Regulatory Toxicology and Pharmacology 59 (2011) 364–374

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

# The impact of CYP2E1 genetic variability on risk assessment of VOC mixtures

Hana R. Pohl *, Franco Scinicariello

*Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Atlanta, GA 30333, USA*

ARTICLE INFO

*Article history:*
Received 14 July 2010
Available online 2 February 2011

*Keywords:*
CYP2E1
Chemical mixtures
VOC interaction
Genetic polymorphism

ABSTRACT

Humans are simultaneously exposed to multiple chemicals in the environment. Many of the chemicals use the same enzymes in their metabolic pathways. Competitive inhibition may occur as one of the possible interactions between the xenobiotics in human body. For example, many volatile organic compounds (VOCs) are metabolized using P450 enzymes, specifically *CYP2E1*. Inheritable gene alterations may result in changes of function of the enzymes in different human subpopulations. Variations in quantity and/or quality of particular isoenzymes may cause differences in the metabolism of VOCs. These variations may cause higher sensitivity in certain populations. Using examples of three different mixtures, this review paper outlines the variances in *CYP2E1* isoenzymes, effect of exposure to such mixtures on sensitive populations, and approaches to mixtures risk assessment.

Published by Elsevier Inc.

## 1. Introduction

Volatile organic compounds (VOCs) are pollutants that are often associated with human activities. They are widely encountered in our environment at low levels in air, water, and even soil. For example, when the US Geological Survey tested samples of untreated groundwater from 1255 domestic drinking water and 242 public supply wells, VOCs were detected in 44% of the samples (Squillace et al., 2002). VOCs are also frequently found at hazardous waste sites. In fact, of the thousands of chemicals found at hazardous waste sites, 6 of the first 10 most often detected chemicals were VOCs (Fay and Mumtaz, 1996). However, indoor air pollution is usually first to come to mind when discussing VOCs. Indeed, VOC concentrations measured in the indoor air often exceed (up to 10 times) concentrations in the outdoor air (EPA, 2010). The Agency for Toxic Substances and Disease Registry (ATSDR) prepared numerous toxicological profiles that summarized and interpreted current information about individual VOCs (www.atsdr.cdc.gov/toxpro2.html). However, people are more likely exposed to VOC mixtures than to single chemicals. Therefore, issues of possible chemical interactions must be addressed. The interactions of VOCs were discussed in three ATSDR's interaction profiles (www.atsdr.cdc.gov/interactionprofiles/) that evaluated the following mixtures:

- 1,1,1-trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloroethylene;
- chloroform, 1,1-dichloroethylene, trichloroethylene, and vinyl chloride;
- benzene, toluene, ethylbenzene, and xylenes.

In these interaction profiles, the mixtures of VOCs were evaluated according to the ATSDR Guidance Manual (ATSDR, 2004). Briefly, the evaluation of the first two mixtures used an additivity approach (i.e., hazard index) based on the components of the mixtures. The results were qualitatively augmented by the weight-of-evidence (WOE) evaluation method (Mumtaz and Durkin, 1992). The third mixture was evaluated based on the physiologically based pharmacokinetic (PBPK) model (see farther for more details). The ATSDR's methodology was also described in detail in several publications (Pohl et al., 2009; Pohl and Abadin, 2008).

This paper will discuss concerns regarding exposures to mixtures of VOCs in relation to sensitive populations. The VOCs in the above mixtures are metabolized using P450 enzymes, specifically CYP2E1. Inheritable gene alterations may result in changes of function of the enzymes in different subpopulations (Daly et al., 1993). Variations in quantity and/or quality of particular isoenzymes may cause differences in metabolism of chemicals seen among species, sexes, age groups, etc. These differences may cause higher sensitivity in certain population groups.

## 2. VOC mixtures

### 2.1. Mixture of 1,1,1-trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloroethylene

#### 2.1.1. Background information

Chemicals in this mixture have been widely used in dry cleaning and textile-processing (tetrachloroethylene), vapor degreasing

* Corresponding author. Address: Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine, 1600 Clifton Road, F-62 Atlanta, GA 30333, USA. Fax: +1 770 488 3470.
*E-mail address:* hpohl@cdc.gov (H.R. Pohl).

0273-2300/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.yrtph.2011.01.013

of fabricated metal parts (tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane), or in manufacturing other chemical products such as vinyl chloride and high vacuum rubber (1,1-dichloroethane).

The mixture of 1,1,1-trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloroethylene was found in water at 26 sites of the 1608 on the US National Priority List (NPL) of hazardous waste sites recorded in the ATSDR's HazDat database. It was also the most frequently occurring mixture of four volatile organic chemicals (Pohl et al., 2010). Combinations of three of the chemicals were found in waters at even more sites. Trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane were reported together at 50 sites in 60 completed exposure pathways (i.e., people were actually exposed to the mixture). Trichloroethylene, 1,1,1-trichloroethane, and 1,1-dichloroethane were reported in water at 36 sites in 48 completed exposure pathways. Therefore, this mixture became a priority for ATSDR.

Trichloroethylene is the only one of the four chemicals that is extensively metabolized; the remaining three are predominately excreted unmetabolized in exhaled air. None of the chemicals persist in the body for long periods of time. The portions that are metabolized are utilizing the P450 enzymes. Several isozymes may be involved. However, experiments with human liver microsomes and a CYP2E1-specific inhibitor indicate that CYP2E1 is the predominant CYP isozyme involved in metabolism of 1,1,1-trichloroethane and other chlorinated hydrocarbon solvents (Guengerich et al., 1991).

Neurotoxicity is the critical health effect in humans and is mostly caused by the parent chemicals. Effects of trichloroethylene on the central nervous system may involve not only the parent chemical, but also metabolites such as trichloroethanol. Reactive metabolites are linked to exposure-related liver and kidney effects, and cancer. In the liver, the production of free radical metabolic intermediates formed by the oxidative catalytic action of CYP isozymes is responsible for the tissue damage via cleavage of the carbon–chlorine bond. The free radicals are thought to react with unsaturated lipids and proteins in the endoplasmic reticulum of hepatocytes leading to morphological and functional changes in the organelle and eventually to cellular dysfunction (triglyceride accumulation) and necrosis (Plaa, 1986). This mechanism of action is shared by all chemicals in the mixture.

#### 2.1.2. Evaluation

No pertinent studies on the toxicity or on the interactions (measured or modeled) of complete mixture or any of the tertiary submixtures were located. Most of the chemicals in the mixture are of low potency because they are poorly metabolized (Kaneko et al., 1994; McCall et al., 1983; Mitoma et al., 1985; Nolan et al., 1984). Mild liver or kidney effects observed in rodents from these chemicals are caused by reactive metabolic intermediates formed via CYP2E1 or 2B1/2 catalysis. However, trichloroethylene is metabolized to a much greater extent (Kaneko et al., 1994; Lash et al., 2000). Metabolism of all these chemicals can be influenced, to certain degree, by induction of CYP isozymes. For example, CYP induction by ethanol increased metabolism of inhaled 1,1,1-trichloroethane in rats, but most of the chemical was still eliminated unmetabolized (Kaneko et al., 1994). Similarly, pretreatment of rats with phenobarbital or ethanol increased 1,1-dichloroethane metabolism in liver microsomes (McCall et al., 1983; Sato et al., 1980). Although phenobarbital induction of CYP has been associated with enhancement of acute high level trichloroethylene hepatotoxicity (Allemand et al., 1978; Carlson, 1974; Moslen et al., 1977; Nakajima et al., 1990), CYP induction by ethanol has not produced consistent potentiation of acute high-level 1,1,1-trichloroethane hepatotoxicity (Carlson, 1973; Cornish et al., 1973). Explanation of these differences can be found in a series of Japanese studies. First, it was reported that ethanol markedly enhanced the metabolism of trichloroethylene and increased its hepatotoxicity in rats at trichloroethylene exposures of 2000 ppm or lower (Nakajima et al., 1988). In contrast, pretreatment with phenobarbital increased the metabolism of trichloroethylene only at 4000 ppm or higher and the hepatotoxicity was markedly enhanced at higher levels, as well. Histopathological changes showed differences between ethanol and phenorbarbital pretreated rats (Okino et al., 1991). Trichlorothylene-induced necrosis in centrilobular hepatocytes in phenobarbital-pretreated animals, whereas ballooning degeneration of hepatocytes in midzonal areas was reported in those pretreated with ethanol. A comparative study on the contribution of CYP2E1, CYP2C11/6, CYP2B1/2, and CYP1A1/2 to the formation of chloral hydrate from trichloroethylene was tested in microsomes from control, ethanol-, phenobarbital-, and 3-methylcholantrene-pretreated rats using monoclonal antibodies to the respective P450 isozymes (Nakajima et al., 1992). The results indicated that all isozymes are involved in this metabolic step; however, the CYP2E1 involvement in the trichloroethylene oxidation was much greater than that of the other isozymes. CYP2E1 has the low-$K_m$ (i.e., high affinity) for trichloroethylene at low exposure levels.

Interaction studies regarding chemicals in this mixture are scarce. Koizumi et al. (1982) reported that tetrachloroethylene inhibited the rates of urinary excretion of a 1,1,1-trichloroethane metabolite, trichloroethanol, in rats exposed by inhalation to a mixture of 350 ppm 1,1,1-trichloroethane and 100 ppm tetrachloroethylene. Goldsworthy and Popp (1987) studied the joint effect of trichloroethylene (1000 mg/kg/day) and tetrachloroethylene (1000 mg/kg/day) on peroxisome proliferation in the livers and kidneys of rats and mice. In each case except rat kidneys, the response of the peroxisome proliferation marker to the binary mixture was less than the sum of the responses to the individual chemicals. In contrast, Stacey (1989) investigated the joint toxic action of trichloroethylene, 1,1,1-trichloroethane, and tetrachloroethylene on renal and hepatic endpoints in rats *in vivo* and *in vitro* in isolated hepatocytes, The results from the *in vivo* and *in vitro* studies demonstrated that exposure to binary and ternary mixtures of the chemicals, at doses that were below individual thresholds, produced mild toxic hepatic or renal responses, and that the responses to the ternary mixtures were generally greater than responses to the binary mixtures. These observations support the premise that additivity occurs at lower exposure doses and inhibition at high doses. Occupational exposures are much higher than environmental exposures. OSHA's PELs (TWA) are 350 ppm, 100 ppm, 100 ppm, and 100 ppm for 1,1,1-trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloroethylene, respectively (NIOSH, 2006).

### 2.2. Mixture of chloroform, 1,1-dichloroethylene, trichloroethylene, and vinyl chloride

#### 2.2.1. Background information

Industrial production and use is the primary source of chloroform, 1,1-dichloroethylene, trichloroethylene, and vinyl chloride in the environment. Chloroform is mainly used to manufacture the refrigerant HCFC-22 and is also generated during the process of disinfecting water with chlorine. The primary source of 1,1-dichloroethylene is manufacturing polyvinylidene chloride copolymers for plastics, flexible wraps, and flame retardant coatings. Trichloroethylene may be found in numerous industrial applications as well as in paint remover, adhesives, and spot removers. Vinyl chloride is mainly used in the production of polyvinyl chloride (PVC) polymers.

These chemicals are among the top 10 found in water around hazardous waste sites. They are at the 9th, 7th, 1st, and 8th place,