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TOXICOKINETICS AND METABOLISM

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Toxicokinetic interaction of 2,5-hexanedione and methyl ethyl ketone

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Abstract Co-exposure to methyl ethyl ketone (MEK) potentiates the neurotoxicity of *n*-hexane in humans as well as in animals. This effect is associated with increased persistence of 2,5-hexanedione (2,5-HD) in blood, probably due to inhibition of 2,5-HD phase II biotransformation by MEK. There is no previous quantitative toxicokinetic model to describe this interaction. In this study we constructed a toxicokinetic model to depict the inhibition of 2,5-HD metabolism and elimination by MEK. Experimental data on 2,5-HD blood concentrations in rats from a published study were used to estimate model parameters. Three different inhibition mechanisms were evaluated: competitive, uncompetitive, and noncompetitive inhibition. Extrapolation from high to low doses was made to assess the interactive effects of MEK on 2,5-HD beyond experimental conditions. The models developed successfully described the toxicokinetic behavior of 2,5-HD when inhibited by MEK. The competitive inhibition model yielded a much lower estimate for the constant (65.5 mg/l) of 2,5-HD inhibition by MEK than did the uncompetitive and noncompetitive models (403 and 440 mg/l, respectively). The apparent half-life of 2,5-HD appeared to be a linear function of the Michaelis-Menten constant, and 2,5-HD and MEK concentrations in rats. The area under the curve of 2,5-HD in blood of rats was a nonlinear function of 2,5-HD and MEK concentrations in the blood. This study highlights the importance of the interactive effect of MEK on deactivation and elimination of 2,5-HD, and further illustrates the advantage of toxicokinetic modeling to investigate chemical interactions associated with exposure to multiple chemical agents.

Keywords Chemical mixture · Toxicokinetic interaction · Metabolic inhibition · 2,5-Hexanedione · Methyl ethyl ketone

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Introduction

Humans are often exposed to multiple chemical agents in environmental and occupational settings. There is limited research on chemical interactions that result in enhancement or attenuation of toxicological outcomes. These interactions can be broadly divided into toxicokinetic or toxicodynamic phases. As seen in Fig. 1 (Mehendale 1994; La and Swenberg 1996; Perera 1996), toxicokinetic interactions may occur during the process of absorption, metabolic activation and deactivation, and elimination. Toxicodynamic interactions take place during cellular and molecular processes of binding to macromolecules or cellular membranes, repair and dissociation of macromolecular adducts, damage, and recovery or regeneration of target cells. The focus of this research was to investigate the toxicokinetic interactions responsible for the enhancement of potential health effects due to co-exposure to 2,5-hexanedione (2,5-HD, molecular weight 114) and methyl ethyl ketone (MEK, molecular weight 72.1).

n-Hexane is known to cause neurotoxicity among occupationally exposed individuals and there are numerous reports of polyneuropathies associated with ''glue sniffing'' of hexane-containing solvents and glues (Spencer et al. 1980; Abou-Donia et al. 1982; Graham et al. 1995). The neurotoxic effect is characterized by

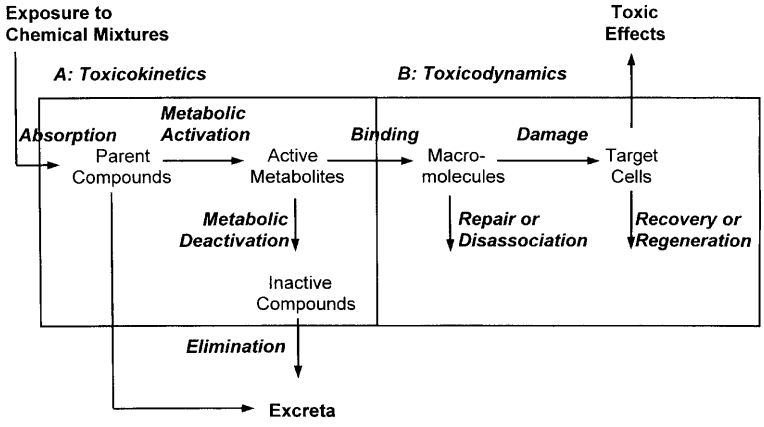

Fig. 1 Paradigm of potential toxicological interactions of chemical mixtures (adapted from Mehendale 1994; La and Swenberg 1996; Perera 1996)

loss of sensation and distal reflexes in the feet and hands after prolonged exposure to *n*-hexane. The current basis for explaning this pathogenesis is the formation of a pyrrole from a hexane metabolite, 2,5-HD, and subsequent reaction of the oxidized pyrrole with protein nucleophiles (Graham et al. 1995). In addition to central–peripheral distal axonopathy, experimental animals exposed to 2,5-HD exhibit testicular atrophy (Chapin et al. 1982; Boekelheide et al. 1989), possibly resulting from 2,5-HD-induced apoptosis of the germ cells (Allard and Boekelheide 1996; Blanchard et al. 1996).

Metabolism of *n*-hexane involves complex pathways (Fig. 2). *n*-Hexane is first metabolized by the hepatic mixed-function oxidase system to form 2-hexanol. The latter is metabolized further to either methyl *n*-butyl ketone (MnBK) or 2,5-hexanediol and then to 5-hydroxy-2-hexanone. Oxidation of 5-hydroxy-2-hexanone leads to the formation of 2,5-HD (Krasavage et al. 1980), which may be excreted from urine in free form or undergoes further metabolism to produce 4,5-dihydroxy-2-hexanone which is excreted from urine as the glucuronide (Fedtke and Bolt 1987). 2,5-HD is considered the ultimate toxic metabolite, and the neurotoxic potency of *n*-hexane is strongly correlated with the area under the serum concentration-time curve (AUC) of 2,5-HD, and not with the applied dose of *n*-hexane (Krasavage et al. 1980; Ralston et al. 1985).

MEK is often used as a solvent in the manufacturing of colorless synthetic resins, artificial leather, rubbers, lacquers, varnishes, and glues. It is usually found in mixtures with other solvents, including *n*-hexane (ATSDR 1992). Neurotoxicity increases as a result of co-exposure to MEK and either *n*-hexane, MnBK, or 2,5-HD in human studies, in vivo, and in vitro studies. MEK metabolism potentially interferes with *n*-hexane metabolism with implications for toxicokinetic interaction. MEK is first metabolized to either 2-butanol, a minor process, or to 3-hydroxy-2-butanone, a microsomal ω-1 oxidation and the limiting step of MEK metabolism. The latter metabolite is further metabolized to 2,3-butanediol (Diez et al. 1981; DiVincenzo et al. 1976). Because of similarities between the biotransformation of MEK and *n*-hexane it is plausible there may be competition between the two pathways.

Co-exposure to MEK may affect more than one step in the metabolism of *n*-hexane. Previous experiments suggest the concentration of 2,5-HD in blood decreases more slowly when rats are simultaneously exposed to MnBK and MEK (Abdel-Rahman et al. 1976), *n*-hexane and MEK (Robertson et al. 1989), and 2,5-HD and MEK (Yasui et al. 1995). The AUC of 2,5-HD in blood

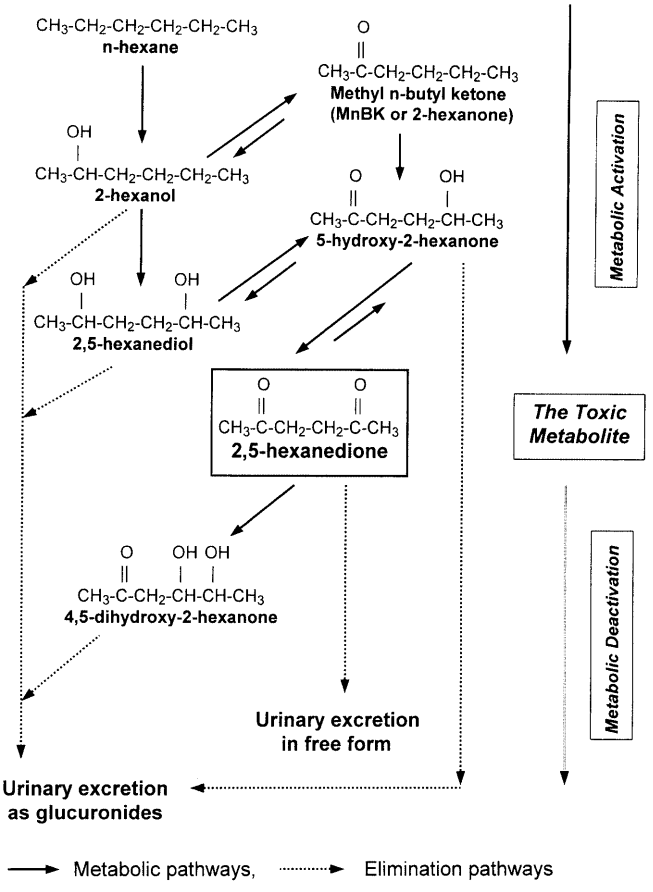

Fig. 2 Major metabolic pathways of *n*-hexane and its metabolites (adapted from Krasavage et al. 1980; Fedtke and Bolt 1987; Manini et al. 1998)

is greater in rats treated with 2,5-HD and MEK than in those treated with 2,5-HD alone (Ralston et al. 1985). Urinary excretion of 2,5-HD increases in guinea pigs co-administered both MnBK and MEK (Couri et al. 1978), but decreases in rats co-exposed to *n*-hexane and MEK (Iwata et al. 1983, 1984; Shibata et al. 1990a). Krishnan et al. (1994a) suggest that *n*-hexane exposure is an example of a single chemical that results in concomitant exposure to many chemicals because of the complex biotransformation processes. Co-exposure to MEK further complicates these interactions. Currently, there is no information in the literature describing these complex interactions. To investigate such complexity in a manageable manner it is necessary to limit the scope of the study by the use of simplifying assumptions.

The objective of this study was to evaluate the effectiveness of toxicokinetic modeling in investigation of the toxicological interaction between 2,5-HD and MEK. A toxicokinetic interaction model has been developed. Its kinetic parameters were estimated on the basis of fitting the model to published experimental data. Three different toxicokinetic mechanisms of interaction were evaluated: competitive, noncompetitive, and uncompetitive inhibition. The model was extrapolated from high to low doses to examine the effect of co-exposure to MEK on the AUC for 2,5-HD.

Materials and methods

Materials

Published data of Yasui et al. (1995), which investigated the blood concentration of 2,5-HD as influenced by co-exposure to 2,5-HD and MEK, were selected to estimate model parameters. The authors injected subcutaneously 2.6 mmol/kg 2,5-HD alone or 2.6 mmol/kg 2,5-HD plus MEK at 2.6 mmol/kg or 13 mmol/kg to male Wistar rats. Following treatment for 0.5, 1, 2, 4, 8, and 16 h, they killed the animals and determined the blood concentrations of 2,5-HD.

A model of toxicokinetic interaction between 2,5-HD and MEK

We addressed three issues on the toxicokinetic behavior of 2,5-HD as influenced by MEK: toxicokinetics of 2,5-HD alone, toxicokinetics of MEK alone, and the interaction between 2,5-HD and MEK. A single saturable pathway was used to describe the deactivation and elimination of 2,5-HD (Fig. 3). We used a one-compartmental model in which 2,5-HD was instantaneously available to the systemic circulation in rats administered 2,5-HD via subcutaneous injection. After the treatment, the mass balance governing 2,5-HD was

$$\frac{dM_{HD}}{dt} = -\frac{V_{\max,HD} \cdot C_{HD}}{K_{m,HD} + C_{HD}}, \tag{1}$$

where M_{HD} = total mass of 2,5-HD (mg), $C_{\mathrm{HD}} = M_{\mathrm{HD}}/V_{\mathrm{HD}}$ = concentration of 2,5-HD (mg/l), V_{HD} = volume of distribution of 2,5-HD (l), $V_{\max,\mathrm{HD}}$ = maximum elimination rate of 2,5-HD (mg/h), and $K_{\mathrm{m,HD}}$ = apparent Michaelis constant for 2,5-HD kinetics (mg/l).

Similarly, a single compartment model was used to model the kinetics of MEK (Fig. 3), with parameters $V_{\max,\mathrm{MEK}}$ and $K_{\mathrm{m,MEK}}$. Following the treatment of MEK, the mass balance governing MEK in the compartment was

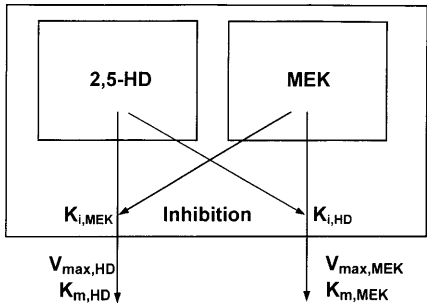

Fig. 3 A diagram of the toxicokinetic interaction model of 2,5-hexanedione (2,5-HD) and methyl ethyl ketone (MEK). The large compartment represents the overall model that consists of two distinct compound-specific compartments: 2,5-HD and MEK. Elimination of the compound is characterized by Michaelis-Menten kinetic parameters $V_{\max,\mathrm{HD}}$ and $K_{\mathrm{m,HD}}$ for 2,5-HD, and $V_{\max,\mathrm{MEK}}$ and $K_{\mathrm{m,MEK}}$ for MEK

$$\frac{dM_{MEK}}{dt} = -\frac{V_{\max,MEK} \cdot C_{MEK}}{K_{m,MEK} + C_{MEK}}, \tag{2}$$

where M_{MEK} = total mass of MEK (mg), $C_{\mathrm{MEK}} = M_{\mathrm{MEK}}/V_{\mathrm{MEK}}$ = concentration of MEK (mg/l), V_{MEK} = volume of distribution of MEK (l), $V_{\max,\mathrm{MEK}}$ = maximum elimination rate of MEK (mg/h), and $K_{\mathrm{m,MEK}}$ = apparent Michaelis constant for MEK kinetics (mg/l).

Fig. 3 depicts the interaction between 2,5-HD and MEK. The interaction may occur by one of three inhibition modes: competitive, uncompetitive, or noncompetitive. Competitive inhibition occurs when an inhibitor competes directly with a normal substrate for the same binding sites available on the enzyme. If MEK competitively inhibits 2,5-HD kinetics, Eq. (1) can be modified (Krishnan et al. 1994b), as follows,

$$\frac{dM_{HD}}{dt} = -\frac{V_{\max,HD} \cdot C_{HD}}{K_{m,HD} \cdot \left[1 + {\mathrm{CMEK}}/{\mathrm{Ki,\ MEK}}\right] + C_{HD}}, \tag{3}$$

where $K_{\mathrm{i,MEK}}$ (mg/l) in Eq. (3) refers to the constant for competitive inhibition of 2,5-HD by MEK.

Uncompetitive inhibition results from an inhibitor binding to the enzyme-substrate complex to produce an inactive enzyme–substrate–inhibitor complex. The presence of the inhibitor molecule not only affects formation of the final product but also influences production of the enzyme–substrate complex. If the interaction of 2,5-HD and MEK occurred under this mechanism, Eq. (1) can be modified (Krishnan et al. 1994b), as follows

$$\frac{dM_{HD}}{dt} = -\frac{C_{HD} \cdot \left[\dfrac{V_{\max,HD}}{1+{\mathrm{CMEK}}/{\mathrm{Ki,\ MEK}}}\right]}{C_{HD} + \left[\dfrac{K_{m,HD}}{1+{\mathrm{CMEK}}/{\mathrm{Ki,MEK}}}\right]}, \tag{4}$$

where $K_{\mathrm{i,MEK}}$ (mg/l) in Eq. (4) refers to the constant for uncompetitive inhibition of 2,5-HD by MEK.

In noncompetitive inhibition, an inhibitor binds not only to the free enzyme to form an enzyme–inhibitor complex but also to the enzyme–substrate complex to generate an inactive enzyme–substrate–inhibitor complex. If the interaction of 2,5-HD and MEK operated in this mode, Eq. (1) can be modified (Krishnan et al. 1994b), as follows

$$\frac{dM_{HD}}{dt} = -\frac{C_{HD} \cdot \left[\dfrac{V_{\max,HD}}{1+{\mathrm{CMEK}}/{\mathrm{Ki,\ MEK}}}\right]}{K_{m,HD} + C_{HD}}, \tag{5}$$

where $K_{i,MEK}$ (mg/l) in Eq. (5) refers to the constant for noncompetitive inhibition of 2,5-HD by MEK.

Effects of chemical interaction on half-life of chemical mixtures

The apparent half-life of 2,5-HD ($T_{1/2,HD}$) as inhibited by MEK can, by definition (Gibaldi and Perrier 1982), be described as:

$$T_{1/2},HD = \frac{0.693 \cdot V_{HD}}{\left(-\frac{dM_{HD}}{dt}/C_{HD}\right)}, \quad (6)$$

where V_{HD}, is volume distribution of 2,5-HD and the denominator $\left(-\frac{dM_{HD}}{dt}/C_{HD}\right)$ is the clearance of 2,5-HD (Gibaldi and Perrier 1982). In the case of MEK competitively inhibiting 2,5-HD, substitution of Eq. (3) into Eq. (6) and rearrangement of the latter equation gives

$$T_{1/2},HD = \frac{0.693 \cdot V_{HD}}{V_{max,HD}}(K_{m,HD} + C_{HD} + \frac{K_{m,HD}}{K_{i,MEK}} \cdot C_{MEK}). \quad (7)$$

In the cases of uncompetitive and noncompetitive inhibition of 2,5-HD by MEK, the $T_{1/2,HD}$ can be written in Eqs. (8) and (9), respectively, as follows:

$$T_{1/2},HD = \frac{0.693 \cdot V_{HD}}{V_{max,HD}}(K_{m,HD} + C_{HD} + \frac{C_{HD}}{K_{i,MEK}} \cdot C_{MEK}), \quad (8)$$

and

$$T_{1/2},HD = \frac{0.693 \cdot V_{HD}}{V_{max,HD}}(K_{m,HD} + C_{HD} + \frac{K_{m,HD} + C_{HD}}{K_{i,MEK}} \cdot C_{MEK}). \quad (9)$$

Estimation of model parameters

Liira et al. (1990) report the values of $V_{max,MEK}$ and $K_{m,MEK}$ for humans, 30 μmol/min (130 mg/h) and 2 μM (0.14 mg/l), respectively. In this study, we assumed the value of $K_{m,MEK}$ of rats was the same as that in the study of Liira et al. (1990), namely 0.14 mg/l. Based on the approach described by Gargas et al. (1986), the value of $V_{max,MEK}$ was extrapolated from humans to rats, as follows

$$\begin{aligned} V^{rat}_{max,MEK} &= V^{human}_{max,MEK} \times \left(\frac{BW^{rat}}{BW^{human}}\right)^{0.7} \\ &= 130mg/h \times \left(\frac{0.272kg}{71kg}\right)^{0.7} = 2.64mg/h, \end{aligned}$$

where body weights (BW) of rats and humans were based on data reported by Yasui et al. (1995) and Liira et al. (1990), respectively. Table 1 shows the equivalent volume of distribution of MEK to blood (V_{MEK} = 280 ml), which was estimated from physical volumes of the major physiological compartments (including fat, liver, slow-perfusion tissue group, rich-perfusion tissue group, and liver) of rats and the tissue/blood partition coefficients of MEK in these compartments.

The AR program of BMDP statistical software (Dixon 1990) was used to fit experimental concentration data of Yasui et al. (1995) and estimate kinetic parameters. The program is a derivative-free nonlinear regression procedure that estimates the parameters for a wide variety of nonlinear functions by the method of least squares (equivalent to the method of maximum likelihood under the assumption of Gaussian errors) using a pseudo-Gauss-Newton iterative algorithm. It simultaneously estimated four unknown model parameters: $V_{max,HD}$, $K_{m,HD}$, V_{HD}, and $K_{i,MEK}$. The AR program was run for three separate models that assumed the toxicokinetic interaction between 2,5-HD and MEK were competitive, uncompetitive, or noncompetitive inhibition, respectively. The natural logarithms of the predicted concentration were fitted to the logarithms of the observed data by unweighted least squares. The AR program reported the estimates and asymptotic standard errors of kinetic parameters for each model under the assumption of constant coefficient of variation for measurement error.

Table 1 Estimation of distribution volume of methyl ethyl ketone (MEK). Fat, liver, slow-perfusion tissue group (*SPG*), and rich-perfusion tissue group (*RPG*) consist of 4, 7, 5, and 75% of tissue volume, respectively (Arm and Travis 1988). Tissue/blood partition coefficients were estimated by Perbellini et al. (1984) for humans

Compartment	Volume (ml)	Tissue/blood coefficient	Volume (ml, blood equivalents)
Fat	19.0	0.88	17
Liver	10.9	0.98	11
SPG	204.0	1.16	237
RPG	13.6	1.12[a]	15
Total			280

Model simulations

Simulation studies were carried out to extrapolate the effect of co-exposure to MEK on the AUC of 2,5-HD from high to low doses. We ran a total of 186 simulations, involving all possible combinations of six regimens for 2,5-HD (0.1, 0.3, 0.6, 1.2, 2.0 and 3.0 mmol/kg) and 31 regimens for MEK (0 to 3.0 mmol/kg, with an increment of 0.1 mmol/kg). In each simulation experiment, numeric values were integrated with a time increment interval of 0.01 h by an Excel worksheet program. The AUC of 2,5-HD was estimated by the trapezoid rule, an approximate numeric integration algorithm (Gibaldi and Perrier 1982), to a time when the predicted concentration of 2,5-HD was less than 0.01 mg/l.

Results

The model, which assumed MEK competitively inhibited 2,5-HD metabolism and elimination, successfully fitted the experimental data of Yasui et al. (1995) (Fig. 4). The estimates of the kinetic parameters were $V_{max,HD}$ = 7.6 mg/h, $K_{m,HD}$ = 32.2 mg/l, V_{HD} = 264 ml, and $K_{i,MEK}$ = 65.5 mg/l. The model not only predicted the behavior of 2,5-HD in the blood of the rats exposed to 2.6 mmol/kg 2,5-HD alone (the line labeled with *HD* in Fig. 4) reasonably well, but also the exposures to 2.6 mmol/kg 2,5-HD plus 2.6 mmol/kg MEK (the line labeled with *HD + MEK*) and 2.6 mmol/kg 2,5-HD plus 13 mmol/kg MEK (the line labeled with *HD + 5MEK*). This toxicokinetic model demonstrated that the persistence of 2,5-HD in rats was MEK-dependent; the higher the MEK dose, the greater the persistence of 2,5-HD.

Figures 5 and 6 show the uncompetitive and noncompetitive inhibition models also fit reasonably well to the experimental data. The goodness-of-fit was not substantially different from that of the competitive model (Fig. 4). When the kinetic parameters were compared, the values of V_{HD}, $V_{max,HD}$, and $K_{m,HD}$ were not substantially different (Table 2). The mean squared error (MSE) obtained from the competitive model (MSE = 0.008124) was smaller than, but not substantially different from, those of the uncompetitive (MSE = 0.008747) and noncompetitive (MSE = 0.008424) models. In comparison,

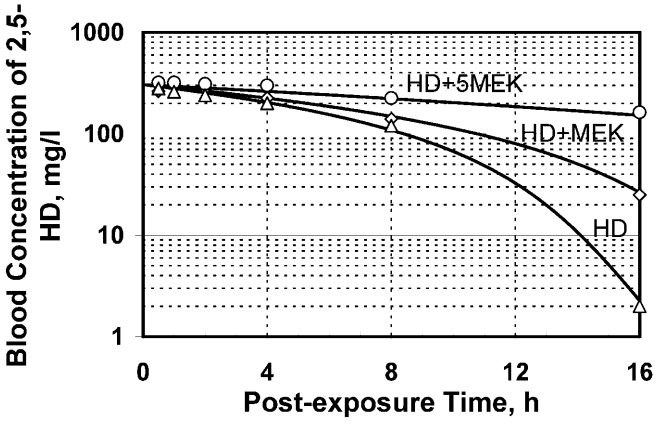

Fig. 4 Time course of 2,5-hexanedione (2,5-HD) concentration in the serum of male Wistar rats injected subcutaneously with 2.6 mmol/kg 2,5-HD alone (*HD*), or 2.6 mmol/kg 2,5-HD plus 2.6 mmol/kg methyl ethyl ketone (*HD + MEK*) or 13 mmol/kg MEK (*HD + 5MEK*). The *solid lines* are predictions of the competitive inhibition model [see Eq. (3) for model specification and Table 2 for model parameters]. The symbols represent experimental data from Yasui et al. (1995)

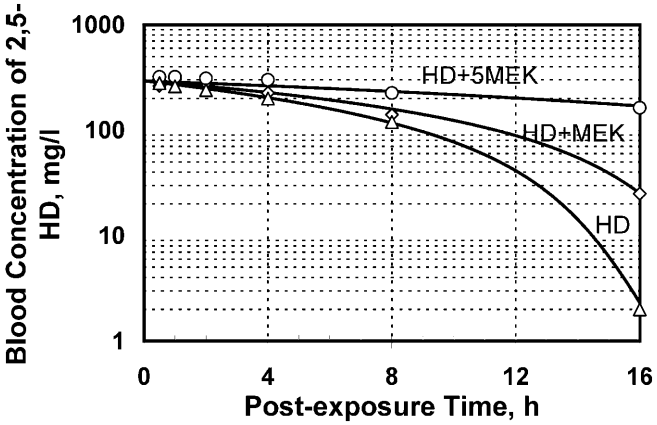

Fig. 5 Time course of 2,5-hexanedione (2,5-HD) concentration in the serum of male Wistar rats injected subcutaneously with 2.6 mmol/kg 2,5-HD alone (*HD*), or 2.6 mmol/kg 2,5-HD plus 2.6 mmol/kg methyl ethyl ketone (MEK) (*HD + MEK*) or 13 mmol/kg MEK (*HD + 5MEK*). The solid lines are predictions of the uncompetitive inhibition model [see Eq. (4) for model specification and Table 2 for model parameters]. The symbols represent experimental data from Yasui et al. (1995)

the inhibition constant for the competitive inhibition model ($K_{i,MEK} = 65.5 \pm 8.5$ mg/l) was substantially lower than those for noncompetitive inhibition (403 ± 86.5 mg/l) and uncompetitive inhibition (440 ± 77.1 mg/l).

The competitive inhibition model was used to extrapolate the behavior of 2,5-HD in rats as influenced by MEK from high to low doses. Figure 7 depicts the AUC of 2,5-HD as a function of the co-exposure dose of MEK when rats are co-administered 2,5-HD at 0.1, 0.3, 0.6, 1.2, 2.0, and 3.0 mmol/kg. The figure shows the $\log_{10}$(AUC) increases in an approximately linear manner with increases in MEK dose ranging from 0 to 3 mmol/kg. This corresponds to an increase in AUC by a factor of approximate 10^{β} per unit dose of MEK, where β is the slope of the linear relationship.

Table 3 summarizes the approximate slopes of the linear relationships and the corresponding values of 10^{β} at various doses of 2,5-HD. The slopes decreased from 0.399 to 0.09 [$\log_{10}$(AUC) per mmol/kg MEK] with increases in 2,5-HD doses from 0.1 to 3.0 mmol/kg. For every increment of 1 mmol/kg MEK, the AUC increases approximately 2.5-fold in the rat co-administered 0.1 mmol/kg 2,5-HD. In comparison, the AUC increases only by approximately 1.23-fold at 3.0 mmol/kg 2,5-HD (Table 3). These results suggested that the 2,5-HD AUC enhancement by co-exposure to MEK increased slower at high doses than at low doses of 2,5-HD, probably as a result of increasing saturation of metabolism and elimination of 2,5-HD.

Equations (7)–(9) show that apparent $T_{1/2,HD}$ is a linear function of $K_{m,HD}$, C_{HD} and C_{MEK}. Figure 8 depicts the $T_{1/2,HD}$ as a function of MEK concentration for various 2,5-HD concentrations of 10, 75, 150, 250 and 350 mg/l. At 10 mg/l 2,5-HD, which is approximately equal to the predicted initial 2,5-HD concentration in rats injected subcutaneously with 0.1 mmol/kg, the $T_{1/2,HD}$ was 1 h when there was no inhibition by MEK. In comparison, at 350 mg/l, which is approximately equal to the initial 2,5-HD concentration in rats exposed to 3 mmol/kg, the apparent half-life of 2,5-HD in rats was about 9 h. When MEK was co-administered to rats, the $T_{1/2,HD}$ increased approximately 1.2 h for every increment of 100 mg/l MEK.

Discussion

The objective of this study was to use toxicokinetic modeling to investigate the mechanistic basis for the interaction of MEK with 2,5-HD. The model focused upon the metabolism and elimination of 2,5-HD, without considering the complex interactions of intermediate metabolites (Figs. 1 and 2). The agreement between model predictions and experimental data suggested reasonable model specification and parameter estimation, as well as a possible mechanism for the toxicokinetic interaction between 2,5-HD and MEK. The model, although not physiologically based, captured the essential feature of the interaction of 2,5-HD with MEK and demonstrated the utility of modeling in investigating toxicokinetic interactions between chemical mixtures. It highlighted the significance of inhibiting deactivation and elimination of a toxic metabolite on its persistence in the body.

Inhibition of metabolic and elimination pathways increased the persistence of 2,5-HD in rats co-exposed to 2,5-HD and MEK. Ralston et al. (1985) found concomitant administration of MEK and 2,5-HD increased the (2–9 h) AUC of 2,5-HD from 456 to 811 μg h/ml in rats orally exposed once to 2,5-HD alone and 2,5-HD plus MEK, respectively. In an experiment using rats

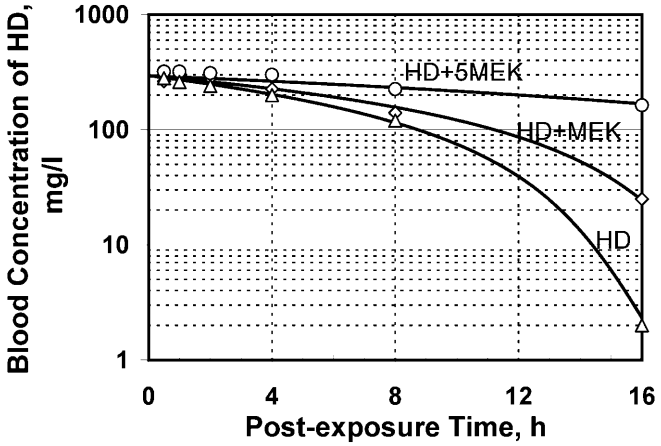

Fig. 6 Time course of 2,5-hexanedione (2,5-HD) concentration in the serum of male Wistar rats subcutaneously injected with 2.6 mmol/kg 2,5-HD alone (*HD*), or 2.6 mmol/kg 2,5-HD plus 2.6 mmol/kg methyl ethyl ketone (MEK) (*HD+MEK*) or 13 mmol/kg MEK (*HD+5MEK*). The *solid lines* are predictions of the noncompetitive model [see Eq. (5) for model specification and Table 2 for model parameters]. The symbols represent experimental data from Yasui et al. (1995)

Table 2 Parameters used to fit models of different inhibition mechanisms of methyl ethyl ketone (MEK) on 2,5-hexanedione (2,5-HD). Kinetic parameters are shown as regression estimates ± asymptotic standard error (V_{HD} volume of distribution of 2,5-HD, $V_{max,HD}$ maximum rate of 2,5-HD metabolism and elimination, $K_{m,HD}$ apparent constant of Michaelis-Menten kinetics for 2,5-HD, $K_{i,MEK}$ constant of 2,5-HD inhibition by MEK)

Parameter	Inhibition mechanism		
	Competitive	Uncompetitive	Noncompetitive
V_{HD} (ml)	264.3 ± 6.9	275 ± 7.0	273.9 ± 6.9
$V_{max,HD}$ (mg/h)	7.6 ± 0.7	6.7 ± 0.6	6.9 ± 0.6
$K_{m,HD}$ (mg/l)	32.2 ± 8.7	20.7 ± 7.0	22.8 ± 7.7
$K_{i,MEK}$ (mg/l)	65.5 ± 8.5	403.0 ± 86.5	440.2 ± 77.1
Mean squared error	0.008124	0.008747	0.008428

repeatedly exposed for 7 days, the AUC increased from 549 to 860 μg h/ml in groups receiving 2,5-HD alone and 2,5-HD plus MEK, respectively. Ralston et al. (1985) postulated that the increased persistence of 2,5-HD observed in rats was due to competitive inhibition of 2,5-HD by MEK. Yasui et al. (1995) show similar results in rats injected subcutaneously with 2,5-HD and MEK at different doses. However, the kinetic mechanism had not been elucidated by these experiments. We employed a toxicokinetic model to test the hypothesis that inhibition of 2,5-HD metabolism and elimination was likely to be responsible for the increased persistence of 2,5-HD in rats co-exposed to 2,5-HD and MEK. Our results suggest that a single compartment model with saturable elimination kinetics adequately describes the effects of MEK on the metabolism and elimination of 2,5-HD in rats. MEK inhibits the metabolism and elimination of 2,5-HD so that 2,5-HD persists in rats substantially greater in the co-exposure groups than in the group receiving 2,5-HD alone.

2,5-HD in rats undergoes further phase II metabolism and is partially excreted in free form by the kidneys. Manini et al. (1998) found that 2-hexanol, 5-hydroxy-2-hexanone, and 4,5-dihydroxy-2-hexanone are major glucuronide-conjugated metabolites excreted in the urine of rats exposed to *n*-hexane. The 'total' 2,5-HD, determined after strong acid treatment of urine samples, is composed of 2,5-HD converted from 5-hydroxy-2-hexanone and 4,5-dihydroxy-2-hexanone glucuronides and from a small fraction of 'free' 2,5-HD (Fedtke and Bolt 1987; Manini et al. 1998). The amounts of 2,5-HD determined after complete acid hydrolysis minus those of free 2,5-HD in rats exposed to *n*-hexane are about 10 times higher than those of freely extractable 2,5-HD (Fedtke and Bolt 1987). As shown in Fig. 2, 2,5-HD may be converted into 5-hydroxy-2-hexanone and 4,5-dihydroxy-2-hexanone (Fedtke and Bolt 1987; Soriano et al. 1996). These studies suggest disappearance of 2,5-HD in rats probably results from phase II metabolism to form 5-hydroxy-2-hexanone and 4,5-dihydroxy-2-hexanone glucuronides, which are the predominant pathways of 2,5-HD detoxification, and from elimination of free 2,5-HD, a relatively minor pathway. Following *n*-hexane administration, the rates of appearance in urine of both 4,5-dihydroxy-2-hexanone and free 2,5-HD appear to be saturable (Fedtke and Bolt 1987), which is consistent with our model assumptions.

The method of goodness-of-fit did not clearly identify the mode of metabolic inhibition in this study although it has been used to elucidate the inhibitory mechanism of metabolic interactions between chemicals in other studies (Andersen et al. 1987; Purcell et al. 1990; Sato et al. 1991; Tardif et al. 1993; Barton et al. 1995; El-Masri et zal. 1996). In most cases, there is clear evidence that a particular inhibitory mechanism provides better goodness-of-fit than others in toxicokinetic modeling. However, as shown by the similar fits among Figs. 4, 5 and 6, it was difficult to discern among the three inhibition mechanisms. The mean squared error for the competitive inhibition model was only slightly smaller than those for the uncompetitive and the noncompetitive models (Table 2), and the differences could not be statistically tested because the three models were not nested. These results suggest that the goodness-of-fit of toxicokinetic modeling to experimental data may not always result in clearly differentiating mechanisms of inhibition in metabolic interaction studies. The reason why the goodness-of-fit method works in other studies but not here was not clear. It may be due to the differences in study design with respect to doses and sampling times.

The competitive inhibition model yielded a much lower estimate for the inhibition constant $K_{i,MEK}$ than did the uncompetitive and noncompetitive models (Table 2). Fitting the experimental data of Yasui et al. (1995) to the competitive inhibition model gave the lowest estimate of the inhibition constant among the

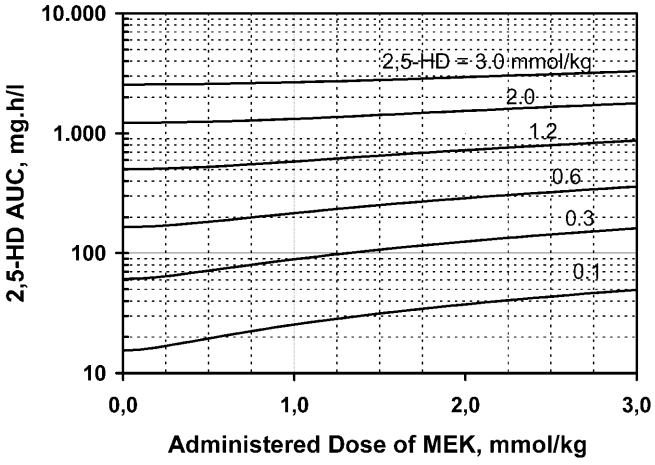

Fig. 7 Effects of co-exposure to methyl ethyl ketone (MEK) on the area under the serum concentration-time curve (AUC) for 2,5-hexanedione (2,5-HD) at various doses. The competitive inhibition model [see Eq. (3) for model specification and Table 2 for model parameters] predicts the values of 2,5-HD AUC, based on the assumption that rats were treated subcutaneously with 2,5-HD and MEK

three inhibition mechanisms. This value (65.5 mg/l) was also much closer to the estimate of the corresponding Michaelis-Menten constant (32.2 mg/l) than those estimated by the uncompetitive (403 vs 20.7 mg/l) or the noncompetitive model (440 vs 22.8 mg/l). These results were similar to those found in the study of Thrall and Poet (2000), in which the inhibition constants of toluene and trichloroethylene in the competitive model were reported to be smaller than those under the uncompetitive and noncompetitive models. The authors argued that competitive inhibition was the most plausible type of metabolic interaction between these two solvents because the inhibition constants are closest to the corresponding Michaelis-Menten constants. Based on similar reasoning, we favor competitive inhibition to represent the most appropriate mechanistic model for the chemical interaction between 2,5-HD and MEK, although uncompetitive and noncompetitive inhibition modes could not be entirely ruled out.

Extrapolation of the behavior of 2,5-HD AUC in the blood from high to low doses of 2,5-HD and MEK was a major application for the toxicokinetic model developed in this study. The experimental data of Yasui et al. (1995) focused on the behavior of 2,5-HD as influenced by MEK at high dose. The concave nature of the 2,5-HD concentration profiles highlights the saturation of 2,5-HD metabolism (*HD* lines in Figs. 4, 5 and 6) and increased persistence of 2,5-HD by co-administration of MEK (*HD+MEK* and *HD+5MEK* lines). Since 2,5-HD AUC has been shown to correlate with the neurotoxic potency of *n*-hexane (Krasavage et al. 1980; Ralston et al. 1985), the AUC was extrapolated from the high dose region, more often used in toxicological experiments, to low dose, which is relevant to the exposure levels found in the environment or the workplace. Figure 7 depicts the predicted 2,5-HD AUC in

Table 3 The slope (β) and the corresponding 10^{β} of the linear relationship between $\log_{10}$ [area under the serum concentration-time curve (AUC)] for 2,5-hexanedione (2,5-HD) and methyl ethyl ketone (MEK) at various doses of 2,5-HD

2,5-HD dose (mmol/kg)	β [$\log_{10}$(AUC) per MEK dose]	10^{β}
0.1	0.399	2.50
0.3	0.340	2.19
0.6	0.278	1.90
1.2	0.198	1.58
2.0	0.136	1.37
3.0	0.090	1.23

rats co-administered 2,5-HD and MEK subcutaneously at a variety of dose ranges under the competitive inhibition model.

The apparent 2,5-HD $T_{1/2}$ is quantitatively related to the kinetic parameters of metabolic inhibition. Yasui et al. (1995) showed that MEK is capable of prolonging the 2,5-HD $T_{1/2}$ in the blood, which was thought to relate to the potentiation of 2,5-HD neurotoxicity by MEK. Zhao et al. (1998) showed that MEK (as well as acetone and toluene) decreased the elimination rate constants of 2,5-HD in co-administration groups. These empirical observations consistently suggest a qualitative relationship between 2,5-HD $T_{1/2}$ and 2,5-HD and MEK concentrations. However, there have been no quantitative relationships documented in the literature. In this study, we showed that the apparent 2,5-HD $T_{1/2}$ following co-administration of 2,5-HD and MEK was a linear function of the apparent Michaelis-Menten constant and the concentrations of 2,5-HD and MEK, and depended on the nature of the metabolic inhibition [Eqs. (7)–(9)]. The limiting conditions of these relationships are discussed below:

1. At low concentrations of 2,5-HD ($C_{\mathrm{HD}} \to 0$) and no MEK inhibition ($C_{\mathrm{MEK}} = 0$), $T_{1/2,\mathrm{HD}}$ in Eqs. (7)–(9) is reduced to a constant

$$T_{1/2,HD} = \frac{0.693 \cdot V_{HD} \cdot K_{m,HD}}{V_{\max,HD}},$$

   a limiting condition of linear kinetics of metabolism.
2. When C_{HD} concentrations are relatively high and no MEK inhibition occurs ($C_{\mathrm{MEK}} = 0$), Eqs. (7)–(9) can be reduced to

$$T_{1/2,HD} = \frac{0.693 \cdot V_{HD}}{V_{\max,HD}}(K_{m,HD} + C_{HD}),$$

   a nonlinear kinetic condition of 2,5-HD metabolism that is consistent with the derivation by Gibaldi and Perrier (1982).
3. When the blood concentration of MEK is high, in addition to $K_{\mathrm{m,HD}}$ and C_{HD}, $T_{1/2,\mathrm{HD}}$ is also a function of the MEK concentration that is multiplied by a factor $\left(\frac{K_{m,HD}}{K_{i,MEK}}\right)$, $\left(\frac{C_{HD}}{K_{i,MEK}}\right)$, or $\left(\frac{K_{m,HD}+C_{HD}}{K_{i,MEK}}\right)$ for competitive, uncompetitive, or noncompetitive inhibition, respectively.

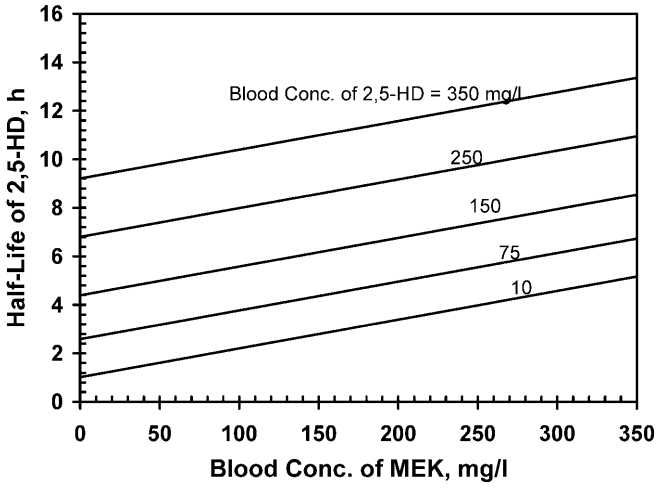

Fig. 8 Effects of co-exposure to methyl ethyl ketone (MEK) on the apparent half-life of 2,5-hexanedione (2,5-HD) at various blood concentrations. The latter is a linear combination [see Eq. (7)] of the apparent Michaelis-Menten constant, and the concentrations of 2,5-HD and MEK in blood

Although 2,5-HD and MEK can theoretically interact with each other (Fig. 3), inhibition of MEK metabolism by 2,5-HD may be relatively unimportant. We conducted a sensitivity analysis to examine the impact of the competitive inhibition constant of MEK by 2,5-HD ($K_{i,HD}$) on other kinetic parameters and mean squared errors. A modification of Eq. (2), multiplying the $K_{m,MEK}$ by $\left(1 + \mathrm{CHD}/\mathrm{Ki,HD}\right)$, was used in the model. Our analyses found that the models including $K_{i,HD}$ above 600 mg/l did not improve the goodness-of-fit at all in comparison to the model including $K_{i,HD}$ at 600 mg/l. Under the latter condition, other kinetic constants were estimated to be: V_{HD} 264.5 ml, $V_{max,HD}$ 7.6 mg/h, $K_{m,HD}$ 32.2 mg/l, and $K_{i,MEK}$ 66.0 mg/l. These values were very close to those estimated when $K_{i,HD}$ was not included in the model (V_{HD} 264.3 ml, $V_{max,HD}$ 7.6 mg/h, $K_{m,HD}$ 32.2 mg/l, and $K_{i,MEK}$ 65.5 mg/l; Table 2). The mean squared errors under these two conditions differed by only 1.5%. In contrast, when $K_{i,HD}$ was less than 600 mg/l, the kinetic parameters gradually departed from the values shown in Table 2. The mean squared errors also increased substantially, suggesting poor goodness-of-fit under the conditions of $K_{i,HD} < 600$ mg/l. Because inclusion of $K_{i,HD}$ did not improve the fit, we eventually eliminated $K_{i,HD}$ from the model to simplify it without loss of descriptive power.

Metabolic interactions occurring in the process of *n*-hexane activation to 2,5-HD could affect the metabolism of *n*-hexane in the body. Andersen and Clewell (1983) considered multiple competitive interactions between hexane, MnBK and 2,5-HD. They used a physiologically based pharmacokinetic model to describe the kinetics of the three compounds and suggested that at high concentrations or continuous exposure the formation of 2,5-HD may be inhibited in part because 2,5-HD and MnBK might inhibit hexane metabolism. In a study of Iwata et al. (1983), rats inhaled *n*-hexane plus MEK at 1000 ppm for 8 h. Forty-eight-hour urinary excretion of 2-hexanol decreased in the animals exposed to *n*-hexane and MEK, suggesting that production of 2-hexanol was inhibited by MEK. In another study (Iwata et al. 1984), rats were exposed to hexane and MEK at 500 ppm, 8 h per day for 33 weeks. The authors demonstrated similar decreases in elimination of 2-hexanol. The studies of Shibata et al. (1990a, 1990b) showed that serum level of 2-hexanol and its elimination in urine decreased in animals exposed to mixtures of hexane and MEK, compared with those exposed to hexane only. In a study of human volunteers exposed to a mixture of hexane and MEK (Van Engelen et al. 1997), the serum concentration of 2,5-HD decreased and the time to reach maximum 2,5-HD concentration increased. Previous studies show that hydroxylation of hexane is inhibited by the presence of MEK as a result of competition for the CYP2E1 enzyme that is required for biotransformation of hexane (Nakajima et al. 1991) and MEK (Brady et al. 1989; Raunio et al. 1991). In brief, many steps in the activation of *n*-hexane to 2,5-HD, as outlined in Fig. 2, can be affected by co-exposure to MEK. Our study could not address this issue.

There is no compelling evidence to indicate that MEK affects the toxicity of hexane or MnBK at the toxicodynamic level. In one relevant report (Ralston et al. 1985), rats were treated for 3 weeks with 2.2 mmol/kg per day 2,5-[^{14}C]HD alone or in combination with 2.2 mmol/kg per day MEK. Total ^{14}C activity was examined in peripheral nerve crude homogenate, neurofilament-enriched fraction of peripheral nerve, spinal cord crude homogenate, and neurofilment-enriched fraction of spinal cord. The binding of radiolabeled 2,5-HD to the nervous tissues and their corresponding neurofilament-enriched fractions in rats exposed to 2,5-HD plus MEK were not consistently different from those in animals exposed to 2,5-HD alone. These results indicate that enhancement of *n*-hexane-induced neurotoxicity by MEK does not result from increased binding of 2,5-HD to neurofilament at toxicodynamic level.

Although our study applied toxicokinetic modeling to shed new light on the impact of MEK on 2,5-HD metabolism and elimination, we did not take into consideration species extrapolation (rats to humans), differences in routes of administration (subcutaneous injection to inhalation), or exposure regimen (acute to chronic) for the developed toxicokinetic model. Further studies are needed to address these issues.

We conclude that the persistence of 2,5-HD in rats results from saturable inhibition of 2,5-HD metabolism and elimination by MEK. The estimated values of kinetic parameters provide a quantitative basis for description of 2,5-HD deactivation and elimination processes in the presence of MEK. The apparent half-life of 2,5-HD is a linear function of the apparent Michaelis-

Menten constant, and 2,5-HD and MEK concentrations in rats. The AUC of 2,5-HD in rats is a nonlinear function of 2,5-HD and MEK concentrations in the blood. This study demonstrates the utility of toxicokinetic modeling in the investigation of metabolic interactions between chemical mixtures.

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