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Comparative metabolism and disposition of trichloroethylene in *Cyp2e1*-/- and Wild-Type Mice

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Abstract

Trichloroethylene (TCE)[1] is an important environmental contaminant, a well-established rodent carcinogen, and a "probable human carcinogen." Metabolism of TCE occurs primarily via cytochromes P450 (CYPs)-dependent oxidation. *In vitro* studies suggested that CYP2E1 is the principal high affinity enzyme responsible for TCE metabolism. The objective of the present work is to more directly assess the role of CYP2E1 in the metabolism and disposition of 1,2-^{14}C-TCE administered at 250 or 1000 mg/kg (gavage) using *Cyp2e1*-/- (KO) vs. wild-type (WT) mice. After dosing, animals were individually placed in glass metabolism cages that allowed the collection of expired air, urine, and feces. Exhalation of TCE-derived $^{14}CO_2$ increased in a dose-dependent manner in mice of both genotypes and was significantly higher in WT vs. KO mice. A significantly greater % of the dose was exhaled in KO vs. WT mice as organic volatiles (mainly as TCE). Urinary excretion was the major route of TCE metabolism in WT mice and the % of dose eliminated in urine was significantly higher at the 250 vs. 1000 mg/kg dose. Further, urinary excretion and CO_2 exhalation significantly decreased in KO vs. WT mice. Pretreatment with 1-aminobenzotriazole clearly inhibited TCE metabolism as evident from increased exhalation of parent TCE, and decreased urinary excretion and CO_2 exhalation in mice of both genotypes. In conclusion, these data showed that while CYP2E1 plays an important role in TCE metabolism and disposition, other CYPs also play a significant role and may explain earlier results that showed TCE causes lung damage in KO and WT mice.

Introduction

Trichloroethylene (TCE) is a nonflammable solvent that is used as a metal degreasing agent and an ingredient in the manufacturing of glue, paint, and spot removers (Davidson and Beliles, 1991; Gist and Burg, 1995). TCE is a common contaminant of ground and surface water as well as soil and air. (Westirck et al., 1984). Owing to its volatility and lipophilicity, TCE is readily absorbed through the lungs and gastrointestinal tract and distributed to various tissues. Exposure to TCE causes a variety of tumors depending on species and tissues (Clewell and Anderson 2004; Green, 2000). In mice, TCE causes lung and liver tumors following inhalation or gavage exposure (Rhomberg, 2000). Further, exposure to TCE induces kidney and lung toxicity, and inhibits male fertility (Green et al., 1997; DuTeaux et al., 2004; Forkert et al., 2006). Human exposure to TCE was associated with elevated risks of tumors at numerous sites (Raaschou-Nielsen et al., 2002), and was classified as a "probable human carcinogen" (IARC, 1995; NTP, 2002).

TCE metabolism occurs via conjugation with glutathione via glutathione-S-transferase (GST) and oxidation via the cytochromes P450 (CYPs) enzymes (Lash et al., 2000). GST-dependent conjugation of TCE is a minor pathway and occurs in the liver and kidney. Subsequent metabolism via the ß-lyase enzyme leads to the conversion of S-(1,2-dichlorovinyl)-L-cysteine to S-1,2-dichlorovinyl thiol, which is implicated in TCE-induced nephrotoxicity in rats (Elfarra et al., 1986; Lash et al., 2001). Although TCE conjugation is distinguishable from its oxidation in terms of the metabolites produced and target organ specificity, this pathway is not considered quantitatively significant. However, at elevated exposure levels of TCE when the high affinity oxidative pathway is saturated, the role of conjugation may become more significant (Lash et al., 1998).

CYP-dependent oxidative metabolism of TCE occurs primarily in the liver and in other organs, such as lung, kidney, and reproductive tract. Although different isozymes (CYP1A1/2, CYP2B1/2. CYP2C11/6, and CYP2E1) have been identified as playing a role in TCE metabolism (Nakajima et al., 1993), CYP2E1 is regarded a major high affinity isozyme in the oxidation of TCE. Recent reports showed that CYP2E1, CYP2F, and CYP2B1 are the main enzymes involved in the pulmonary bioactivation of TCE (Forkert et al., 2005 and 2006). The initial proposed step in the oxidation of this chemical is the conversion to TCE epoxide (Fig. 1), which may react with DNA or proteins (Anders and Jakobson, 1985). Alternatively, it was proposed that an oxygenated TCE-O-CYP adduct could contribute to the formation of chloral (Fig. 1) (Miller and Guengerich, 1982). Although the oxidation of TCE has not been fully characterized, it has been presumed that it proceeds via epoxide formation which may subsequently lead to the formation of CO, CO_2, dichloroacetic acid (DCA), chloroacetic acid (MCA), and another intermediate (TCE-O-CYP) that may undergo chloride migration to produce chloral followed by oxidation and reduction to trichloroacetic acid (TCA) and trichloroethanol (TCOH), respectively (Fig. 1). Metabolism is thought to play a critical role in TCE-induced toxicity in various organs. Chloral is thought to contribute to the induction of mouse lung tumors (Green et al., 2000). TCA and DCA were also shown to cause liver tumors in rats and mice through induction of peroxisome proliferation in hepatocytes (Herren-Freund et al., 1987). Spermatoxicity studies in rats showed that DCA causes delayed spermiation and morphological abnormalities in residual bodies and testes (Linder et al., 1997). Further, it was reported that CYPs-dependent formation of TCE reactive metabolites and the subsequent formation of dichlororacetyl protein adducts were associated with pulmonary (Forkert et al., 2006) and reproductive (DuTeaux et al., 2003) toxicities. Kidney damage might be attributed to TCOH or

to metabolites originating from TCE conjugation with GSH (Green et al., 2003; Elfarra et al., 1986).

Metabolism of TCE is influenced by many factors, including gender, tissue, and dose (Lash et al., 2000; Rhomberg 2000) and is closely related to the expression and distribution of enzymes involved in the oxidation and GSH conjugation pathways. Therefore, understanding the enzymatic basis of TCE metabolism is critical for scrutinizing the mechanisms of action and the human risks to this chemical. In the current work, the metabolism and disposition of TCE were investigated to assess the role of CYP2E1 and other CYPs using *Cyp2e1*-/- and wild-type mice. Doses were selected for the current work because similar TCE doses were reported to cause cancer and lung injury in mice and the fact that TCE metabolism and toxicity were well investigated using similar doses (Clewell and Anderson, 2004; Forkert et al., 2005 and 2006).

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Materials and Methods

Chemicals. Trichloroethylene-1,2-^{14}C, specific activity 5.4 mCi/mmol, was purchased from Sigma-Aldrich (St. Louis, MO). The radiochemical purity (99+%) was determined using high performance liquid chromatography (HPLC). Tween 80, trichloroethylene (TCE), trichloroacetic acid (TCA), dichloroacetic acid (DCA), chloroacetic acid (MCA), trichloroethanol (TCOH), chloral hydrate, 1-aminobenzotriazole (ABT) and ß-glucuronidase (Type VII-A, 25000 units) were obtained from Sigma-Aldrich (St. Louis, MO). Ethanol was obtained from Pharmaco (Trinidad, WI), and ethanolamine and ethylene glycol monomethyl ether were from Fisher (Fair Lawn, NJ). HPLC-grade water and acetonitrile were purchased from Caledon Ltd (Georgetown, Canada).

Animals and Dose Preparation. Male *Cyp2e1*+/+ (wild-type, WT) and *Cyp2e1*-/- (*Cyp2e1* knock-out, KO) mice were 3-4 months old and ranged in weight from 21-36 g, were first produced at the National Cancer Institute, Bethesda, MD (Lee et al., 1996) and were re-derived and bred at Charles River Laboratories (Wilmington, DE) as previously described (Hoffler et al., 2003). Dosing solutions were freshly prepared prior to administration by diluting 1,2-^{14}C-TCE with unlabeled TCE in 1 % aqueous Tween 80 solution. TCE was administered by gavage at 250 or 1000 mg/kg to deliver 40-300 µCi/kg in a dose volume of 10 ml/kg. Pretreatment with ABT at 50 mg/2.5 ml saline/kg (i.p.) was performed 1 hr before TCE administration. Food and water were available ad libitum throughout the experiments. All animal care and procedures were carried out according to the National Institutes of Health guidelines (U.S. Department of Health and Human Services, 1985).

Disposition Experiments. Disposition studies were basically similar to methods described by Ghanayem et al. (1999) and Hoffler et al. (2003) with minor modifications. Groups

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of WT and KO mice were treated in groups as follows: 1) WT and KO mice (n=4) received a single dose of 250 mg of 1,2-^{14}C-TCE/kg by gavage and were euthanized 24 hr later; 2) WT and KO mice received a single dose of 1000 mg TCE/kg by gavage and were euthanized 24 hr (n=7) or 48 hr (n=4) later; and 3) WT and KO mice (n=8) were administered 50 mg ABT/kg (i.p.) followed 1 hr later by a single dose of 1000 mg TCE/kg by gavage and were euthanized 24 hr after TCE administration. Immediately after TCE administration, mice were housed in individual glass metabolism cages (Wyse Glass Specialties, Inc. Freeland, MI) to separately collect urine, feces, and exhaled radioactivity over 24 or 48 hr. CO_2 was trapped using ethanolamine/ethylene glycol monomethyl ether (3:7) trapping solutions and organic volatiles were trapped using charcoal traps. Tissue, fecal, and charcoal samples were combusted using a sample oxidizer and counted. All other steps and procedures were followed as previously described (Ghanayem et al., 1999; Hoffler et al., 2003).

HPLC Analysis of Urine, Hydrolyzed Urine, and Charcoal Traps Extracts. Metabolites in urine and exhaled organic volatiles retained in the charcoal traps were extracted, separated, and identified using a Waters HPLC System (Waters Corp., Milford, MA) connected to a UV detector and an on-line 515T radiomatic flow scintillation analyzer (PerkinElmer Life Science). The activated charcoal (~150 mg) taken after breaking the glass traps was extracted three times with acetonitrile (200 µl each). The extract was injected into an HPLC system equipped with Microsorb MV column (C_{18}, 4.6 × 250 mm, Rainin Instruments Co., Woburn, MA) with a mobile phase of 50:50 mixture of 0.1 % phosphoric acid in water and acetonitrile for 10 min at a flow rate of 0.4 ml/min, then a linear gradient to 100 % acetonitrile over 15 min, where it was held for 35 min. The extract was further analyzed by nuclear magnetic resonance spectroscopy (NMR, 300MHz, Varian Inc., Palo Alto, CA). Urine samples were syringe-filtered

through a 0.22 µm filter and directly injected into the HPLC system using an Aminex HPX-87H column (Bio-Rad Laboratories, Richmond, CA) maintained at 40°C with 0.008 M H_2SO_4 at a flow rate of 0.7 ml/min. Characterization of the urinary metabolites was based on the method described by Green and Prout (1985) and was modified for this study. Each urine sample was incubated with ß-glucuronidase (2000 units/ml of urine) in 0.1 M phosphate buffer (pH 7.0) at 37°C for 4 hr. The hydrolyzed urine samples were filtered and analyzed by the HPLC method described above. The metabolite profile of the hydrolyzed samples was compared with that of the same urine samples incubated without ß-glucuronidase in phosphate buffer. All peaks were detected using the online radiomatic and UV detectors at 210 nm.

Macromolecular binding of TCE-derived radioactivity in the Liver: Fractionation of liver homogenates to determine binding was based on the method described by Ghanayem et al. (1987) and was modified for this study. Liver specimens (weighing ~ 200 mg each) from mice (n=3 each was performed in duplicates) administered 250 mg TCE/kg were homogenized in ~ 400 µl 0.25 M cold sucrose using an Omni µH homogenizer (Omni International, Marietta, GA). To the homogenates, ~400 µl of 10 % TCA was added, mixed, and placed in a refrigerator overnight to facilitate the precipitation of proteins. The homogenates were centrifuged at 10,000 rpm for 10 min and the supernatants were transferred into a pre-weighed vial. Precipitants were washed twice with 5% TCA, and the supernatants were combined to the previous solution (unbound radioactivity). The pellets were extracted with ethanol and a mixture of ethanol and ethyl ether (3:1), and the radioactivity contents of the supernatants represented the lipid-bound radioactivity. To the pellets, 10 % NaCl was added and boiled in a water bath for 20 min to prepare the supernatants that contains the nucleic acids-bound radioactivity. Precipitates were

digested overnight using 1.0 N NaOH. Triplicate samples of each prepared fraction (50 µl) were mixed with EcoLume and the radioactivity was determined using scintillation counting. The TCE-derived radioactivity in the pellet represented the protein binding. HPLC analysis of the unbound radioactivity (soluble fraction) was performed using the same method described above to analyze the urine.

Statistical Analysis. All values are presented as the mean ± standard error. Group mean comparisons were conducted using the Student's *t* test. Values were considered statistically significant at $p \leq 0.05$.

Results

Exhalation of TCE-Derived Organic Volatiles: TCE-derived organic volatiles were calculated for each time point by combining exhaled radioactivity that was retained in the ethanol and charcoal traps. Charcoal traps retained most of the exhaled organic volatiles. Exhalation was mostly complete within 24 hr after a single TCE dose in mice of both genotypes (Fig. 2). Exhalation of TCE-derived organic volatiles was genotype- and dose-dependent (Fig. 2-A). Twenty-four hours after administration of 250 or 1000 mg TCE/kg, WT mice eliminated ~14 and ~23 % of the dose, respectively, as organic volatiles (Fig. 2-A and Table 1). In comparison, a significantly greater % of the dose was exhaled as organic volatiles by KO mice and accounted for ~34 and ~49 % of the 250 and 1000 mg TCE/kg dose, respectively (Fig. 2-A and Table 1). Pretreatment of mice with ABT, a universal CYP inhibitor, resulted in significant increases in the % of TCE dose exhaled as organic volatiles in mice of both genotypes in comparison to mice untreated with ABT (Fig. 2-B). WT and KO mice pretreated with ABT exhaled relatively similar amounts of TCE-derived organic volatiles (~73 and ~79 % of the dose, respectively) (Fig. 2-B and Table 1). HPLC analysis of the exhaled organic volatiles extracted from the charcoal traps of both WT and KO mice produced comparatively similar profiles, displaying one peak at ~24 min, corresponding to the retention time of TCE (Fig. 3). Subsequently, NMR analysis supported this finding showing that unchanged TCE was the only detectable chemical exhaled as organic volatiles (data not shown).

Exhalation of TCE-Derived $^{14}CO_2$: Exhalation of ^{14}C-TCE-derived $^{14}CO_2$ continued over the 48 hr monitoring period and was dose-dependent in mice of both genotypes (Fig. 4). WT and KO mice treated with 250 mg TCE/kg exhibited a decrease in the % of dose exhaled as $^{14}CO_2$ in comparison to mice administered 1000 mg TCE/kg (Fig. 4-A). Further, there was a

significantly greater % of the TCE dose exhaled as $^{14}CO_2$ in WT vs. KO mice (Fig. 4-A and Table 1). When WT and KO mice were pretreated with ABT, 1 hr before treatment with 1000 mg TCE/kg, the % of dose exhaled as $^{14}CO_2$ drastically decreased (Fig. 4-B). ABT pretreatment apparently rendered WT and KO mice metabolically similar, and CO_2 exhalation accounted for ~4 % of the dose.

Analysis and Characterization of TCE-Derived Urinary Metabolites: Urine is the primary route of the excretion of TCE-derived metabolites and a significantly higher % of the dose was excreted in the urine of WT vs. KO mice (Table 1). Twenty-four hours after the administration of 1000 mg TCE/kg, ~ 38 % of the dose was excreted in the urine of WT mice compared to ~16 % of the dose excreted in urine of KO mice (Table 1). The cumulative % of dose excreted in the urine of mice of both genotypes that received 250 mg TCE/kg was significantly higher than that excreted in the urine of mice treated with 1000 mg TCE/kg (accounted for ~57 and ~34 %, respectively) (Table 1). Most of the excretion of TCE-derived urinary metabolites occurred in the first 24 hr after dosing and minimal excretion occurred between 24 and 48 hr. Similar to its effect on $^{14}CO_2$ exhalation, pretreatment of mice with ABT caused a drastic decrease in the excretion of TCE-derived radioactivity in the urine of WT and KO mice when compared to excretion in the absence of ABT (Table 1). HPLC profiles of TCE-derived metabolites in the urine, however, were qualitatively similar in mice of both genotypes, even with ABT pretreatment, showing three peaks with an approximate retention times of approximately 7, 12, and 15 min (Fig. 5).

Based on HPLC analysis and co-elution with authentic standard (^{14}C-TCA), the metabolite eluting at ~7 min was tentatively identified as TCA and accounted for ~14 % of the total urinary metabolites excreted in WT and KO mice that received the high dose (Fig. 5 and

Table 2). However, significant reduction of TCA excretion (calculated as % of dose) in the urine was found in KO vs. WT mice treated with either dose of TCE (Table 2). The major TCE metabolite eluting at ~12 min was characterized as TCOH-glucuronide which is in agreement with an earlier report by Green and Prout (1985). Urine was incubated with ß-glucuronidase to convert TCOH-glucuronide to free TCOH and analyzed using HPLC (Fig. 5). After the urines of WT or KO mice were incubated with ß-glucuronidase, the major peak eluting at ~12 min disappeared and a new major peak emerged at ~36 min which is consistent with the retention time of authentic TCOH (Fig. 5). This clearly confirmed that this major metabolite was TCOH-glucuronide. The TCA peak was not affected by incubation with ß-glucuronidase, nor did any new peak appear in the chromatogram. Although the minor peak that eluted at ~15 min was not identified, it can be concluded that it is neither DCA (t_R = ~9 min) nor MCA (t_R = ~17 min) (Fig. 5). Interestingly, when 250 mg TCE/kg was administered to mice, a small peak corresponding to free TCOH was detected in the urine. At the 1000 mg TCE/kg dose, the urinary metabolites profiles showed differences in mice of both genotypes as a function of time (Table 2). The proportion of TCA in the urine of mice of both genotypes collected between 24-48 hr vs. 0-24 hr increased significantly, whereas that of TCOH-glucuronide declined. At the 250 mg TCE/kg dose, the proportion of TCA was significantly higher in WT vs. KO mice (Table 2). In contrast, the proportion of TCA was significantly higher in KO vs. WT mice pretreated with ABT (Table 2).

TCE-derived Radioactivity in Feces, Blood, and Tissues. Fecal excretion of TCE-derived radioactivity accounted for 2-5 % of the dose in WT and KO mice (Table 1). Pretreatment with ABT caused a significant decrease in the excretion of TCE-derived radioactivity in the feces and accounted for ~2 and 1 % of the dose in WT and KO mice,

respectively (Table 1). TCE-derived radioactivity was widely distributed in the tissues of WT and KO mice and retention was dose-dependent, with the highest levels found in the forestomach, liver, and kidney of mice of both genotypes (Table 3). Twenty-four hours after exposure to 1000 mg TCE/kg, tissues collected from WT mice retained higher levels of TCE-derived radioactivity than tissues from KO mice. Overall, ABT pretreatment caused a notable decrease in the concentration of TCE-derived radioactivity in tissues of mice of both genotypes (Table 3).

Macromolecular Binding of TCE-Derived Radioactivity in the Liver: Characterization of the macromolecular binding in the liver of treated mice showed that ~79 and 72% of the total TCE-derived radioactivity was bound to liver macromolecules of WT and KO, respectively (Table 4). Binding of TCE-derived radioactivity to lipids and nucleic acids was comparatively minimal and was similar in KO and WT mice (Table 4). Most interesting, however, is the fact that ~61 and 68 % of the total TCE-derived radioactivity was bound to the protein fraction of the liver homogenates in KO and WT mice, respectively (Table 4). The concentration of radioactivity in the unbound fraction (supernatant) was low and therefore, HPLC analysis showed no distinct peaks and identification of the soluble metabolites was inconclusive.

Discussion

TCE is a chlorinated solvent that is heavily used in the preparation of consumer and industrial products (Gist and Burg, 1995; ATSDR, 1997). Although the uses of TCE have diminished due to its toxicity, TCE remains a common environmental contaminant (ATSDR, 1997). Bioactivation of TCE is considered a pre-requisite for the development of TCE-induced toxicity and carcinogenicity. Oxidation via cytochromes P450 (CYPs) is considered the primary pathway of TCE metabolism and metabolites formed via this pathway are implicated in the pulmonary and reproductive toxicities as well as the carcinogenicity of this chemical (DuTeaux et al., 2003; IARC 1995; Forkert et al., 2006). Earlier studies suggested that while CYP2E1 is the main high affinity enzyme responsible for TCE oxidation, other CYPs, including CYP2F, CYP2B1/2, CYP1A1/2, and CYP2C11 are also involved (Nakajima et al., 1992; Lipscomb et al., 1997; Forkert et al., 2005 and 2006). Using KO mice, the current study was undertaken to assess the enzymatic basis of TCE metabolism and disposition *in vivo*.

Earlier studies (Green and Prout, 1985; Prout et al., 1985; Larson and Bull, 1992; Fisher, 2000; Keys et al., 2003) demonstrated that TCE is rapidly absorbed and metabolized in animals and humans. In agreement, current work confirmed that after gavage administration, TCE was rapidly absorbed and distributed to all major tissues. Excretion of TCE-derived radioactivity occurred primarily in the urine, expired air (as parent compound and CO_2), and feces. The importance of CYP2E1 in TCE metabolism was evident when comparing the exhalation of organic volatiles, which showed that significantly greater % of the dose was exhaled in KO vs. WT mice. This indicated that in the absence of CYP2E1, TCE metabolism is inhibited, resulting in retention of parent compound, which is subsequently eliminated via exhalation. Increased exhalation of the parent compound in KO vs. WT mice was also associated with significant

decreases in CO_2 exhalation and urinary excretion of TCE-derived metabolites in KO mice. In an earlier study, Prout et al. (1985) reported that the major route of excretion of TCE-derived radioactivity was in the urine and there was no significant effect of dose on the urinary excretion in mice (57, 53, and 52 % of dose at 10, 500, and 1000 mg TCE/kg, respectively). In contrast, our study showed that urinary elimination is both dose- and genotype-dependent. Urinary excretion was the primary route of TCE metabolism at the 250 mg/kg dose and the % of dose excreted in the urine of KO and WT mice had significantly declined at the high dose. This finding suggested that enzymes involved in the production of TCE metabolites that are subsequently excreted in the urine might be saturated at the high dose.

Recently, the catalytic efficiencies (V_{max}/K_m) were determined using recombinant rat CYP2E1 (0.79), CYP2F4 (0.27), and CYP2B1 (0.07), mouse CYP2F2 (0.11), and human CYP2E1 (0.02) in TCE metabolism to chloral hydrate (Forkert et al., 2005). Subsequently, it was reported that TCE causes bronchiolar Clara cell injury in WT and KO mice in association with dichloroacetyl protein adducts formation (Forkert et al., 2006). It was suggested that TCE oxidation to chloral hydrate in murine lungs was mediated by CYP2E1 and other CYPs including CYP2F2. In an attempt to assess the involvement of CYPs other than CYP2E1, WT and KO mice were pre-treated with ABT (a universal CYPs inhibitor). Generally, ABT pretreatment rendered mice of both genotypes metabolically similar. The drastic increase in TCE exhalation in KO mice pretreated with ABT vs. KO mice untreated with ABT was attributed to the contribution of CYPs other than CYP2E1. Further, the decline in the exhalation of TCE-derived CO_2 and excretion of TCE-derived urinary metabolites in ABT pretreated KO mice may be attributed to CYPs other than CYP2E1. Collectively, this work as well as earlier reports demonstrated that multiple CYPs are responsible for TCE metabolism. However, the

contribution of various CYPs may be dependent on the organ in question and may also be affected by the absence of CYP2E1 in the KO mice. In comparison to WT mice, inhibition of TCE metabolism in the absence of CYP2E1 in KO mice may lead to an increase the substrate (TCE) concentration. Hence, the metabolic contribution of other CYPs may change due to the increase in the substrate (TCE) concentration in KO mice. However, it remains to be confirmed if the metabolic activities of CYP2F and CYP2B enzymes increase at high blood TCE levels in KO. Furthermore, TCE exhalation over an extended period in conjunction with the presence of CYPs including CYP2E1, CYP2F, and CYP2B1 in the respiratory tract (Forkert et al., 2005 and 2006) may lead to the in situ metabolism of TCE to reactive intermediates; which may explain the pulmonary toxicity of this chemical.

Chlorinated acetic acids derived from TCE are considered a major source of CO_2 exhalation in treated mice (Figure 1; Green and Prout, 1985; Larson and Bull, 1992). CYP2E1 was reported to be involved in the conversion of chloral to TCA (Ni et al., 1996). This finding infers that the absence of CYP2E1 should lead to less TCA formation in KO vs. WT mice, which was corroborated by the fact that the proportion of TCA in the urine of KO and WT mice accounted for ~8 vs. 17 % at the low dose of TCE, respectively. Interestingly, the % of TCA in the urine increased in KO mice pretreated with ABT; which may imply that TCA accumulation could occur in mice devoid of CYP2E1. Further, although there was no direct evidence that CYP2E1 is responsible for the metabolism of TCA and/or DCA, relevant studies revealed that dechlorination of 1,1,1-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)-ethane (Kitamura et al., 2002) and debromination of ethylene dibromide (Doty et al., 2000) may be mediated by CYP2E1. It is therefore possible that CYP2E1 may be involved in TCA metabolism.

In comparing the urinary metabolite profiles in WT and KO mice, the proportion of various metabolites changed as a function of time. TCA proportion of total urinary metabolites increased and TCOH-glucuronide decreased in the urine of mice of both genotypes collected between 24-48 hr vs. urine collected between 0-24 hr. This change in the urinary metabolite profiles might be due to enterohepatic recirculation of TCOH-glucuronide that is transformed to TCOH and then to TCA, which results in retaining TCA in the blood (Stenner et al., 1997). The long half-life of TCE-derived TCA could provide an explanation for CO_2 formation over a 48 hr period after TCE administration and may contribute to the hepatocarcinogenicity of TCE through proliferation of hepatic peroxisomes in rodents (Herren-Freund et al., 1987).

The pattern of distribution of TCE-derived radioactivity showed that CYP2E1-mediated metabolism might be linked to retention of TCE-derived radioactivity in tissues. In comparison to WT mice, KO mice exhaled a significantly greater portion of the administered TCE unchanged and excreted less TCE-derived metabolites in the urine. However, the concentrations of TCE-derived radioactivity in tissues of KO mice were not distinctly different from those of WT mice. Interestingly, current work showed that most of the TCE-derived radioactivity in the liver of mice of both genotypes was bound to macromolecules, and protein binding accounted for 60-70% of the total binding. This finding is in agreement with a recent report that demonstrated significant formation of dichloroacetyl lysine protein adducts in the lungs of mice of both genotypes (Forkert et al., 2006). Current work also demonstrated that the total bound and the protein bound TCE-derived radioactivity were significantly higher in WT vs. KO mice. Collectively, this data confirmed that in addition to CYP2E1, other CYPs play an important role in TCE metabolism to reactive intermediates and subsequent formation of protein adducts.

In conclusion, the current *in vivo* study confirmed that while CYP2E1 plays an important role in the metabolism and disposition of TCE administered by gavage at 250 or 1000 mg TCE/kg, other CYPs perform a significant function as well. While this conclusion is derived from studies conducted at doses that are higher than the reported Km value for this chemical, the contribution of various CYP isoforms to the metabolism of TCE may vary at lower doses. In the absence of CYP2E1, inhibition of TCE metabolism occur leading to increased substrate (TCE) concentration. It is possible that other CYPs may metabolize TCE at an enhanced rate and compensate for the loss of CYP2E1 in KO animals. This premise is in agreement with the fact that TCE caused comparatively similar bronchiolar Clara cells damage in both KO and WT mice. It is also in agreement with finding that substantial macromolecular binding of TCE–derived metabolites occurs in mice of both genotypes. Further investigation of the role of CYP2E1 in the metabolism of TCE metabolites such as TCA and DCA will be required to further understand the role of various CYPs in TCE metabolism and toxicity.

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Footnotes

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Figure Legend

Fig. 1. A proposed scheme of trichloroethylene (TCE) metabolism in rodents.

Fig. 2. Exhalation of TCE-derived organic volatiles (presented as cumulative % of dose) in WT and KO mice as a function of dose and time. TCE was administered at 250 or 1000 mg kg (A) and 1000 mg/kg with or without ABT pretreatment (B). Values are the mean ± standard error of 4-11 mice.. [a] Denotes values in KO mice that are significantly different from values in similarly treated WT mice at $p \leq 0.05$. [b] Denotes values in mice treated with 1000 mg TCE/kg that are significantly different from values in mice of the same genotype that received 250 mg TCE/kg at $p \leq 0.05$. [c] Denotes values in mice treated with ABT+1000 mg TCE/kg that are significantly different from values in mice that received 1000 mg TCE/kg without ABT at $p \leq 0.05$.

Fig. 3. Representative HPLC radiochromatograms of charcoal trap extracts collected at 12 hr after TCE administration.

Fig. 4. Exhalation of TCE-derived CO_2 (presented as cumulative % of dose) in WT and KO mice as a function of dose and time. TCE was administered at 250 or 1000 mg/kg (A) and 1000 mg/kg with or without ABT pretreatment (B). Values are the mean ± standard error of 4-11 mice. [a] Denotes values in KO mice that are significantly different from values in similarly treated WT mice at $p \leq 0.05$. [b] Denotes values in mice treated with 1000 mg TCE/kg that are significantly different from values in mice of the same genotype that received 250 mg TCE/kg at $p \leq 0.05$. [c] Denotes values in mice treated with ABT+1000 mg TCE/kg that are significantly different from values in mice that received 1000 mg TCE/kg without ABT at $p \leq 0.05$.

Fig. 5. Representative HPLC radiochromatograms of authentic standards (A), urinary metabolites at the 1000 mg TCE/kg dose (B), and urinary metabolites after incubation with ß-

glucuronidase (C). Metabolite 1, trichloroacetic acid; 2, trichloroethanol-glucuronide; 3, unknown; and 4, trichloroethanol.

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Table 1: A summary table showing the disposition of TCE in WT and KO mice

	(mg/kg)	Time (hr)	WT	KO	WT-w/ABT	KO-w/ABT
CO_2	250	24	12.2 ± 0.7	8.7 ± 0.1 [a]		
	1000	24	20.1 ± 1.3 [b]	17.6 ± 1.0 [a,b]	4.3 ± 0.5 [c]	3.6 ± 0.5 [a,c]
		48	26.3 ± 0.3	22.6 ± 0.8 [a]		
Volatiles	250	24	13.8 ± 0.5	34.4 ± 4.7 [a]		
	1000	24	22.6 ± 1.3 [b]	48.6 ± 1.3 [a,b]	73.2 ± 1.6 [c]	78.8 ± 1.3 [a,c]
		48	22.9 ± 1.7	50.2 ± 2.6 [a]		
Urine	250	24	57.2 ±1.2	33.8 ±0.4 [a,]		
	1000	24	37.7 ± 2.5 [b]	16.1 ± 1.6 [a,b]	11.3 ± 0.5 [c]	3.5 ± 0.3 [a,c]
		48	40.2 ± 2.1	17.0 ± 0.6 [a]		
Feces	250	24	5.0 ± 1.1	3.8 ± 0.6		
	1000	24	4.1 ± 1.3	1.8 ± 0.2 [b]	1.7 ± 0.2 [c]	0.7 ± 0.1 [a,c]
		48	5.2 ± 1.2	2.1 ± 0.5		
Total	250	24	88.2	80.7		
	1000	24	84.5	84.1	90.5	86.6
		48	94.6	91.9		

All values are cumulative % of the dose and are presented as the mean ± standard error of 4-11 mice. [a] Denotes values in KO mice that are significantly different from values in similarly treated WT mice at $p \leq 0.05$. [b] Denotes values in mice treated with 1000 mg TCE/kg that are significantly different from values in mice of the same genotype that received 250 mg TCE/kg at $p \leq 0.05$. [c] Denotes values in mice treated with ABT+1000 mg TCE/kg that are significantly different from values in mice that received 1000 mg TCE/kg without ABT at $p \leq 0.05$.

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Table 2: Effect of dose, time, and ABT pretreatment on the major urinary metabolites of TCE in WT and KO mice

Time (hr)	Treatment Dose (mg/kg)	TCA		TCOH-glucuronide	
		WT	KO	WT	KO
0-24	250	16.7 ± 1.3 (8.4 ± 0.4)	8.3 ± 0.4 [a] (2.3 ± 0.3 [a])	78.2 ± 2.2 (39.7 ± 3.2)	84.1 ± 3.0 (23.1 ± 2.9 [a])
0-24	1000	13.9 ± 0.9 [d] (3.6 ± 0.5 [b,d])	13.6 ± 0.6 [b,d] (1.7 ± 0.1 [a,b,d])	82.2 ± 1.0 [d] (23.1 ± 2.7 [b,d])	85.1 ± 0.5 [d] (10.8 ± 0.9 [a,b,d])
24-48	1000	34.0 ± 3.6 (1.9 ± 0.2)	27.7 ± 3.1 (0.6 ± 0.1 [a])	66.0 ± 3.6 (4.1 ± 1.2)	72.3 ± 3.1 (1.8 ± 0.3)
0-24	ABT+1000	9.1 ± 0.7 [c] (0.8 ± 0.1 [c])	21.8 ± 1.1 [a,c] (0.4 ± 0.0 [a,c])	87.6 ± 0.8 [c] (7.3 ± 0.4 [c])	78.2 ± 1.1 [a,c] (1.5 ± 0.2 [a,c])

Values are presented as % of total TCE-derived urinary metabolites (mean ± standard error of 4-8 mice). Values of metabolites in parentheses are % of the administered TCE dose (mean ± standard error of 4-8 mice). [a] Denotes values in KO mice that are significantly different from values in similarly treated WT mice at $p \leq 0.05$. [b] Denotes values in mice treated with 1000 mg TCE/kg that are significantly different from values in mice of the same genotype that received 250 mg TCE/kg at $p \leq 0.05$. [c] Denotes values in mice treated with ABT+1000 mg TCE/kg that are significantly different from values in mice that received 1000 mg TCE/kg without ABT at $p \leq 0.05$. [d] Denotes values in mice treated with 1000 mg TCE/kg at 24 hr that are significantly different from values in mice of the same genotype treated with the same dose at 48 hr at $p \leq 0.05$.

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Table 3: Effect of dose and ABT pretreatment on the concentration of TCE-derived radioactivity in tissues of WT and KO mice

	250 mg		1000 mg		1000 mg- w/ABT	
	WT	KO	WT	KO	WT	KO
Blood	42.6 ± 2.0	61.5 ± 6.5[a]	342.8 ± 35.2	285.6 ± 12.5	88.7 ± 4.5	85.7 ± 2.3
Kidneys	64.9 ± 1.2	72.3 ± 2.1[a]	333.6 ± 22.6	269.4 ± 17.3 [a]	222.9 ± 6.1	190.1 ± 13.5[a]
Thymus	24.9 ± 0.2	25.29 ± 0.8	135.9 ± 24.6	107.9 ± 16.5	52.1 ± 4.2	44.1 ± 2.5
Heart	14.9 ± 0.8	17.4 ± 1.0	131.6 ± 10.9	103.2 ± 12.3	29.1 ± 0.7	23.3 ± 1.3[a]
Forestomach	204.8 ± 54.3	254.3 ± 75.1	942.4 ± 105.4	567.2 ± 23.5[a]	351.4 ± 34.3	359.5 ± 25.4
Glandular stomach	44.74 ±3.8	47.9 ± 5.6	395.9 ± 57.2	296.6 ± 36.6	136.7 ± 21.6	126.2 ± 12.2
Fat	3.8 ±0.5	3.4 ± 0.3	32.1 ± 6.0	20.2 ± 0.6	16.2 ± 0.3	16.9 ± 0.9
Liver	123.6 ±5.7	123.1 ± 6.8	928.2 ± 82.3	803.5 ± 43.4	203.8 ± 3.1	194.8 ± 4.9
Spleen	20.9 ± 0.8	29.1 ± 1.8[a]	228.6 ± 25.0	213.5 ± 9.7	50.4 ± 2.3	45.7 ± 2.0
Lung	21.6 ± 1.8	30.0 ± 1.7[a]	202.4 ± 14.1	181.3 ± 9.4	42.1 ± 1.7	36.2 ± 1.4[a]
Testes	8.1 ± 0.5	12.1 ± 0.5[a]	85.7 ± 10.2	60.3 ± 1.2[a]	17.6 ± 1.0	16.4 ± 1.3
Brain	4.1 ± 0.4	6.0 ± 0.5[a]	43.8 ± 4.9	27.6 ± 2.1[a]	8.9 ± 0.5	7.7 ± 0.4[a]

Values are presented as the mean ± standard error of 4-8 mice expressed as µg TCE equivalents/g tissue. TCE was administered by gavage and mice were sacrificed 24 hr after treatment. [a] Denotes values in KO mice that are significantly different from values in similarly treated WT mice at $p \leq 0.05$. Tissue concentrations of TCE-derived radioactivity in mice treated with 1000 mg TCE/kg are significantly different in mice of the same genotype that received 250 mg TCE/kg at $p \leq 0.05$. Tissue concentrations of TCE-derived radioactivity in mice treated with ABT+1000 mg TCE/kg are significantly different from values in mice that received 1000 mg TCE/kg without ABT at $p \leq 0.05$.

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Table 4. Macromolecular binding of TCE-derived radioactivity in the liver of WT and KO mice

	WT	KO
Unbound	20.8 ± 1.7	28.3 ± 2.5[a]
Lipid-bound	5.5 ± 0.9	6.5 ± 0.3
Nucleic acid-bound	3.7 ± 0.5	3.7 ± 0.2
Protein-Bound	68.1± 1.4	61.1 ±1.5[a]

Values are presented as % of total TCE-derived radioactivity in the liver and are the mean ± standard error of 3 mice in each treatment group. This study was repeated using the same mice at a later time. Mice received 250 mg TCE/kg by gavage and were sacrificed at 24 hr after dosing. [a] Denotes values in KO mice that are significantly different from values in similarly treated WT mice at $p \leq 0.05$.

Fig. 1

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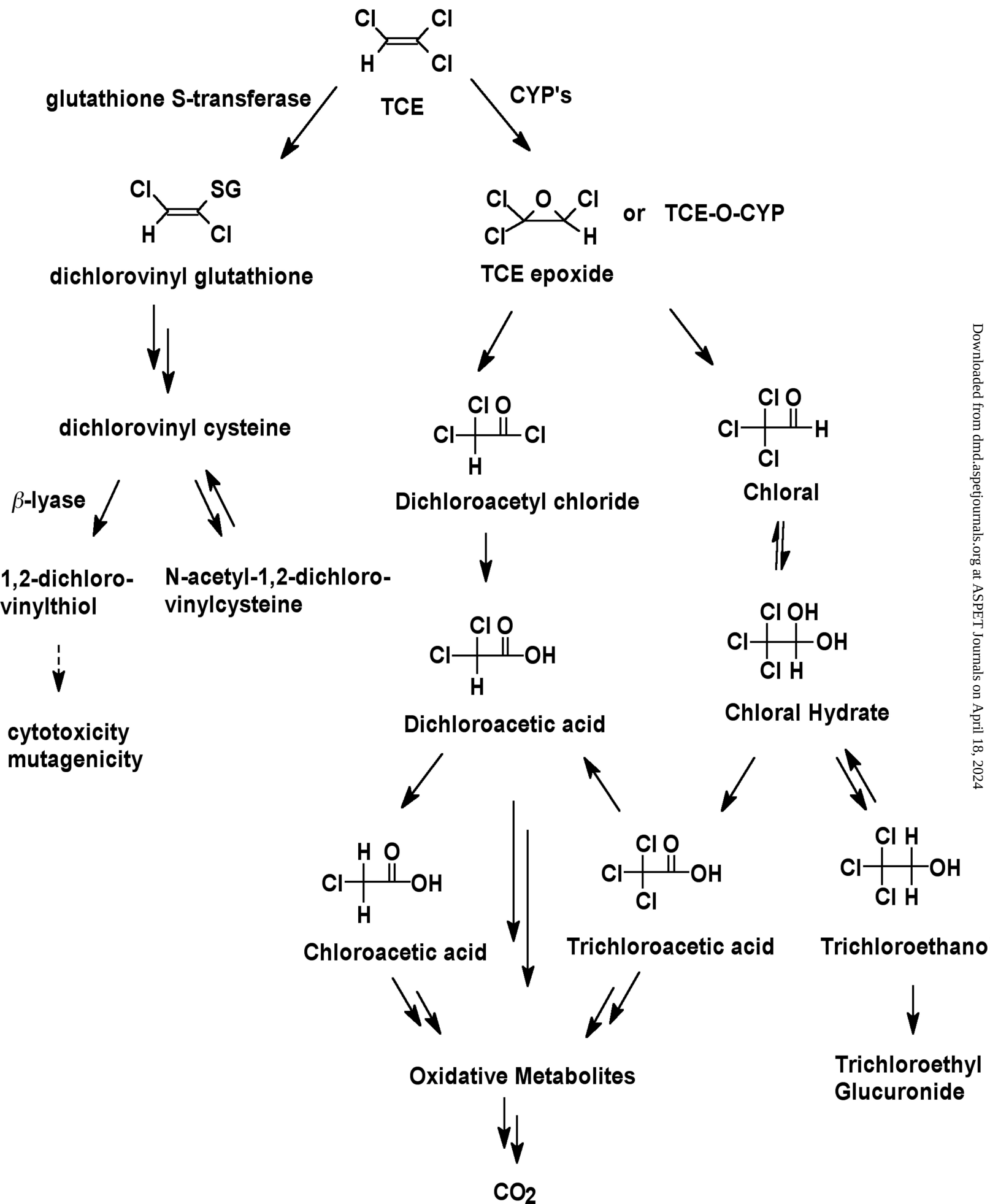

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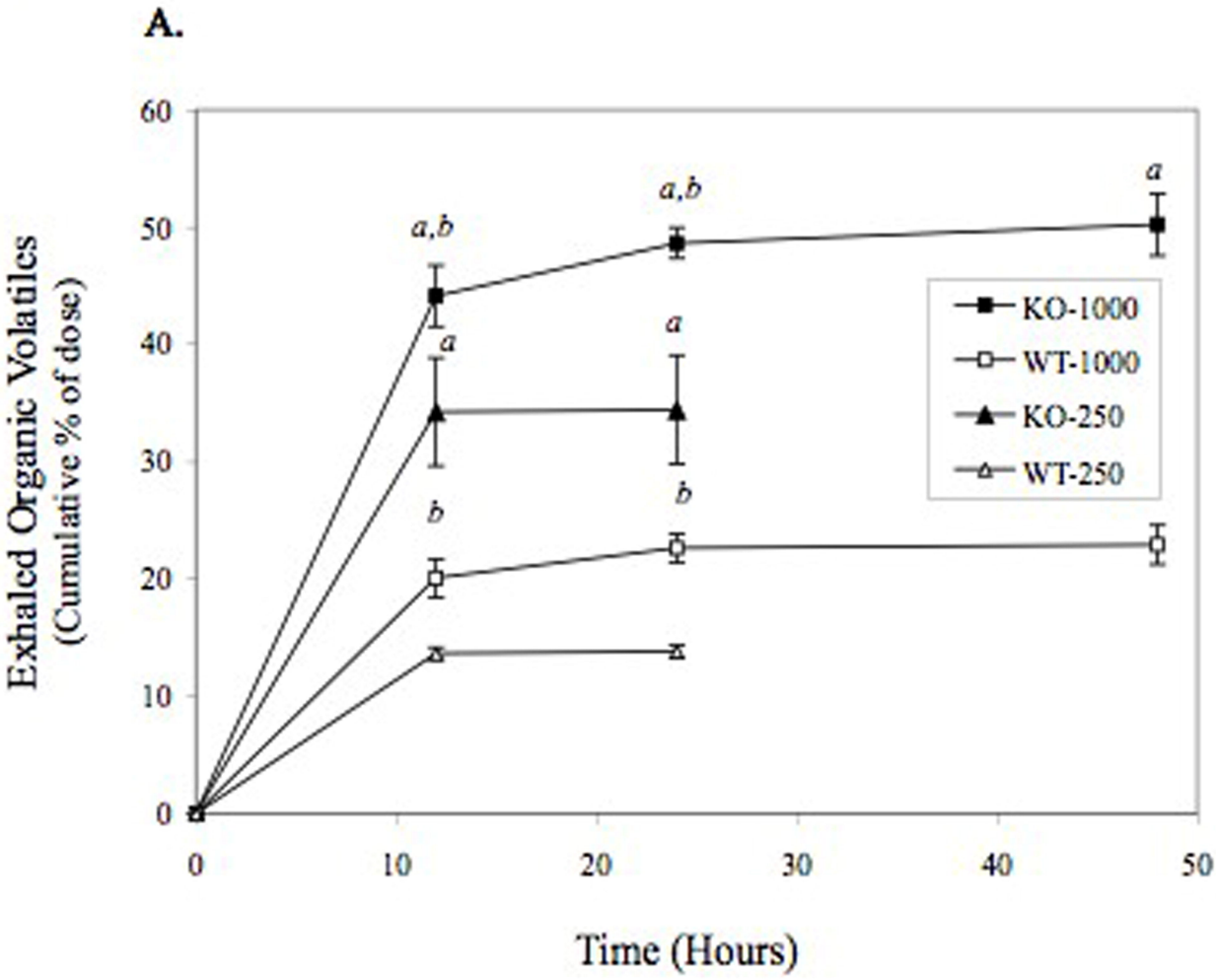
Fig. 2



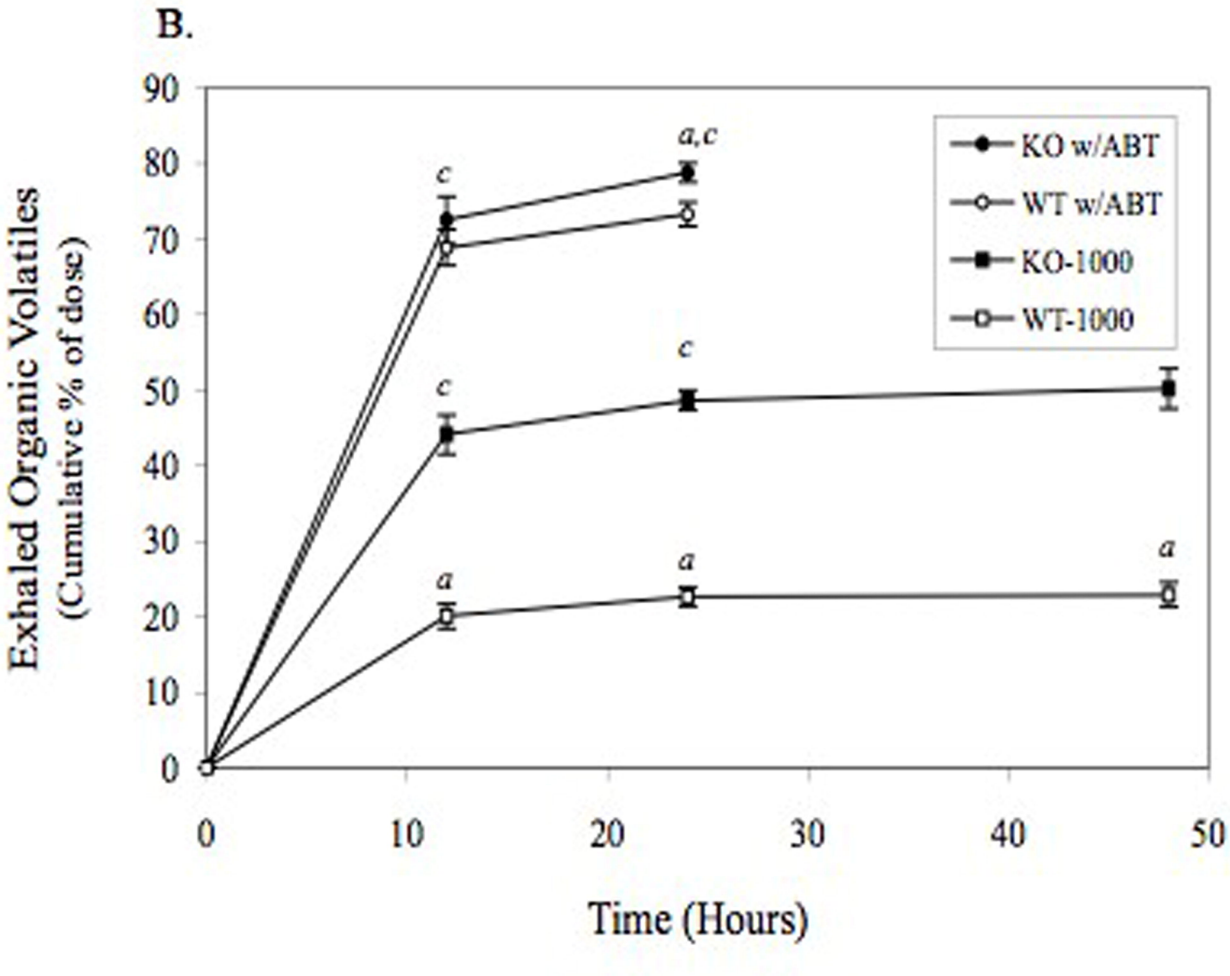


Fig. 3

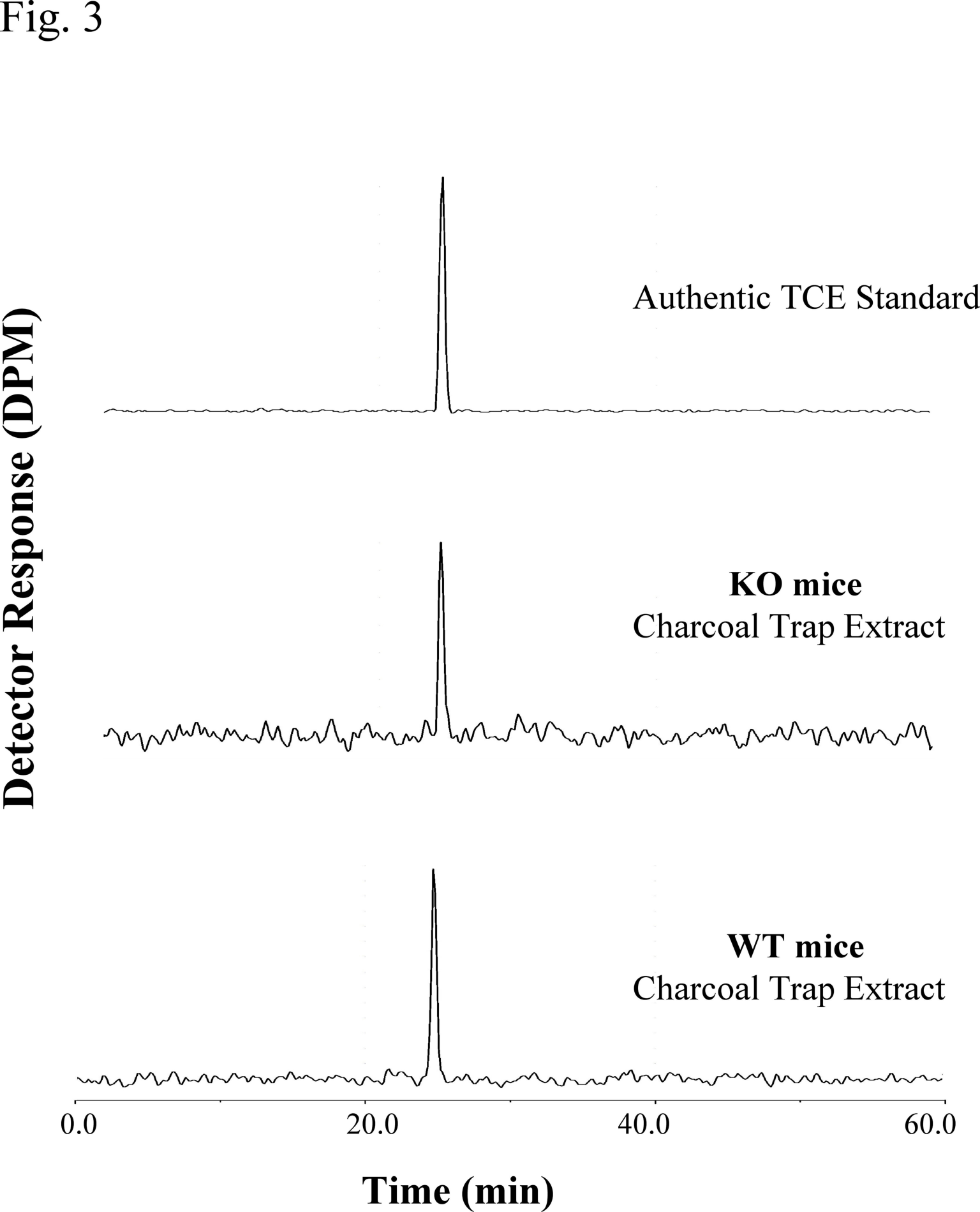

Authentic TCE Standard
KO mice
Charcoal Trap Extract
WT mice
Charcoal Trap Extract
Detector Response (DPM)
0.0
20.0
40.0
60.0
Time (min)

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Fig. 4

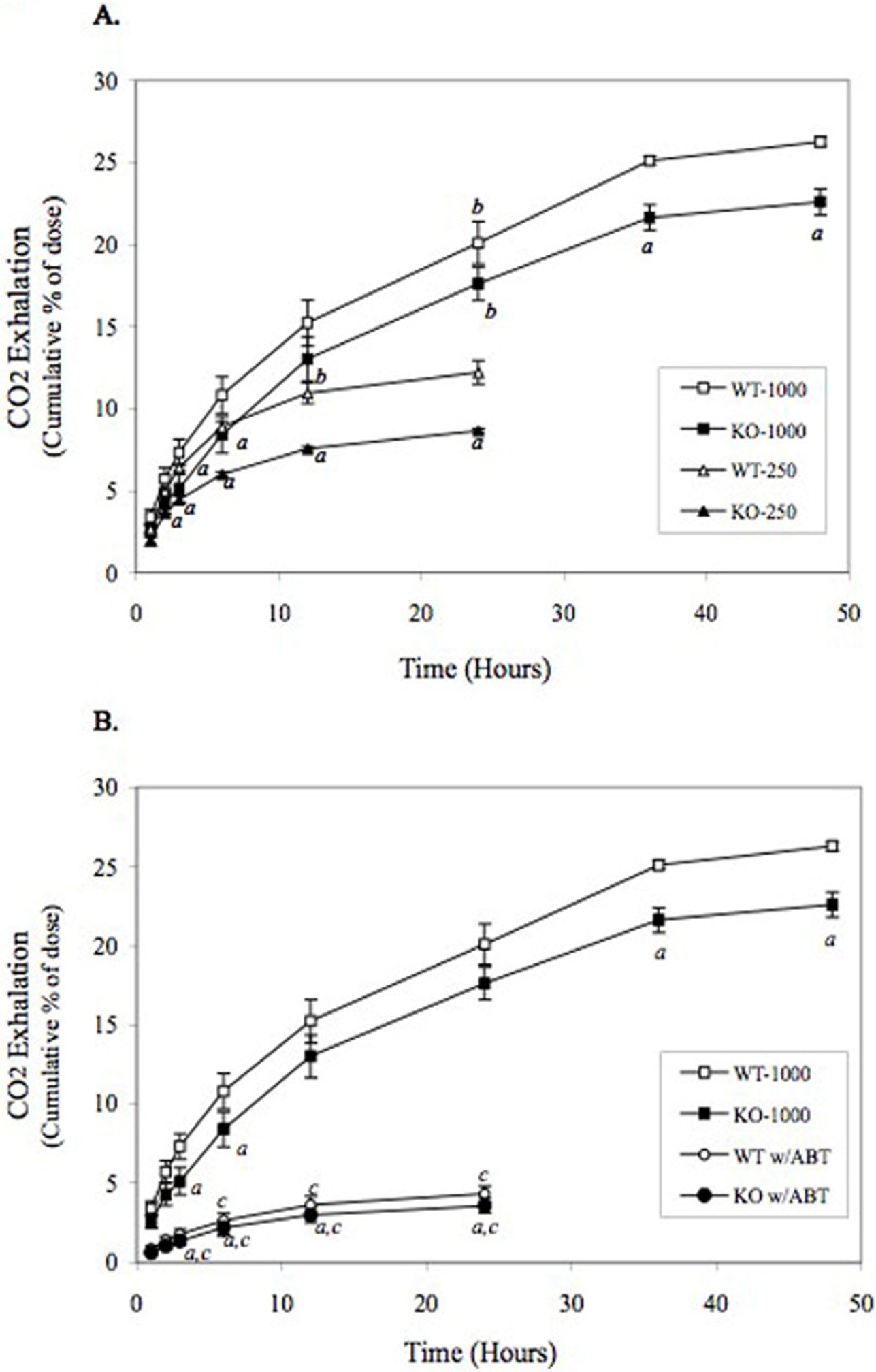
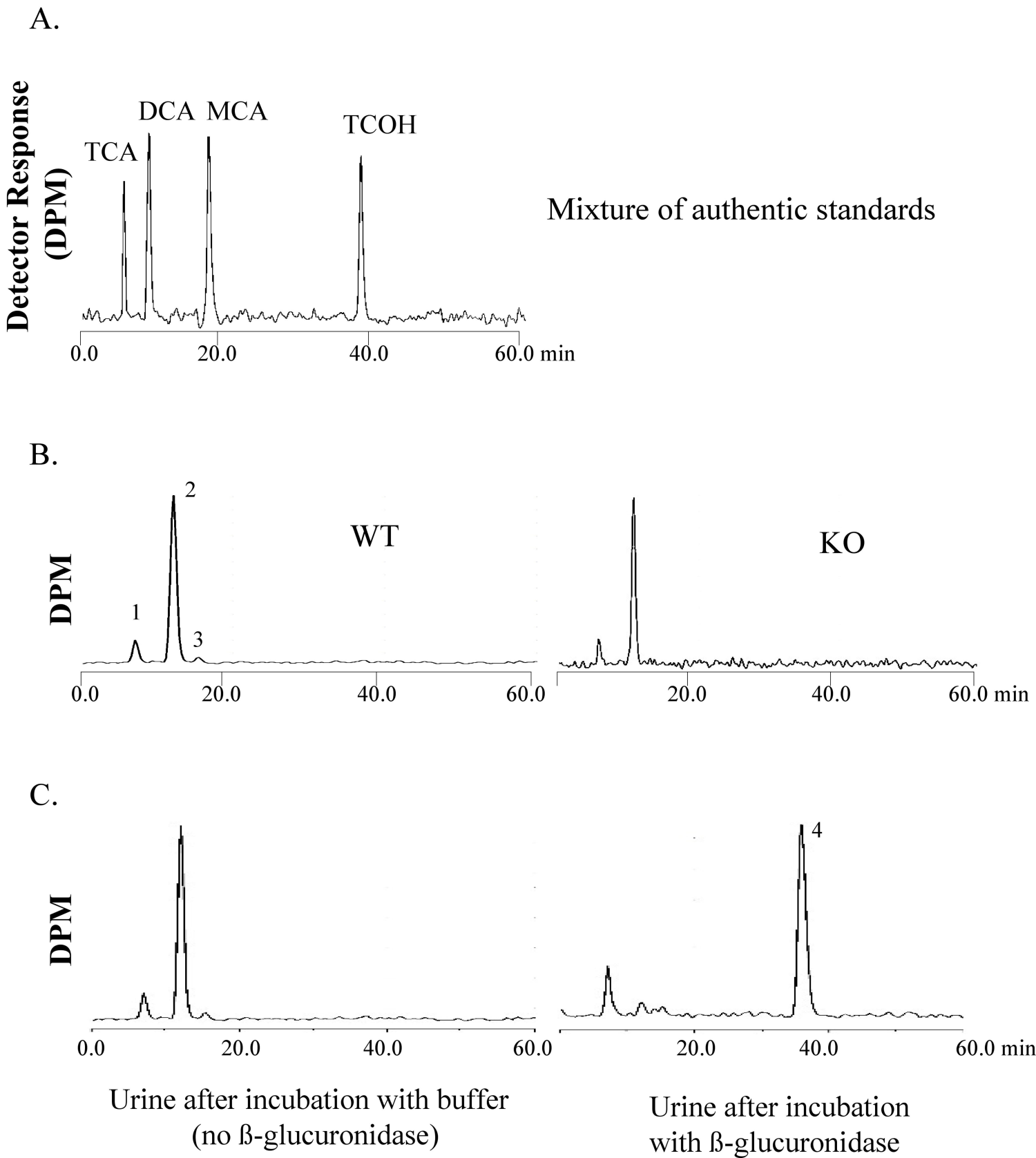


Fig. 5


A.
Detector Response (DPM)
TCA
DCA
MCA
TCOH
0.0
20.0
40.0
60.0 min
Mixture of authentic standards
B.
DPM
1
2
3
WT
KO
0.0
20.0
40.0
60.0
20.0
40.0
60.0 min
C.
DPM
4
0.0
20.0
40.0
60.0
20.0
40.0
60.0 min
Urine after incubation with buffer (no ß-glucuronidase)
Urine after incubation with ß-glucuronidase