Original Article

Metabolic Interactions between Organic Solvents — with Highly and Poorly Metabolized Compounds

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Abstract: Metabolic interactions between organic solvents were clarified with highly and poorly metabolized compounds. m-Xylene (m-X) and trichloroethylene (TRI) were used as examples of highly metabolized compounds. 1,1,1-Trichloroethane (1,1,1) was used as an example of a poorly metabolized compound. Adult male Wistar rats were exposed to various concentrations of m-X, TRI or 1,1,1 for 6 hours singly or as a combination of 2 compounds (m-X and TRI; m-X and 1,1,1). The blood concentrations of parent compounds (m-X, TRI or 1,1,1) at the end of the exposure and the urinary cumulative excretion of their metabolites (m-methylhippuric acid and/or trichloroethanol & trichloroacetic acid) during and after exposure were determined. In vitro metabolic interactions between m-X and TRI and those between m-X and 1,1,1 were also determined with microsomes from rat livers. The in vivo experiment showed that the metabolic interactions occur only when the exposure concentration of a coexistent solvent is higher than the metabolic saturation point. The in vitro experiment demonstrated that the patterns of metabolic interactions between the solvents used in this experiment approximated those of noncompetitive inhibition.

Key words: Metabolic interaction, m-Xylene, Trichloroethylene, 1,1,1-Trichloroethane, Cytochrome P450

INTRODUCTION

Clearance of foreign chemicals by hepatic metabolism depends on the relationship between intrinsic metabolic clearance (Vmax/Km, maximum velocity/Michaelis constant) and the hepatic blood flow (QH), and the metabolic clearance of foreign chemicals is often rate limited by QH independently of enzyme capacity (Vmax) when the exposure concentration is low (perfusion limited metabolism)\(^1\)). Compounds whose Vmax/Km is larger than QH are highly metabolized substrates\(^2\)). These compounds are almost completely metabolized while passing through the liver, as long as the concentration in the hepatic tissue (Gt) is low. Thus QH limits the extent of their metabolism. However, when the concentration in the inhaled air raises so that the metabolic clearance expressed in terms of Vmax/(Km+Gt) becomes smaller than QH, enzyme capacity becomes a factor limiting the metabolism (capacity limited metabolism)\(^1\)). On the other hand, for those solvents whose Vmax/Km is smaller than QH (poorly metabolized substrates), the enzyme activity limits the metabolism independently of the level of exposure (capacity limited metabolism)\(^2\)).

Xylene is a clear, colorless, aromatic liquid. This chemical is widely used as a solvent for paints, glues, printing inks, pesticides and adhesives, as a component of industrial and household products, and in the laboratory\(^3\)). The
A technical grade of mixed xylenes is a mixture of all 3 xylene isomers (o-, m-, p-), in which m-Xylene (m-X) is the major component. m-X is categorized as a highly metabolized compound. Trichloroethylene (TRI) and 1,1,1-trichloroethane (1,1,1) are industrial solvents used mainly for the degreasing of metal parts. These compounds are found in printing inks, varnishes, adhesives, paints, and lacquers. These substances have attracted great attention among the general public because of leakage from industrial settings into the general environment. TRI is also categorized as a highly metabolized compound. On the other hand, 1,1,1 is categorized as a poorly metabolized compound.

Most of the solvents used in industrial fields are a mixture of several compounds. When the metabolism of a chemical in the mixture is evaluated, the effect of the coexistent solvent has to be considered. The goal of the present study was to evaluate the metabolic interaction between highly metabolized organic solvents (m-X and TRI) and that between a highly metabolized organic solvent (m-X) and a poorly metabolized organic solvent (1,1,1). Our reasons for selecting these compounds are as follows: (1) we have performed animal experiments investigating the pharmacokinetics of these substances, (2) since the metabolites of these compounds are not physiologically excreted into the urine, each metabolite serves as an index of exposure.

In this experiment, we compared the metabolism of an organic solvent in a mixture with that of its respective single exposure. First, we determined the metabolic interactions in vivo. Next, we determined the metabolic interactions in vitro with the same organic solvents, and the type of inhibition (competitive, noncompetitive, or uncompetitive) was clarified.

### MATERIALS AND METHODS

#### Reagents

All the reagents used in this experiment were the highest grade (> 99.9%, Wako Pure Chemicals, Osaka) except for NADP, glucose-6-phosphate (both from Oriental Yeast, Tokyo) and glucose-6-phosphate dehydrogenase (Sigma Chemicals, U.S.A.).

#### Animals

Eighty 8-week-old male Wistar rats (Shizuoka Laboratory Animal Center, Shizuoka) were housed individually in metabolism cages in an air-conditioned room (22 ± 2°C) with artificial lighting from 06:00 to 18:00. They were maintained on unlimited pellet chow (Clea CE-2, Nippon Clea, Tokyo) and water ad libitum. Seventy rats were subjects for the in vivo study and 10 rats for the in vitro study. The animal experiments were performed in accordance with Guidelines for Animal Experiments, Yamanashi Medical University.

1. **in vivo study**

At 10 weeks of age, the rats were randomly divided into 14 groups. They were exposed to air containing m-X, TRI or 1,1,1 for 6 hours from 10:00 to 16:00. The inhalation exposure was performed in a dynamic flow exposure chamber as described previously. In the preliminary experiment performed in our laboratory, 6-hour exposure to these compounds resulted in metabolic saturation over a concentration of about 100–200 ppm. Moreover, according to the simulation study, a linear relationship between exposure concentration and internal dose (blood concentrations of parent compounds and urinary excretions of the metabolites) exists only when the exposure concentration remains below 100 ppm. Therefore, a low concentration should be less than 100 ppm.
pm, and a high concentration should be more than 200 ppm to clarify the dose-response effect on the metabolism of these compounds. In this experiment, exposure concentrations for each solvent were set at 35 ppm (a low concentration) and 350 ppm (a high concentration, 10 times the low concentration). This low concentration reflects those actually found in the industrial fields.

The patterns of exposure were as follows: (1) an exposure of 35 ppm \(m\)-X alone, (2) an exposure of 35 ppm TRI alone, (3) an exposure of 35 ppm 1,1,1 alone, (4) an exposure of 350 ppm \(m\)-X alone, (5) an exposure of 350 ppm TRI alone, (6) an exposure of 350 ppm 1,1,1 alone, (7) a combined exposure of 35 ppm \(m\)-X with 35 ppm TRI, (8) a combined exposure of 35 ppm \(m\)-X with 350 ppm TRI, (9) a combined exposure of 350 ppm \(m\)-X with 35 ppm TRI, (10) a combined exposure of 350 ppm \(m\)-X with 350 ppm TRI, (11) a combined exposure of 35 ppm \(m\)-X with 35 ppm 1,1,1, (12) a combined exposure of 35 ppm \(m\)-X with 350 ppm 1,1,1, (13) a combined exposure of 350 ppm \(m\)-X with 35 ppm 1,1,1, and (14) a combined exposure of 350 ppm \(m\)-X with 350 ppm 1,1,1.

At the end of the 6-hour exposure, a small blood sample (20\(\mu\)l) was taken with a micropipette from a cut in the tail, and the \(m\)-X, TRI or 1,1,1 concentration in the blood was measured by the vial-equilibration method. The operating conditions of gas chromatograph were as follows: 3 mm \(\times\) 2 m glass column packed with PEG-400 on Uniport B (GL Sciences, Tokyo) at 80°C; injection port temperature, 100°C; carrier gas, \(N_2\) at 70 ml/min; \(H_2\) at 20 ml/min.

Urine samples collected during a 12-hour period beginning at the start of exposure were diluted with distilled water to 100 ml, then centrifuged at 1,800 g for 5 min. The supernatant was analyzed for \(m\)-methylhippuric acid (\(m\)-MHA, a metabolite of \(m\)-X), trichloroethanol (TCE, a metabolite of TRI and 1,1,1), and trichloroacetic acid (TCA, a metabolite of TRI and 1,1,1). \(m\)-MHA was assayed with HPLC (Hitachi L-6200, Tokyo) equipped with a UV detector (Hitachi L-4000) and an integrator (Hitachi D-2500). The analytic conditions of HPLC were: column, Hitachi ODS, 4.6 mm \(\times\) 150 mm; mobile phase, acetonitrile: distilled water: acetic acid: \(\beta\)-cyclodextrine (200:800:15:15) at a flow rate of 1.0 ml/min; detection wavelength, 228 nm. TCE and TCA were analyzed essentially according to the head space gas chromatographic method originally described by Breimer et al. In brief, 0.1 ml of diluted urine was placed in a head space vial (Perkin-Elmer; U.S.A., 22 ml in volume) containing 0.05 ml methanol and 0.25 ml concentrated \(H_2SO_4\). The vial, capped with a Teflon lined stopper, was kept at 85°C to make the methyl ester of TCA and to hydrolyze TCE-glucuronide. The head space gas was transferred to a gas chromatograph (Hitachi 263–30) equipped with an electron capture detector, and analyzed for TCA and TCE. This series of procedures was performed with an autosampler (Perkin-Elmer Headspace Sampler HS40) and an integrator (Hitachi D-2500). Operating conditions of the autosampler were: thermostat temperature, 85°C; thermostat time, 65 min; transfer temperature, 120°C; pressurization time, 0.5 min; injection time, 4.8 seconds. The analytic conditions of the gas chromatograph were: column, 0.32 mm \(\times\) 30 m TC-WAX capillary (GL Sciences); column temperature, 130°C; carrier gas, \(N_2\) at a flow rate of 2.0 ml/min; split ratio, 40:1.

Rats from each exposure group were decapitated 6 hours after the end of exposure, and the liver was removed. The hepatic microsomal cytochrome P450 (CYP) content was deter-
mined spectrophotometrically according to the method described elsewhere\textsuperscript{11}).

2. \textit{in vitro} study

At 10 weeks of age, 10 male Wistar rats were killed by decapitation at 10:00, and the livers were removed. A 25\% (w/v) liver homogenate in 1.15\% KCl-0.01 M phosphate buffer (pH 7.4) was centrifuged at 10,000 g at 0°C for 10 min. The supernatant was further centrifuged at 105,000 g for 60 min to obtain microsomal pellets. The pellet was suspended in the solution and centrifuged again at 105,000 g for 60 min. The washed microsomal pellet was resuspended with the buffer solution, and the protein content was measured according to the method described by Lowry \textit{et al.}\textsuperscript{12}). The protein content was adjusted to a concentration of 10 mg/ml with the buffer solution, aerated with N\textsubscript{2} and stored frozen at −85°C until use.

\textit{m}-X, TRI or 1,1,1 metabolisms \textit{in vitro} were assessed by measuring the rate of substrate disappearance according to the method described by Sato and Nakajima\textsuperscript{13}) with the slight modification that the final volume of the reaction mixture was reduced to 0.5 ml, containing 0.1 ml of microsomal solution, 0.3 ml of cofactor solution, and 0.1 ml of substrate solution. The reaction mixture (0.5 ml) contained a final concentration of 1.0 mM NADP, 50 mM MgCl\textsubscript{2}, 20 mM glucose-6-phosphate, 2 units of glucose-6-phosphate dehydrogenase, substrate solution, and 50 mM K/K-phosphate buffer (pH 7.4), in addition to microsomes corresponding to 1.0 mg protein. Substrate concentrations for each solvent were set at 0.02, 0.04, 0.10 and 0.50 mM. In the case of a mixture, the concentrations of a mixed solvent, \textit{i.e.}, those of \textit{m}-X to TRI and TRI to \textit{m}-X and those of \textit{m}-X to 1,1,1 and 1,1,1 to \textit{m}-X, were set at 0.02 and 0.50 mM, which correspond to unsaturable and saturable concentrations in our preliminary experiment, respectively.

Statistics

The results were expressed as means ± SD. Means were tested by the Student-Newman-Keuls’ test\textsuperscript{14}). The 0.05 level of probability was chosen as significant.

RESULTS

1. \textit{in vivo} study

The blood concentrations of \textit{m}-X, TRI or 1,1,1 at the end of 6-hour exposure to each solvent alone and in combinations are shown in Table 1. There were no significant differences between each single exposure and the corresponding combined exposure in both the combination of \textit{m}-X and TRI and that of \textit{m}-X and 1,1,1 when the coexistent solvent concentration was low (35 ppm). However, significant differences were observed between each single exposure and the corresponding combined exposure in both the combination of \textit{m}-X and TRI and that of \textit{m}-X and 1,1,1 when the coexistent solvent concentration was high (350 ppm).

The cumulative amounts of \textit{m}-X, TRI or 1,1,1 metabolites (\textit{m}-MHA and/or TCE & TCA) in the urine during the 12 hours after the start of exposure to each solvent alone and in combinations are shown in Table 2. There were no significant differences between each single exposure and the corresponding combined exposure in both the combination of \textit{m}-X and TRI and that of \textit{m}-X and 1,1,1 when the coexistent solvent concentration was low (35 ppm). However, significant differences were observed between each single exposure and the corresponding combined exposure in both the combination of \textit{m}-X and TRI and that of \textit{m}-X and 1,1,1 when the coexistent solvent concentration was high (350 ppm).

The hepatic microsomal CYP contents after each combined exposure were not significantly
Table 1. Blood concentration of parent compounds at the end of 6-hour inhalation exposure.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>m-X (µM)</th>
<th>TRI (µM)</th>
<th>1,1,1 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-X: 35 ppm</td>
<td>7.40 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>TRI: 35 ppm</td>
<td>--</td>
<td>2.35 ± 0.4</td>
<td>--</td>
</tr>
<tr>
<td>1,1,1: 35 ppm</td>
<td>--</td>
<td>--</td>
<td>2.71 ± 0.2</td>
</tr>
<tr>
<td>m-X: 350 ppm</td>
<td>149 ± 20</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>TRI: 350 ppm</td>
<td>--</td>
<td>28.4 ± 1.2</td>
<td>--</td>
</tr>
<tr>
<td>1,1,1: 350 ppm</td>
<td>--</td>
<td>--</td>
<td>32.1 ± 2.2</td>
</tr>
<tr>
<td>m-X: 35 ppm + TRI: 35 ppm</td>
<td>7.69 ± 0.7</td>
<td>2.35 ± 0.4</td>
<td>--</td>
</tr>
<tr>
<td>m-X: 35 ppm + TRI: 350 ppm</td>
<td>9.47 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.2 ± 2.2</td>
<td>--</td>
</tr>
<tr>
<td>m-X: 350 ppm + TRI: 35 ppm</td>
<td>151 ± 10</td>
<td>3.48 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>--</td>
</tr>
<tr>
<td>m-X: 350 ppm + TRI: 350 ppm</td>
<td>209 ± 16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.0 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>--</td>
</tr>
<tr>
<td>m-X: 35 ppm + 1,1,1: 35 ppm</td>
<td>8.51 ± 0.8</td>
<td>--</td>
<td>2.83 ± 0.2</td>
</tr>
<tr>
<td>m-X: 35 ppm + 1,1,1: 350 ppm</td>
<td>9.93 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>--</td>
<td>33.3 ± 3.9</td>
</tr>
<tr>
<td>m-X: 350 ppm + 1,1,1: 35 ppm</td>
<td>175 ± 25</td>
<td>--</td>
<td>3.12 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>m-X: 350 ppm + 1,1,1: 350 ppm</td>
<td>190 ± 27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>--</td>
<td>38.4 ± 3.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values represent the mean ± SD for 5 rats.

<sup>b</sup> Significantly different from the corresponding single exposure at the same exposure concentration (p < 0.05).

Table 2. Cumulative amounts of urinary metabolites during 12 hours after the beginning of 6-hour inhalation exposure.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>m-MHA (µmol)</th>
<th>TCA (µmol)</th>
<th>TCE (µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-X: 35 ppm</td>
<td>20.3 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>TRI: 35 ppm</td>
<td>--</td>
<td>0.60 ± 0.06</td>
<td>6.55 ± 0.37</td>
</tr>
<tr>
<td>1,1,1: 35 ppm</td>
<td>--</td>
<td>0.019 ± 0.004</td>
<td>0.24 ± 0.04</td>
</tr>
<tr>
<td>m-X: 350 ppm</td>
<td>102 ± 6.5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>TRI: 350 ppm</td>
<td>--</td>
<td>4.43 ± 0.45</td>
<td>37.3 ± 4.0</td>
</tr>
<tr>
<td>1,1,1: 350 ppm</td>
<td>--</td>
<td>0.18 ± 0.03</td>
<td>1.76 ± 0.25</td>
</tr>
<tr>
<td>m-X: 35 ppm + TRI: 35 ppm</td>
<td>19.1 ± 1.0</td>
<td>0.56 ± 0.05</td>
<td>6.44 ± 1.4</td>
</tr>
<tr>
<td>m-X: 35 ppm + TRI: 350 ppm</td>
<td>16.9 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.24 ± 0.64</td>
<td>34.2 ± 4.5</td>
</tr>
<tr>
<td>m-X: 350 ppm + TRI: 35 ppm</td>
<td>101 ± 7.9</td>
<td>0.48 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.73 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>m-X: 350 ppm + TRI: 350 ppm</td>
<td>79.0 ± 6.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.42 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.1 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>m-X: 35 ppm + 1,1,1: 35 ppm</td>
<td>17.1 ± 3.0</td>
<td>0.017 ± 0.003</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>m-X: 35 ppm + 1,1,1: 350 ppm</td>
<td>15.2 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.16 ± 0.01</td>
<td>1.66 ± 0.15</td>
</tr>
<tr>
<td>m-X: 350 ppm + 1,1,1: 35 ppm</td>
<td>91.8 ± 8.2</td>
<td>0.014 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>m-X: 350 ppm + 1,1,1: 350 ppm</td>
<td>74.1 ± 12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.40 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values represent the mean ± SD for 5 rats.

<sup>b</sup> Significantly different from the corresponding single exposure at the same exposure concentration (p < 0.05).
different from those after the corresponding single exposure (data not shown). The values were almost the same as those of naive rats reported elsewhere[11).

2. in vitro study

The in vitro experiment indicated the presence of 2 pairs of Vmax and Km at higher (more than 0.10 mM) and lower (less than 0.10 mM) substrate concentrations (Figs. 1 & 2).

When the concentration of the mixed solvent was lower (0.02 mM), the metabolism was slightly inhibited compared with the corresponding single solution in both the combination of m-X and TRI and that of m-X and 1,1,1 (Figs. 1 & 2). However, the inhibition was not statistically significant. On the other hand, the metabolism in the mixture at a higher concentration of the mixed solvent (0.50 mM) was significantly

Fig. 1. Double reciprocal plots of metabolic velocity (v) versus substrate concentration (S). Substrate: A, m-Xylene (m-X) metabolism; B, trichloroethylene (TRI) metabolism. Symbols represent means of 10 determinations. Open square; single exposure. Closed lozenge; + low concentration (0.02 mM) of a mixed solvent. Closed triangle; + high concentration (0.50 mM) of a mixed solvent. Solid line; regression line at lower substrate concentrations. Dotted line; regression line at higher substrate concentrations.

Fig. 2. Double reciprocal plots of metabolic velocity (v) versus substrate concentration (S). Substrate: A, m-xylene (m-X) metabolism; B, 1,1,1-trichloroethane (1,1,1) metabolism. Symbols represent means of 10 determinations. Open square; single exposure. Closed lozenge; + low dose (0.02 mM) of a mixed solvent. Closed triangle; + high dose (0.50 mM) of a mixed solvent. Solid line; regression line at lower substrate concentrations. Dotted line; regression line at higher substrate concentrations.
inhibited compared with the corresponding single solution in both the combination of \(m\)-X and TRI and that of \(m\)-X and 1,1,1 (Figs. 1 & 2). As shown in Fig. 1, the metabolism of \(m\)-X was noncompetitively inhibited by TRI (Fig. 1A), and that of TRI was also noncompetitively inhibited by \(m\)-X (Fig. 1B). Figure 2 shows the metabolic interaction between \(m\)-X and 1,1,1. The inhibition between these 2 compounds was also noncompetitive with each other.

**DISCUSSION**

The present maximum allowable concentration of \(m\)-X recommended by the Occupational Exposure Limit Committee of the Japan Society of Occupational Health is 100 ppm, that of TRI is 50 ppm and that of 1,1,1 is 200 ppm\(^{15}\). In this experiment, the exposure concentrations for each solvent were set at 35 ppm and 350 ppm. The exposures at these concentrations did not show any adverse effect on hepatic enzyme contents. The present study showed that as long as exposure concentration does not exceed the present maximum allowable concentration recommended by the committee, metabolic interaction does not occur. Metabolic interactions between these compounds occur only when the exposure concentrations are higher than the current occupational exposure limit. Moreover, if these compounds are completely metabolized by the common CYP isozymes, their metabolism should be subject to competitive inhibition. However, their metabolism approximated noncompetitive inhibition in the *in vitro* experiment.

\(m\)-X is metabolized primarily to \(m\)-toluic acid, which is then conjugated with glycine and excreted as \(m\)-MHA in the urine\(^{17}\). The pathway from \(m\)-X to 2,4-xylenol accounts for only 1—2% of the total amount metabolized\(^{17}\). Tassaneeyakul *et al.*\(^{18}\) demonstrated that although CYP1A2 appears to be responsible for the formation of the minor metabolites of the xylene isomers, CYP2E1 catalyzed methylhydroxylation is the major determinant of the clearance of this compound. A major portion of TRI is metabolized by CYP isozymes to chloral hydrate, which is then oxidized to TCA or reduced to TCE, and these 2 compounds are excreted in the urine either directly (TCA) or after conjugation reaction (TCE)\(^{19}\). Nakajima *et al.*\(^{20}\) reported that CYP2E1, 2C11/6 and 2B1/2 are involved in the metabolic step from TRI to chloral hydrate, and the first isozyme may be a low-
Km TRI oxidase, and the rest a high-Km one. Moreover, the contribution of CYP2E1 in TRI oxidation was greater than that of CYP2C11/6. On the other hand, about 80% of 1,1,1 is exhaled unchanged, and only slightly metabolized to TCA and TCE by microsomal enzymes. Although the CYP isozymes involved in 1,1,1 metabolism have yet been identified, they appear to be common to those in TRI metabolism from the similarity of the chemical structure. In summary, some CYP isozymes have the same metabolism between \( m \)-X and TRI, and others have different metabolism. This may be true of the metabolism between \( m \)-X and 1,1,1.

Our in vitro experiment showed that the metabolic interactions between \( m \)-X and TRI and that between \( m \)-X and 1,1,1 approximated those of noncompetitive inhibition. These results may be due to the fact that some CYP isozymes have the same metabolism, and other CYP isozymes have different metabolism. In conclusion, metabolic interaction occurs only when the exposure concentration of a coexistent solvent is higher than the metabolic saturation point, and the metabolic inhibition between the solvents used in this experiment approximated that of noncompetitive inhibition.

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