | 26±13* | 1.04±0.09* |

Mice were killed 24 hr after i.p. administration of furan. DTC or CS₂ was administered p.o. 1 hr before furan. Each value represents the mean±S.D. *Significantly higher than the control group at P<0.01. **Significantly lower than the group given furan alone at *P<0.01 and **P<0.05.

Fig. 1. Prevention of furan-induced renal and hepatic necrosis by DTC or CS₂. Refer to the legend of Table 1. Upper panel: kidney. Lower panel: liver. (1-A, E) Control. (1-B, F) Furan (0.2 ml/kg). (1-C, G) DTC (100 mg/kg)+furan. (1-D, H) CS₂ (30 mg/kg)+furan. Magnification: ×68.
were also elevated, accompanied by massive coagulation necrosis of centrilobular parenchymal cells. All of these biochemical and morphologic alterations in the kidney and liver were prevented by pretreatment with DTC (100 mg/kg) or CS₂ (30 mg/kg). Lower doses of DTC and CS₂ were less effective.

In CCl₄-poisoned mice, nephrotoxicity by a lower dose (0.1 ml/kg) of furan was significantly augmented as judged by plasma urea nitrogen and kidney calcium levels (Table 2, left) and by histologic examination (data not shown). This augmented furan nephrotoxicity in CCl₄-poisoned mice was also suppressed by pretreatment with DTC or CS₂.

**Bromobenzene:** DTC and CS₂ completely prevented bromobenzene-induced nephrotoxicity as evidenced by suppression of

### Table 2. Augmentation of furan nephrotoxicity in CCl₄-poisoned mice and its suppression by DTC or CS₂

| Treatment (n) | Plasma urea (mgN/dl) | Kidney calcium (µmol/g) | Plasma GPT (karmen unit) | Liver calcium (µmol/g) |
|--------------|----------------------|-------------------------|--------------------------|------------------------|
| Control (7)  | 20.4±3.3             | 1.39±0.05               | 42±13                    | 0.95±0.04              |
| CCl₄ (0.2 ml/kg, 24 hr) (7) | 19.9±1.8 | 1.39±0.09               | 5150±1080                | 27.3±2.5               |
| CCl₄ (0.2 ml/kg, 48 hr) (7) | 18.5±2.6 | 1.36±0.06               | 189±25                   | 42.6±33.8              |
| Furan (0.1 ml/kg) alone (14) | 16.0±2.2 | 1.66±0.26               | 4260±2120                | 15.7±5.8               |
| CCl₄ (24 hr) + Furan (13/14)* | 47.2±44.5** | 2.79±1.35** | 349±168 | 28.9±21.8 |
| CCl₄ (24 hr) + DTC (100 mg/kg) + Furan (14) | 16.7±1.7 | 1.37±0.13 | 313±212 | 34.1±24.2 |
| CCl₄ (24 hr) + CS₂ (30 mg/kg) + Furan (14) | 18.0±4.0 | 1.36±0.09 | 254±120 | 26.6±20.0 |

Mice were pretreated with CCl₄ (0.2 ml/kg, i.p.) 24 hr before administration of furan (0.1 ml/kg, i.p.). DTC or CS₂ was given p.o. 1 hr before furan, and mice were killed 24 hr after furan administration. Each value represents the mean±S.D. *Significantly higher than the groups given furan alone (no CCl₄ pretreatment) and pretreated with DTC or CS₂ at P<0.01 and **P<0.05. *Number of surviving mice.

### Table 3. Protective effects of DTC, CS₂ and CCl₄-induced liver damage against bromobenzene nephrotoxicity in C57BL/6 mice

| Treatment (n) | Plasma urea (mgN/dl) | Kidney calcium (µmol/g) | Plasma GPT (karmen unit) |
|--------------|----------------------|-------------------------|--------------------------|
| Control (8)  | 20.9±1.8             | 1.31±0.11               | 24±8                     |
| Bromobenzene (1.0 ml/kg) (10) | 57.7±27.2* | 2.88±1.00* | 4060±3230* |
| DTC (100 mg/kg) + Bromobenzene (5) | 32.0±9.4 | 1.39±0.09* | 2040±4380 |
| DTC (300 mg/kg) + Bromobenzene (5) | 23.7±3.4** | 1.33±0.13* | 64±44** |
| CS₂ (30 mg/kg) + Bromobenzene (5) | 19.7±2.6* | 1.35±0.12* | 47±16** |
| CS₂ (100 mg/kg) + Bromobenzene (5) | 23.0±3.5** | 1.32±0.07* | 57±39** |
| CCl₄ (0.2 ml/kg, 24 hr) (4) | 27.5±1.8 | 1.39±0.06 | 8940±1650 |
| CCl₄ (0.2 ml/kg, 48 hr) (4) | 20.5±2.1 | 1.22±0.08 | 1290±630 |
| CCl₄ (24 hr) + Bromobenzene (7) | 25.5±3.5* | 1.27±0.10* | 5420±870 |

Mice were killed 24 hr after i.p. administration of bromobenzene. DTC or CS₂ was given p.o. 1 hr before bromobenzene, and CCl₄ was given 24 hr before bromobenzene. Each value represents the mean±S.D. *Significantly higher than the control at P<0.01. **Significantly lower than the group given bromobenzene alone at *P<0.01 and **P<0.05.
increases in plasma urea nitrogen concentration and kidney calcium content (Table 3). Histologically, bromobenzene produced coagulation necrosis of tubular cells and marked dilatation of tubules occasionally filled with acidophilic casts, which were prevented by pretreatment with DTC or CS₂ (data not shown). The hepatoprotective action of DTC and CS₂ reported in a previous paper (1) was also confirmed.

In CCl₄-poisoned mice, unlike with furan, bromobenzene nephrotoxicity was suppressed biochemically (Table 3, bottom) and morphologically (data not shown).

Cephaloridine: A rather high dose (2.0 g/kg) of cephaloridine was required to produce constant renal damage. Increases in plasma urea nitrogen and kidney calcium levels were small, but significant (Table 4) and morphologic alterations such as dilatation and coagulation necrosis of renal tubules, were quite obvious (Fig. 2-A). No hepatic injury was observed. Unlike with the former two nephrotoxicants, even a high dose of DTC (500 mg/kg) or CS₂ (170 mg/kg) did not prevent cephaloridine nephrotoxicity biochemically (Table 4) or morphologically (data not shown).

In CCl₄-poisoned mice, the nephrotoxicity of cephaloridine was significantly suppressed (Table 4, bottom), and this was confirmed morphologically (Fig. 2-B).

Since epoxidation of the thiophene ring of the drug is suggested to be a cause of cephaloridine nephrotoxicity (6), effects of DTC and CS₂ on thiophene toxicity were examined. Thiophene (0.5 ml/kg, 1.3 times the equimolar dose of 2.0 g/kg of cephaloridine), however, had no significant effect on the biochemical parameters of the kidney, but produced hepatic injury as evidenced by increases in plasma GPT and liver calcium and by massive centrilobular coagulation

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**Table 4.** Effects of DTC, CS₂ and CCl₄-induced liver injury on cephaloridine nephrotoxicity in C57BL/6 mice

| Treatment (n) | Plasma urea (mg N/dl) | Kidney calcium (μmol/g) | Liver Plasma GPT (Karmen unit) |
|--------------|------------------------|-------------------------|-------------------------------|
| Control (7)  | 23.6±2.7               | 1.36±0.13               | 50±12                         |
| Cephaloridine (2.0 g/kg) (8) | 34.5±3.8*             | 2.08±0.22**            | 65±12                         |
| DTC (100 mg/kg) + Cephaloridine (4) | 32.4±5.2             | 1.80±0.37              | 67±17                         |
| DTC (500 mg/kg) + Cephaloridine (4) | 31.3±7.3             | 1.75±0.08**            | 69±6                          |
| CS₂ (30 mg/kg) + Cephaloridine (4) | 29.0±2.0**            | 1.84±0.21              | 62±3                          |
| CS₂ (170 mg/kg) + Cephaloridine (4) | 30.1±1.5             | 2.06±0.07              | 69±10                         |
| Cephaloridine (2.0 g/kg) (10) | 42.0±17.9             | 2.19±0.48              | 64±12                         |
| CCl₄ (0.2 ml/kg, 24 hr) + Cephaloridine (10) | 21.9±5.7*            | 1.40±0.16*             | 1470±770                      |

Mice were killed 24 hr after i.p. administration of cephaloridine. DTC or CS₂ was given p.o. 1 hr before cephaloridine, and CS₂ was given 24 hr before cephaloridine. Each value represents the mean±S.D. *Significantly higher than the control at *P<0.01 and **P<0.02. **Significantly lower than the group given cephaloridine alone at *P<0.01 and **P<0.05.
necrosis accompanying pyknosis and karyorrhexis, all of these biochemical and morphologic alterations being completely prevented by DTC or CS$_2$ (Table 5 and Fig. 3).

**Table 5. Protective effects of DTC and CS$_2$ against hepatic injury induced by thiophene in mice**

| Treatment (n)                  | Plasma GPT (Karmen unit) | Liver calcium (μmol/g) | Plasma urea (mgN/dl) | Kidney calcium (μmol/g) |
|-------------------------------|--------------------------|------------------------|----------------------|------------------------|
| Control (6)                   | 17±3                     | 0.90±0.03              | 19.6±3.9             | 1.15±0.10              |
| Thiophene (0.5 ml/kg) (7/10)* | 8631±2776*               | 4.58±1.36*             | 19.1±3.2             | 1.15±0.09              |
| DTC (30 mg/kg)+Thiophene (6)  | 78±143                   | 1.21±0.56              | 19.9±3.5             | 1.20±0.15              |
| DTC (100 mg/kg)+Thiophene (6) | 18±8                     | 0.89±0.10              | 15.7±1.5             | 1.16±0.01              |
| CS$_2$ (10 mg/kg)+Thiophene (5/6)* | 34±19                   | 1.09±0.18              | 24.8±5.8             | 1.31±0.10              |
| CS$_2$ (30 mg/kg)+Thiophene (6) | 20±10                    | 0.94±0.05              | 16.9±5.0             | 1.31±0.15              |

Mice were killed 24 hr after i.p. administration of thiophene. DTC or CS$_2$ was administered p.o. 1 hr before thiophene. Each value represents the mean±S.D. *Number of surviving mice. **Significantly higher than any other group at P<0.01.

### Fig. 3. Prevention of thiophene-induced hepatic necrosis by DTC or CS$_2$ Refer to the legend of Table 5. (3-A) Thiophene (0.5 ml/kg), (3-B) DTC (100 mg/kg)+thiophene, (3-C) CS$_2$ (30 mg/kg)+thiophene. Magnification: ×68.

necrosis accompanying pyknosis and karyorrhexis, all of these biochemical and morphologic alterations being completely prevented by DTC or CS$_2$ (Table 5 and Fig. 3).

### Discussion

The data obtained in the previous (2, 3) and present studies are summarized in Table 6.

Mitchell et al. (18) and McMurtry and Mitchell (6) found that renal and hepatic necrosis produced by various furan derivatives including furan itself was decreased by pretreatment with cobalt chloride or piperonyl butoxide, inhibitors of the microsomal mono-oxygenase system containing cytochrome P-450, and that there was a positive correlation between tissue necrosis and the covalent binding of toxic metabolites in each organ, suggesting that metabolic activation in the liver and kidney is responsible for necrosis in the respective organ.

In the present study, the nephrotoxicity of furan, like that of CHCl$_3$ and 1,1-DCE, was prevented by pretreatment with DTC or CS$_2$ in normal mice and also in CCl$_4$-poisoned mice in which the nephrotoxicity was augmented. The augmentation of the nephrotoxicity in CCl$_4$-poisoned mice may partly result from an increased renal concentration of the toxicant as a consequence of suppressed metabolism in the liver and partly from the elevated renal mono-oxygenase activity (2). The protective action of DTC and CS$_2$ against this augmented nephrotoxicity in CCl$_4$-poisoned mice suggests that DTC
and CS$_2$ may act on the kidney per se. Thus, these three nephrotoxicants may have a common basic nephrotoxic mechanism, i.e., they require metabolic activation by the microsomal monooxygenase system in the kidney per se, and this bioactivation may be inhibited by DTC or CS$_2$.

The protective action of DTC and CS$_2$ against hepatic necrosis may also be due to inhibition of metabolic activation of furan in the liver.

Bromobenzene nephrotoxicity was also prevented by DTC or CS$_2$ in normal mice. However, unlike with the former three nephrotoxicants, CCl$_4$-poisoning suppressed the bromobenzene renal injury. These observations suggest that bioactivation by the liver microsomal monooxygenase system may be a prerequisite for the development of the nephrotoxicity, which is in agreement with the conclusion of Reid (9) that bromobenzene renal necrosis is caused by a metabolite formed in the liver and transported by the circulation to binding sites in the renal tubules. It is still dubious, however, if unstable metabolites formed in the liver could be transported to the distant organ.

Concerning cephaloridine, McMurtry and Mitchell (6) observed that cobaltous chloride and piperonyl butoxide reduced the nephrotoxicity of this antibiotic in mice and rats and proposed that a reactive metabolite of cephaloridine may be responsible for the nephrotoxicity, although neither a reactive metabolite nor its covalent binding to kidney macromolecules has been demonstrated. According to the recent report by Tune et al. (19), however, cobalt did not prevent cephaloridine nephrotoxicity in rabbits, and they further showed that the prevention of nephrotoxicity by piperonyl butoxide might be due to a reduction of cephaloridine concentration in the renal cortex. Moreover, they reported that cephaloridine nephrotoxicity is mediated through an inhibitory effect on mitochondrial respiration (20). Thus, the bioactivation mechanism of cephaloridine nephrotoxicity is still controversial. In the present study, DTC and CS$_2$ did not prevent cephaloridine nephrotoxicity. This observation is not in favor of the renal bioactivation mechanism theory. It is unknown, however, why the nephrotoxicity was suppressed in CCl$_4$-poisoned mice. On the other hand, it is proposed that epoxidation of the thiophene ring of this antibiotic may be necessary for the nephrotoxicity (6). An approximately equimolar dose of thiophene, however, failed to produce renal injury, but did produce severe hepatic injury in accordance with the report of McMurtry and Mitchell (6). This hepatotoxicity was prevented by DTC or CS$_2$. These differences in the organ toxicity and its suppression by DTC or CS$_2$ observed between cephaloridine and thiophene suggest that the toxic mechanism of cephaloridine may be different from that of thiophene.

Finally, hitherto we have shown that DTC and CS$_2$, first of all, protect mice against a variety of hepatotoxicants, including furan and thiophene in this study, and three nephrotoxicants that require metabolic activation (1-3); secondly, prolong the sleeping time of hexobarbital (1), the paralysis time of zoxazolamine (1) and hypothermia induced by chlorpromazine (our unpublished data) by decreasing the

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### Table 6. Effects of DTC and CS$_2$ on renal injury induced by various nephrotoxicants in normal and CCl$_4$-poisoned mice (Summary)

| Nephrotoxicants | Pretreatment with DTC or CS$_2$ | CCl$_4$-poisoning alone | Pretreatment with DTC or CS$_2$ |
|-----------------|--------------------------------|-------------------------|--------------------------------|
| CHCl$_3$        | Suppressed                      | Augmented               | Suppressed                      |
| 1,1-DCE         | Suppressed                      | Augmented               | Suppressed                      |
| Furan           | Suppressed                      | Augmented               | Suppressed                      |
| Bromobenzene    | Suppressed                      | Suppressed              | Suppressed                      |
| Cephaloridine   | Not suppressed                  |                         |                                |

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in vivo metabolic rate of these drugs; and thirdly, inhibit microsomal drug metabolizing enzyme activities in the liver (1) and kidney (2). In addition, depression by CS\textsubscript{2} of liver microsomal drug metabolizing enzyme activity both in vivo and in vitro has also been reported by several investigators (21–23). The action of DTC may be mediated through CS\textsubscript{2}, particularly when given orally, as discussed previously (1). Thus, CS\textsubscript{2} itself and CS\textsubscript{2}-producing agents may modify the pharmacological and toxicological actions of many drugs and toxicants by inhibiting their metabolism in the microsomal mono-oxygenase system of various organs.

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