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A category approach to predicting the repeated-dose hepatotoxicity of allyl esters

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ABSTRACT

We tested a category approach to predict the hepatotoxic effects of repeated doses of allyl esters using a new database for repeated-dose toxicity. Based on information on hepatotoxic mechanism of allyl acetate, the category was defined as allyl esters that are hydrolyzed to allyl alcohol. Allyl alcohol is readily oxidized to acrolein in the liver, causing hepatotoxicity. Seventeen marketed allyl esters were obtained and grouped into category by identifying or predicting allyl alcohol formation. Allyl esters with a saturated straight alkyl carboxylic acid moiety (allyl acetate, hexanoate and heptanoate as tested species, and allyl butyrate, pentanoate, octanoate, nonanoate and decanoate as untested species) are likely similar in rate of ester hydrolysis, thereby defining subcategory 1. NOAEL and LOAEL for the hepatotoxic effects were estimated at 0.12 and 0.25 mmol/kg/d for the untested species, based on those of allyl acetate. The remaining nine allyl esters with other alkyl or aromatic carboxylic acid moieties were placed in subcategory 2: their hepatotoxicity levels were not predictable due to an unclear match between their degree of structural complexity and rate of hydrolysis. Our results demonstrate the usefulness of the category approach for predicting the hepatotoxicity of untested allyl esters with saturated straight alkyl chains.

1. Introduction

Repeated-dose toxicity (RDT) is one of the key regulatory endpoints in the hazard assessment of chemicals. The latest chemical management policies, including the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) in the European Union, the Toxic Substances Control Act (TSCA) in the US, and the Chemical Substances Control Law (CSCL) in Japan require the toxicological evaluation of marketed but untested chemicals. On the other hand, reduced animal testing is desired for economic and animal welfare reasons. In 2007, the Organisation for Economic Co-operation and Development (OECD) published guidance on grouping chemicals for application of the category approach (OECD, 2007). In this method, chemicals whose toxicological properties are likely to be similar, or follow a regular pattern as a result of structural similarities, are grouped into chemical category. When an untested chemical falls into a category, its toxicity is estimated using experimental data on tested analogs in the same category (data gap filling). Several attempts to achieve category testing have recently been accomplished for complex toxicity endpoints (Fabjan et al., 2006; Wu et al., 2010; Blackburn et al., 2011; Ball et al., 2012; Yamada et al., 2012). Mechanistic interpretation is necessary for regulatory acceptable toxicological assessment, since there are several examples of structurally similar molecules with different toxicities. Chemicals often show toxicity when metabolized, so it is logical to assume that chemicals yielding the same toxic metabolite cause similar toxicity. Category formation based on structural and metabolism similarities is promising for expanding the utilization of category approach.

Allyl esters are approved as food flavoring agents, and are used to simulate various fruit flavors in baked goods, candy, ice cream, gelatin, and condiments (Fenaroli, 1971). Many allyl esters are artificial flavors, while some are reported to be naturally occurring (JECFA, 1991). Allyl acetate, a simple allyl ester, is an important intermediate in the synthesis of many industrial chemicals and has various industrial applications. Several studies since the 1980s have demonstrated that certain allyl esters cause liver injury (NTP, 1983, 1985, 2006). Moreover, allyl esters are shown to be metabolized to yield a toxic metabolite, acrolein, in the liver, and its cytotoxic effects have been studied extensively (Ohno et al., 1985; Jaeschke et al., 1987; Silva and O'Brien, 1989; Cooper et al., 1992; Watanabe et al., 1992; Sun et al., 2006; Tanel and Averill-Bates, 2007). These results suggest that the toxicity of

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various allyl esters can be evaluated together. Because of potential exposure to various allyl esters through foods and consumer products, missing RDT data on particular allyl esters should be filled with available data.

In this study, the category approach was utilized to predict the hepatotoxic effects of allyl esters. A category was built with the allyl esters in the Hazard Evaluation Support System (HESS) and the attached database (HESS DB), which contains data on RDT studies of 500 chemicals and related metabolic and mechanistic information (Hayashi and Sakuratani, 2011). Various marketed allyl esters were then addressed for categorization. Based on their chemical structure, the category chemicals were further grouped into a subcategory with similar toxicokinetic features to estimate their hepatotoxic effects without the need for animal testing. These results clearly demonstrate the usefulness of the category approach in predicting the toxicity of class of chemicals.

2. Materials and methods

2.1. Data set

The HESS DB contains the detailed test data of RDT studies of 500 chemicals, and the HESS incorporates the chemical, metabolic and toxicological information to support the grouping of chemicals (Hayashi and Sakuratani, 2011). Both HESS and HESS DB were utilized together to retrieve toxicity and metabolic information on allyl esters. The HESS and HESS DB can be freely downloaded from the following URL (http://www.safe.nite.go.jp/english/kasinn/qsar/hess-e.html). Three allyl esters (allyl acetate, allyl isovalerate, and diallyl phthalate), and allyl alcohol were obtained as a data set of chemicals for building a category. The other two allyl esters (allyl hexanoate and allyl heptanoate) were selected from a different data source (JECFA, 1991) which includes subchronic toxicity information, to justify this category.

2.2. Development of adverse outcome pathway and category

Literature on metabolism and various *in vitro* and *in vivo* toxicological studies was gathered on allyl alcohol and allyl esters for developing an adverse outcome pathway (AOP). An AOP was built for the hepatotoxic effects of allyl acetate, since this substance has been more fully studied. Another toxic response occurred in the forestomach, characterized as epithelial hyperplasia, after oral administration of the chemical at the same dose at which hepatotoxicity appeared (NTP, 2006). This was, however, out of the scope of this study, since forestomach irritation is a local point of contact effect only anticipated to be found in gavage studies. The current assessment examines potential systemic toxicity. To develop a category, a data matrix was constructed and carefully evaluated in terms of the metabolic formation of allyl alcohol and significant pathological changes in the liver, characterized as periportal hepatocyte hypertrophy, degeneration and necrosis, and bile duct hyperplasia. Hepatotoxic potencies were evaluated as no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) for pathological changes in the liver, since hepatotoxic effects are critical for risk assessment of these chemicals. If pathological data were available for both males and females, the data indicating more severe hepatotoxic effects were utilized. Finally, category definition was described based on the structure and metabolism of the category chemicals.

2.3. Categorization and subcategorization of marketed allyl esters

OECD QSAR Toolbox Ver. 2.1 (http://www.qsartoolbox.org/), which contains several international chemical inventories, was utilized to obtain the structures of various allyl esters. The Ministry of International Trade and Industry (MITI) inventory of Japanese CSCL was selected and screened to obtain substances with allyl carboxylate moieties, as a result of which more than 40 allyl esters were acquired. Production and imported amounts of these chemicals in Japan was then investigated to obtain marketed allyl esters using the Chemical Risk Information Platform (CHRIP, http://www.safe.nite.go.jp/english/db.html), a database that provides information on risk assessment and management of chemical substances for Japanese CSCL. To address the category membership of marketed allyl esters, metabolic hydrolysis was predicted by evaluating the originally documented rat metabolism data on several allyl esters. Subcategorization was carried out so as to group further similar-category chemicals for which only hydrolysis and acrolein formation is considered relevant.

3. Results

3.1. Building an AOP for the hepatotoxic effects of allyl acetate

An AOP is a plausible connection between chemicals, key events at molecular, biochemical and cellular levels and an *in vivo* adverse outcome. Since most studies for allyl ester-induced hepatotoxicity and its mechanism have been performed with allyl acetate, the AOP for allyl acetate-induced hepatotoxic effects was developed by considering strength, consistency, and specificity of the association with the set of experimental evidence shown in Fig. 1.

There is strong evidence that hydrolysis of allyl acetate to allyl alcohol is required for allyl acetate-induced hepatotoxicity. In rats, allyl acetate was shown to be hydrolyzed to allyl alcohol (Kaye, 1973). The hydrolysis was inhibited in the liver homogenates from rats pretreated with carboxylesterase inhibitors (Silver and Murphy, 1978). Allyl acetate-induced hepatotoxicity was significantly reduced by pre-treatment with carboxylesterase inhibitors (Silver and Murphy, 1978). There is a dose-dependent correlation between the hepatotoxic effects of allyl acetate and the urinary concentration of 3-hydroxypropylmercapturic acid (3-HPM), a major metabolite of allyl alcohol (Auerbach et al., 2008). This result further supports evidence for the AOP.

Hepatotoxicity of allyl alcohol in rats is documented (Silver and Murphy, 1978; NTP, 2006). However, evidence exists that allyl alcohol-elicited liver toxicity is not induced by allyl alcohol itself, but by the reactive metabolite: acrolein generated in the liver. It is reported in *in vitro* and *in vivo* studies that allyl alcohol is oxidized to acrolein by ADH (Serafini-Cessi, 1972; Atzori et al., 1989; Ghilarducci and Tjeerdema, 1995). The importance of liver ADH in the toxicity of allyl alcohol has been demonstrated in several studies. Pretreatment of rats with ADH inhibitors almost completely inhibited allyl alcohol-induced hepatotoxicity (Silver and Murphy, 1978). In *in vitro* studies with rat hepatocytes, allyl alcohol-induced cytotoxicity was prevented by ADH inhibitors, but augmented by aldehyde dehydrogenase inhibitors (Silva and O'Brien, 1989; Ohno et al., 1985; Miccadei et al., 1988). Deer–mice of the ADH-negative strain were resistant to allyl alcohol toxicity (Belinsky et al., 1985).

A variety of studies have been performed to explore the mechanism by which acrolein is cytotoxic to hepatocytes. They include GSH depletion, oxygen radical formation, macromolecular adduct formation, mitochondrial dysfunction, and activation of mitochondrial death pathway to apoptosis (Silva and O'Brien, 1989; Cooper et al., 1992; Ohno et al., 1985; Jaeschke et al., 1987; Watanabe et al., 1992; Sun et al., 2006;Tanel and Averill-Bates, 2007). These biochemical events caused by acrolein are believed to be associated with hepatocyte injury. This AOP does not fulfill the full AOP requirements, since the molecular initiating event (MIE) is