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Development of a category approach to predict the testicular toxicity of chemical substances structurally related to ethylene glycol methyl ether

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ABSTRACT

We propose a category approach to assessing the testicular toxicity of chemicals with a similar structure to ethylene glycol methyl ether (EGME). Based on toxicity information for EGME and related chemicals and accompanied by adverse outcome pathway information on the testicular toxicity of EGME, this category was defined as chemicals that are metabolized to methoxy- or ethoxyacetic acid, a substance responsible for testicular toxicity. A Japanese chemical inventory was screened using the Hazard Evaluation Support System, which we have developed to support a category approach for predicting the repeated-dose toxicity of chemical substances. Quantitative metabolic information on the related chemicals was then considered, and seventeen chemicals were finally obtained from the inventory as a shortlist for the category. Available data in the literature shows that chemicals for which information is available on the metabolic formation of EGME, ethylene glycol ethyl ether, methoxy- or ethoxyacetic acid do in fact possess testicular toxicity, suggesting that testicular toxicity is a concern, due to metabolic activation, for the remaining chemicals. Our results clearly demonstrate practical utility of AOP-based category approach for predicting repeated-dose toxicity of chemicals.

1. Introduction

Ethylene glycol methyl ether (EGME) is an important industrial chemical that is widely used in jet fuel and ink, as a plasticizer, in the manufacture of printed circuit boards, and in photographic and dyeing applications. Due to concerns about exposure to this chemical, numerous toxicological studies have been conducted (NIOSH, 1991). Several repeated-dose toxicity studies reveal that EGME produces toxicities in multiple organs associated with the hematopoietic system, immuno system and male reproductive organs (Johanson, 2000). One of the most studied organ toxicities is testicular toxicity, which is characterized by atrophy, degeneration and necrosis of the pachytene spermatocytes, and a decrease in sperm count in rats, mice and rabbits (Foster et al., 1983; Miller et al., 1983; Nagano et al., 1984; NTP, 1993). The same class of analog, ethylene glycol ethyl ether (EGEE), also shows testicular toxicity (Johnson, 2002). Comparative studies have revealed that EGME is more toxic than EGEE in rats and mice (Foster et al., 1984; NTP, 1993). Numerous studies to explore the mechanism have revealed that the testicular toxicity of EGME and EGEE is attributable to their major metabolites, methoxyacetic acid and ethoxyacetic acid, respectively (Foster et al., 1984; Moss et al., 1985). Acetates of EGME and EGEE, which are related chemicals, have also been shown to cause testicular toxicity in mice because acetates are readily hydrolyzed to EGME and EGEE (Johanson, 2000; Johnson, 2002). EGME, EGEE and their acetates are candidates for designation as Substances of Very High Concern (SVHC) in the chemical management policy of Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) in the European Union (ECHA, 2013). It is plausible to assume that testicular toxicity will result if related chemicals are chiefly metabolized to methoxy- or ethoxyacetic acid.

Since repeated-dose toxicity is one of the key items of information for hazard evaluation, chemical regulation policies are increasingly requiring that repeated-dose toxicity data be made available for marketed but as yet untested chemicals. On the other hand, reduced animal testing is desired for both economic and animal welfare reasons. The category approach thus has potential as a useful method to reduce animal testing (Schaafsma et al., 2009;

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van Leeuwen et al., 2009). Several pioneering attempts to develop a category approach have been performed for complex regulatory endpoints (Fabjan et al., 2006; Wu et al., 2010; Blackburn et al., 2011; Ball et al., 2012, 2014; ECETOC, 2012). We have recently developed a Hazard Evaluation Support System (HESS) in which the notion of a category approach is adopted based on OECD guidance on grouping chemicals into categories (Hayashi and Sakuratani, 2011; Sakuratani et al., 2013; OECD, 2014). The HESS includes databases of repeated-dose toxicity studies and metabolic maps in rats for industrial chemicals, and has a metabolism simulator. The system has a supportive function for grouping structural analogs into categories using a category profiler. HESS is compatible to OECD QSAR Toolbox. The data and category profilers of HESS are provided to the Toolbox in a period. On the other hand, HESS is unique in that detailed data can be drawn from the attached database HESS DB, which contains dose–response data of toxicity studies and more mechanistic information (Hayashi and Sakuratani, 2011; Abe et al., 2012). We have successfully tested the category approach using HESS to predict the repeated-dose toxicity of untested chemicals by combining it with the adverse outcome pathway (AOP) concept (Yamada et al., 2012, 2013). More case studies are required, however, before this approach can be applied for regulatory use.

In this study, AOP was developed for testicular toxicity of EGME. Metabolic activation was identified as a key event linked to the adverse outcome. A category was then built based on information on the active metabolite formation and the toxicity data of EGME and structural analogs from HESS. The proposed category was evaluated with data of tested analogs from toxicological literatures outside of HESS. Finally, relevant analogs were identified in the chemical inventory of the Ministry of International Trade and Industry (MITI) of Japanese Chemical Substances Control Law (CSCL), consisting of about 16,000 chemicals by using HESS metabolism simulator and taking into account related empirical metabolic information. The results clearly demonstrated the usefulness of our approach to the primary identification of chemicals with potential testicular toxicity similar to that of EGME.

2. Materials and methods

2.1. Data sets

Table 1 shows the list of data sets of chemicals used in this study. It is the merger of Tables 2 and 3, which were from HESS for forming a category and toxicological literatures for evaluating a category, respectively. The HESS software package can be downloaded free of charge from the following URL (http://www.safe.nite.go.jp/english/kasinn/qsar/hess-e.html). The current version of HESS (version 2.8) has four sub-databases of repeated-dose toxicity studies, from which the HESS Repeated-dose Toxicity Database was selected. It contains a summary of data from about 630 toxicity studies on industrial chemicals, mainly from Japanese regulatory submissions and the National Toxicology Program (NTP). All the reports are in the public domain. Given that EGME, EGEE and their acetates are listed as candidates of SVHC (ECHA, 2013), structures of ethylene glycol alkyl ethers with linear and branched chains and their acetate were manually searched for from HESS database by visual inspections. As a result, six chemicals were chosen for building a category (Table 2). Publicly-available toxicological literature was gathered for toxicity studies of EGME and related chemicals that might be converted to methoxy- or ethoxyacetic acid by examining metabolic information (Nagano et al., 1984; Cheever et al., 1989; Poon et al., 2005). A total of 15 chemicals were retrieved from the three literature reports for category evaluation (Table 3).

2.2. Development of an adverse outcome pathway and a category

Literature on EGME metabolism and various *in vitro* and *in vivo* toxicological studies were compiled for developing an AOP, which was then built for the testicular toxic effects of EGME, since this substance has been more often studied for its mechanism of toxicity. To develop the category, a data matrix was constructed and carefully evaluated in terms of the metabolism and significant pathological changes in the testis. Testicular toxic potencies were evaluated as lowest observed adverse effect level (LOAEL) for pathological changes in the testis. Finally, a category definition was described based on the structure and metabolism of the category chemicals.

2.3. Screening a chemical inventory to obtain chemical structures that form methoxy- or ethoxyacetic acid

The OECD QSAR Toolbox Ver. 3.1 (http://www.qsartoolbox.org/) contains 11 international chemical inventories, including the TSCA (Toxic Substances Control Act), the REACH ECB (European Chemicals Bureau) and HPVC (High Production Volume Chemicals) OECD, etc., of which the Japanese MITI inventory was selected for the case

Table 1
Data set chemicals, their abbreviations and CAS numbers in the present study.

Abbreviation	Chemical name	CAS no.	Data source[a]	Compound no.[b]
EGME	Ethylene glycol methyl ether	109-86-4	H, TL	**1**
EGEE	Ethylene glycol ethyl ether	110-80-5	H, TL	**2**
EGiPE	Ethylene glycol isopropyl ether	109-59-1	H	–
EGPE	Ethylene glycol propyl ether	109-86-4	TL	–
EGtBE	Ethylene glycol *tert*-butyl ether	7580-85-0	H	–
EGBE	Ethylene glycol butyl ether	111-76-2	H, TL	–
EGMEA	Ethylene glycol methyl ether acetate	110-49-6	TL	**3**
EGEEA	Ethylene glycol ethyl ether acetate	111-15-9	TL	**4**
EGDME	Ethylene glycol dimethyl ether	110-71-4	TL	**13**
DEGDME	Diethylene glycol dimethyl ether	111-96-6	TL	**14**
EG	Ethylene glycol	107-21-1	TL	–
EGA	Ethylene glycol acetate	542-59-6	TL	–
EGDA	Ethylene glycol diacetate	111-55-7	TL	–
PGMEA	Propylene glycol methyl ether acetate	108-65-6	H	–
MHE	Methyl heptyl ether	629-32-3	TL	–
EHE	Ethyl hexyl ether	5756-43-4	TL	–
BE	Butyl ether	142-96-1	TL	–
HGDE	Hexamethylene glycol dimethyl ether	13179-98-1	TL	–

[a] These chemicals were obtained from HESS (H) for category development and toxicological literature (TL) for category evaluation.
[b] The compound number is designated in Fig. 2.