

**ENVIRONMENT DIRECTORATE
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ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

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**CASE STUDY ON THE USE OF INTEGRATED APPROACHES TO TESTING
AND ASSESSMENT FOR TESTICULAR TOXICITY OF ETHYLENE GLYCOL
METHYL ETHER (EGME)-RELATED CHEMICALS**

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CASE STUDY ON THE USE OF INTEGRATED APPROACHES TO TESTING AND
ASSESSMENT FOR TESTICULAR TOXICITY OF ETHYLENE GLYCOL METHYL
ETHER (EGME)-RELATED CHEMICALS

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2019

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Forward

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This case study was developed by the United States for illustrating practical use of IATA and submitted to the 2018 review cycle of the IATA Case Studies Project. This case study was reviewed by the project team. The document was endorsed at the 3rd meeting of the Working Party on Hazard Assessment in June 2019.

The following case study was also reviewed in the project in 2018:

1. CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR ESTROGEN RECEPTOR ACTIVE CHEMICALS, ENV/JM/MONO(2019)28, Series on Testing & Assessment No. 309.

These case studies are illustrative examples, and their publication as OECD monographs does not translate into direct acceptance of the methodologies for regulatory purposes across OECD countries. In addition, these cases studies should not be interpreted as official regulatory decisions made by the authoring member countries.

In addition, a considerations document summarising the learnings and lessons of the review experience of the case studies is published with the case studies:

REPORT ON CONSIDERATIONS FROM CASE STUDIES ON INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA) -Fourth Review Cycle (2018) -, ENV/JM/MONO(2019)26, Series on Testing & Assessment No. 307.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

Executive Summary

This case study was developed to demonstrate how read-across can be applied to fill data gaps in reproductive toxicity endpoints for screening assessments under the Japanese Chemical Substances Control Law (CSCL). A category approach was used to assess the testicular toxicity of ethylene glycol methyl ether (EGME)-related chemicals. Based on toxicity information for EGME and related chemicals and possible adverse outcome pathway information on the testicular toxicity of EGME, the category members were defined as chemicals that are metabolised to methoxy- or ethoxyacetic acid, which are responsible for testicular toxicity.

A Japanese chemical inventory was screened using the metabolism simulator of the Hazard Evaluation Support System (HESS) to obtain metabolism information for EGME-related chemicals. This resulted in 15 chemicals being shortlisted for the category. Published data show that chemicals that produce methoxy- or ethoxyacetic acid as metabolites possess testicular toxicity, suggesting that untested chemicals that are predicted to produce these toxic metabolites will also have this effect.

Although the overall uncertainty of the case study was low, some of the original compounds are structurally diverse, and metabolic hydrolysis or dealkylation could produce additional toxic compounds that need to be explicitly considered. However, a database search for toxicity and metabolism information suggested that these possible metabolites do not affect the toxicity levels through different mechanisms of action.

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Introduction

Reproductive and developmental toxicity is one of the key regulatory endpoints in the hazard assessment of chemicals. When performing a risk assessment under the Japanese Chemical Substances of Control Law (CSCL), a screening assessment is first carried out to select Priority Assessment Chemical Substances. Human health endpoints for this assessment include i) repeated-dose toxicity, ii) reproductive and developmental toxicity, iii) genotoxicity, and iv) carcinogenicity. A hazard class for reproductive and developmental toxicity is currently assigned when both reproductive and developmental toxicity data have been obtained through animal testing. However, this means that some chemicals cannot be classified for reproductive and developmental toxicity due to a lack of animal studies on them, in which case the reproductive and developmental toxicity hazard is not further considered for prioritisation. In such situations, the read-across approach can be useful for providing a more valid prioritisation under the CSCL when a target chemical is considered to have a reproductive or developmental toxicity potential based on the toxicity of analogue substances.

Approximately 5 kt of ethylene glycol methyl ether (EGME) is manufactured and imported in Japan each year (METI, 2018), although this is gradually being substituted by other chemicals. Numerous toxicological studies have been conducted on EGME due to concerns about exposure to this chemical (NIOSH, 1991), with testicular toxicity having been particularly well studied. These studies have shown that EGME is associated with atrophy, degeneration, and necrosis of the pachytene spermatocytes, and a decreased sperm count in rats, mice, and rabbits (Foster *et al.*, 1983; Miller *et al.*, 1983; Nagano *et al.*, 1984; NTP, 1993). Substantial new information concerning the human health consequences of exposure to this class of chemicals was summarised in a report by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 2005). Moreover, the read-across approach was used in a proposal for the harmonised classification and labelling of tetraethylene glycol dimethyl ether (tetraglyme), which contains the EGME moiety (Environment Agency Austria, 2017).

Category assessment is not currently utilised in the screening assessment but is recommended for chemical assessment under the CSCL (METI *et al.*, 2012). Therefore, this case study was developed to demonstrate how read-across can be applied to fill data gaps in the screening assessment under the CSCL by focusing on testicular toxicity endpoints for the category assessment of EGME-related chemicals, some of which are used as solvents, plasticisers, and synthetic intermediates. This case study is mostly based on work that was published in a previous paper by Yamada *et al.* (2014) but has been updated with relevant literature.

1. Purpose

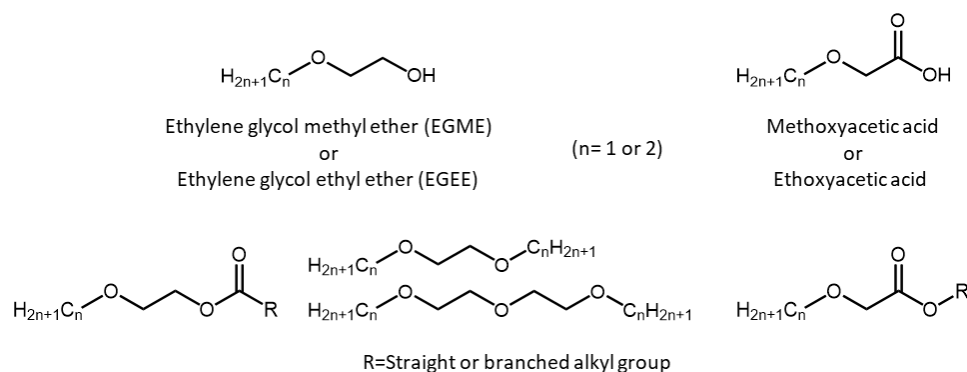
1.1. Purpose of use

The general purpose of this case study is to improve the grouping approaches and guidance provided by the Organisation for Economic Co-operation and Development (OECD) on the Integrated Approach to Testing and Assessment. The specific purpose of this case study is to assess the testicular toxicity of EGME-related chemicals by integrating metabolism information for the category assessment and read-across with toxicity data for EGME-related source chemicals. No-observed-adverse-effect level (NOAEL) values are also derived for hazard classification under the CSCL in Japan.

1.2. Target chemical(s)

EGME is oxidised to methoxyacetic acid, which appears to be responsible for EGME-induced testicular toxicity. Ethylene glycol ethyl ether (EGEE), which is a structural analogue of EGME, is metabolised in a similar way. The target chemicals in this case study are substances that are metabolised to methoxy- or ethoxyacetic acid, which include esters of EGME or EGEE, ethylene glycol dimethyl or diethyl ether, diethylene glycol dimethyl or diethyl ether, and esters of methoxy- or ethoxyacetic acid (Figure 1).

Figure 1. Structures of ethylene glycol methyl ether (EGME) and EGME-related chemicals considered in this study.



1.3. Endpoint(s)

The endpoint of interest is testicular toxicity, which is characterised by weight loss, atrophy, and degeneration of the testis in sub-chronic toxicity studies via the oral route.

2. Hypothesis for the category approach

The oxidation of EGME to methoxyaldehyde in the liver is catalysed by alcohol dehydrogenase, while subsequent oxidation to methoxyacetic acid is catalysed by aldehyde dehydrogenase (Moss *et al.*, 1985). There is strong evidence that the formation of methoxyacetic acid in the liver is a prerequisite for EGME-induced testicular toxicity in rats, as pretreatment with pyrazole, an alcohol dehydrogenase inhibitor, shows a protective effect against the formation of methoxyacetic acid and significantly decreases the testicular toxicity of EGME in rats (Foster *et al.*, 1984; Moss *et al.*, 1985). By contrast, the administration of equimolar amounts of methoxyacetic acid clearly induces testicular toxicity at similar levels to EGME in rats (Foster *et al.*, 1984) and, unlike EGME, also causes cytotoxic effects in primary rat testicular cultures (Gray *et al.*, 1985; Foster *et al.*, 1987).

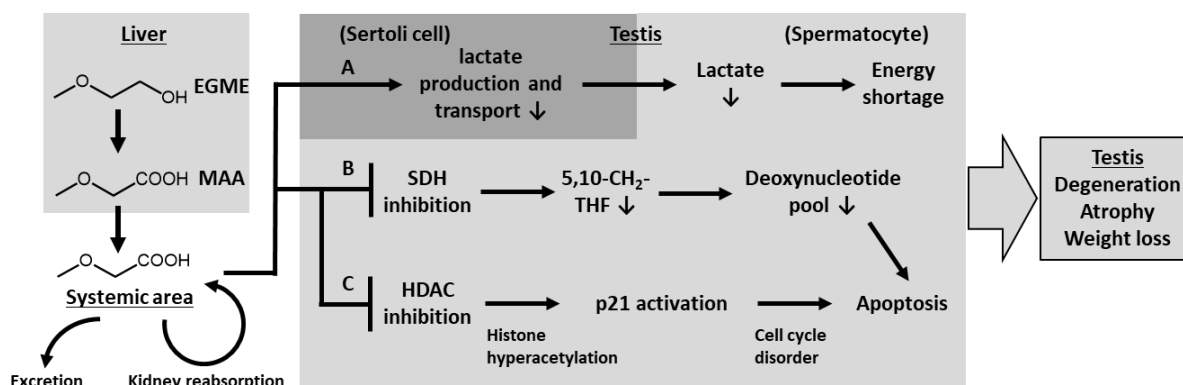
A number of studies have explored the mechanisms of testicular toxicity for methoxyacetic acid (reviewed by Yamazoe *et al.*, 2015; see Figure 2). Examples of these mechanisms include the inhibition of:

- A) Lactate production in Sertoli cells and the transport of lactate to spermatocytes via lactate-carrying monocarboxylate transporters (MCTs), which leads to an energy shortage for developing germ cells and spermatocyte disorders.
- B) Sarcosine dehydrogenase activity, resulting in a decrease in the level of 5,10-methylenetetrahydrofolate pentaglutamate (5,10-CH₂-THF) which supports nucleic acid synthesis through one-carbon incorporation into thymidine. Decreased nucleic acid synthesis coincides with the selective appearance of methoxyacetic acid toxicity in pachytene spermatocytes during the developmental phase.
- C) Histone deacetylase (HDAC) activity in spermatocytes, leading to histone hyperacetylation. Cell cycle disorder is induced by the up-regulation of p21 expression in rapidly dividing germ cells followed by apoptosis.

It should be noted that these are hypothesised adverse outcome pathways (AOPs) and that pathway C is currently under development as AOP 212 entitled “Histone deacetylase inhibition leading to testicular toxicity” (<https://aopwiki.org/aops/212>).

Methoxyacetic acid also exhibits slow plasma clearance ($T_{1/2} = 19.7 \pm 2.3$ h) after intraperitoneal administration in rats (Moss *et al.*, 1985), possibly due to its reabsorption by the kidney through MCTs (Gopal *et al.*, 2004; Yamazoe *et al.*, 2015). Thus, the activation of these pathways accompanied by the longer $T_{1/2}$ of methoxyacetic acid may contribute to its severe toxicity in the testis.

Figure 2. Possible mode of action (MoA) / adverse outcome pathway (AOP) for ethylene glycol methyl ether (EGME)-induced testicular toxicity in rats.



HDAC, histone deacetylase; MAA, methoxyacetic acid; SDH, sarcosine dehydrogenase; THF, tetrahydrofolate pentaglutamate; 5,10-CH₂-THF, 5,10-methylenetetrahydrofolate pentaglutamate.

The EGME analogue EGEE also shows testicular toxicity (Johnson, 2002), although it is less toxic than EGME in rats and mice (Foster *et al.*, 1984; NTP, 1993). Less toxicity information is available for ethoxyacetic acid compared with methoxyacetic acid. However, Foster *et al.* (1987) clearly showed that ethoxyacetic acid causes cellular loss in primary rat testicular cultures, with a close correspondence between the effects of methoxy- and ethoxyacetic acids both *in vivo* and *in vitro*, suggesting that these acids have a similar mode of action, although ethoxyacetic acid was less potent than methoxyacetic acid. Therefore, chemicals that are metabolised to methoxy- or ethoxyacetic acid can induce testicular toxicity in rodents.

Several reports have suggested that the effects of testicular toxicity are of concern for particular populations that are exposed to EGME and EGEE. The findings of these studies are confounded by simultaneous exposures to other chemicals as well as limited information on exposure levels, preventing firm conclusions from being drawn concerning the contribution of the substances to the observed effects (ECETOC, 2005). However, experimental exposure of male volunteers to EGME or EGEE resulted in methoxy- or ethoxyacetic acid being detected in the urine as a major metabolite. The glycine conjugates of methoxy- and ethoxyacetic acid, which are among the major metabolites of EGME and EGEE, respectively, in rats, have not been detected in humans (Groeseneken *et al.*, 1988, 1989) and the elimination half-lives of methoxy- and ethoxyacetic acid in humans are apparently longer than those in rats (Groeseneken *et al.*, 1988, 1989, Aasmoe *et al.*, 1999). Moreover, methoxyacetic acid has been shown to induce germ cell apoptosis in human testicular tissue culture, with spermatocyte cell death occurring within the same concentration range and possibly via similar mechanisms in humans and rats (Li *et al.*, 1996). These toxicokinetic and toxicodynamic findings indicate that the testicular effects of EGME and other members of the same category that have been observed in animals are also relevant to humans.

3. Category members

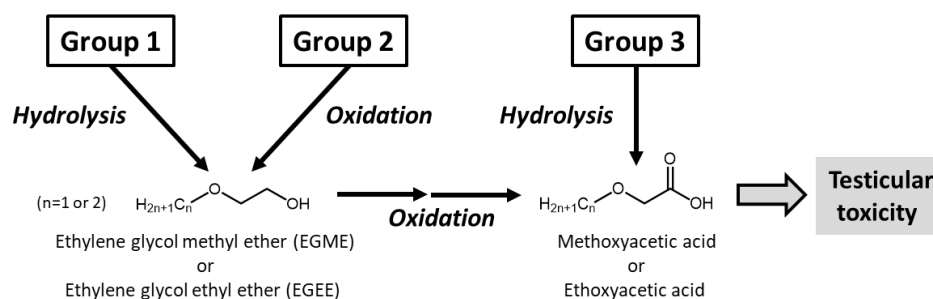
3.1. Identification and selection of category members

Category members were selected using the Hazard Evaluation Support System (HESS), which is freely available software that supports the grouping of chemicals and performs read-across for the repeated-dose toxicity of chemical substances (<http://www.nite.go.jp/en/chem/qsar/hess-e.html>) (Sakuratani *et al.*, 2013). The Japanese MITI inventory, which includes approximately 16,000 substances, was loaded into HESS version 2.8, and potential metabolites were generated using the Rat Cellular Metabolism Simulator, which presents all possible metabolites of a target chemical based on the observed metabolism reactions of similar chemicals. Chemicals that were predicted to be metabolised to EGME, EGEE, or methoxy- or ethoxyacetic acid were then searched for using a custom profiler that defined the structure of these chemicals as a boundary. The profiler was created specifically for this study because careful evaluation of mechanistic information strongly suggested that the formation of their active metabolites is necessary for activating the AOPs. This inventory screening resulted in 40 chemicals being obtained. Some of these chemicals, such as those containing atoms other than carbon, oxygen, and hydrogen, were then eliminated to simplify the analysis, as metabolism and toxicity data are lacking for such chemicals and their analogues. The remaining 20 chemicals were considered for categorisation by evaluating the documented metabolism data of related chemicals in the HESS.

The category was built based on information on the active metabolites that are formed (Figure 3):

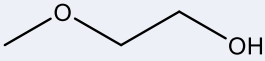
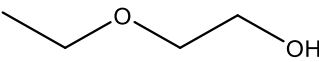


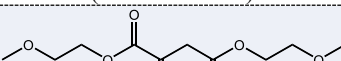
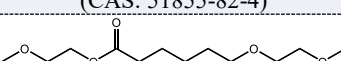

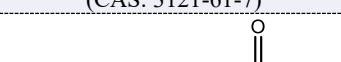


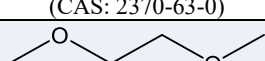
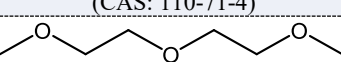
- Group 1: esters of EGME and EGEE that are readily hydrolysed to generate EGME and EGEE.
- Group 2: chemicals that form EGME or EGEE via oxidative O-dealkylation.
- Group 3: chemicals that are hydrolysed to generate methoxy- or ethoxyacetic acid.

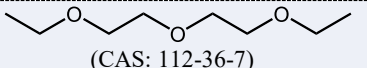
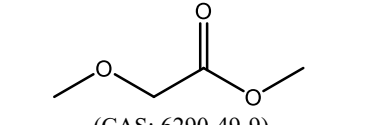
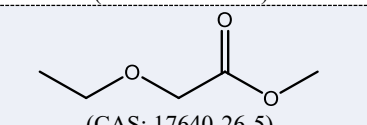
Figure 3. Summary of the major metabolic routes that generate methoxy- or ethoxyacetic acid.



3.2. List of category members

Table 1. List of ethylene glycol methyl ether (EGME)-related category members.

No.	Group	Substance structure (CAS No.)	Chemical name	Molecular formula	Molecular weight
1	-	 (CAS: 109-86-4)	Ethylene glycol methyl ether (EGME)	C ₃ H ₈ O ₂	76.095
2	-	 (CAS: 110-80-5)	Ethylene glycol ethyl ether (EGEE)	C ₄ H ₁₀ O ₂	90.122
3	Group 1	 (CAS: 110-49-6)	Ethylene glycol methyl ether acetate (EGMEA)	C ₅ H ₁₀ O ₃	118.132
4	Group 1	 (CAS: 111-15-9)	Ethylene glycol ethyl ether acetate (EGEEA)	C ₆ H ₁₂ O ₃	132.159
5	Group 1	 (CAS: 51855-82-4)	1,4-Bis(2-methoxyethyl) 2-methylidenebutanedioate	C ₁₁ H ₁₈ O ₆	246.259
6	Group 1	 (CAS: 106-00-3)	Bis(2-methoxyethyl) adipate	C ₁₂ H ₂₂ O ₆	262.302
7	Group 1	 (CAS: 3121-61-7)	2-Methoxyethyl acrylate	C ₆ H ₁₀ O ₃	130.143
8	Group 1	 (CAS: 106-74-1)	2-Ethoxyethyl acrylate	C ₇ H ₁₂ O ₃	144.17
9	Group 1	 (CAS: 6976-93-8)	2-Methoxyethyl methacrylate	C ₇ H ₁₂ O ₃	144.17
10	Group 1	 (CAS: 2370-63-0)	2-Ethoxyethyl methacrylate	C ₈ H ₁₄ O ₃	158.197
11	Group 2	 (CAS: 110-71-4)	Ethylene glycol dimethyl ether (EGDME)	C ₄ H ₁₀ O ₂	90.122
12	Group 2	 (CAS: 111-96-6)	Diethylene glycol dimethyl ether (DEGDME)	C ₆ H ₁₄ O ₃	134.175

13	Group 2	 (CAS: 112-36-7)	Bis(2-ethoxy ethyl) ether	C ₈ H ₁₈ O ₃	162.229
14	Group 3	 (CAS: 6290-49-9)	Methyl methoxyacetate	C ₄ H ₈ O ₃	104.105
15	Group 3	 (CAS: 17640-26-5)	Methyl 2-ethoxyacetate	C ₅ H ₁₀ O ₃	118.132

The selected category members are listed in Table 1 and fall into three groups:

- **Group 1:** This group is defined as esters of EGME and EGEE that are readily hydrolysed to generate EGME and EGEE. It is possible that lipases and/or carboxylesterases participate in the primary hydrolytic reaction, both of which have broad substrate specificity. Members 3–10 fall into this group.
- **Group 2:** This group is defined as chemicals that form EGME or EGEE via oxidative O-dealkylation. It has previously been shown that member 12 (diethylene glycol dimethyl ether; DEGDME) is converted to 2-(2-methoxyethoxy)ethanol and EGME in rats. Although methoxyacetic acid is a minor product that is generated by rat liver and excreted in the urine, this is associated with the testicular toxicity of member 12 (Richards *et al.*, 1993). It also seems possible that members 11 and 13 are transformed to methoxy- and ethoxyacetic acid, respectively.
- **Group 3:** This group is defined as esters of methoxy- and ethoxyacetic acid, which are included in a different group because they do not meet the definition of Groups 1 or 2, which lead to the metabolic formation of EGME or EGEE. It is logical to assume that the simple esters of members 14 and 15 are readily converted to methoxy- or ethoxyacetic acid.

Table 2. List of analogue chemicals that were not included in the category.

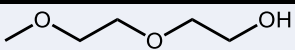
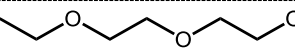
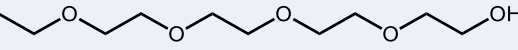
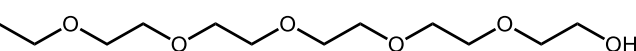

No.	Chemical name (CAS No.)	Structure
16	Diethylene glycol methyl ether (CAS: 111-77-3)	
17	Diethylene glycol ethyl ether (CAS: 111-90-0)	
18	3,6,9,12-Tetraoxatetradecan-1-ol (CAS: 5650-20-4)	
19	3,6,9,12,15-Pentaoxaheptadecan-1-ol (CAS: 4353-29-1)	
20	2,5,8,11,14,17-Hexaoxonadecan-19-ol (CAS: 23601-40-3)	

Table 2 shows analogue chemicals that were not included in this category. These chemicals consist of di- to oligo ethylene glycol methyl or ethyl ethers. Although it is possible that they undergo oxidative cleavage by P450 like member 12, it has been reported that diethylene glycol butyl ether (CAS No. 112-34-5), which is a prototype of this class of chemicals, and its acetate (CAS No. 124-17-4) are mainly converted to the corresponding

carboxylic acid through oxidation of the terminal alcohol group, presumably by alcohol dehydrogenase, and the glucuronide is also a major metabolite. By contrast, butoxyacetic acid, an oxidative cleavage product, was only seen in trace amounts or was not present (Deisinger *et al.*, 1989; Boatman *et al.*, 1993). Therefore, given that alcohol dehydrogenases have a much higher turnover than P450s, it is assumed that only trace, if any, amounts of methoxy- or ethoxyacetic acid are formed by this group of chemicals.

Thus, it is hypothesised that members 1–15 in Table 1 fall into the proposed category.

4. Justification of data gap filling

4.1. Data gathering

Publicly available toxicity data for EGME and related chemicals were collected using the HESS toxicity database since most of the toxicity studies that are included in the HESS have been performed in compliance with the OECD test guidelines and good laboratory practice (GLP). Data from additional toxicity studies were searched for in TOXNET (<http://toxnet.nlm.nih.gov/>), Google (<https://www.google.co.jp/>), PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), and the European Chemicals Agency (ECHA) database (<https://echa.europa.eu/>). The toxicity profiles and data sources for these studies are summarised in Annexes I and II.

Metabolism data were obtained from the HESS metabolism database, which contains metabolism maps for substances listed in the HESS toxicity database or their structural analogues according to papers published in peer-reviewed journals. In this case study, all available metabolism data were used regardless of the route of administration. The metabolism maps, study summaries, and data sources are provided in Annex III.

4.2. Justification

Datasets for six chemicals were selected from HESS, based on variations in the alkyl chain: EGME, EGEE, ethylene glycol isopropyl ether (EGiPE), ethylene glycol *tert*-butyl ether (EGtBE), ethylene glycol butyl ether (EGBE), and propylene glycol methyl ether acetate (PGMEA).

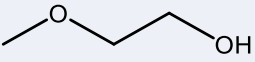
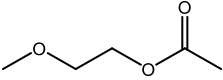
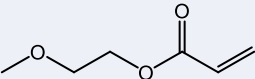
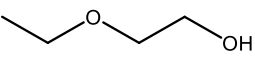
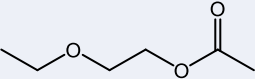
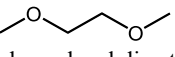
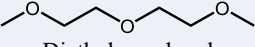
Table 3 shows a matrix that compares the metabolism and testicular toxicity effects of each of these chemicals in rats.

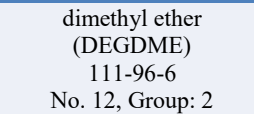
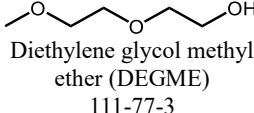
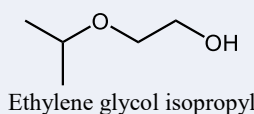
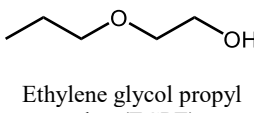
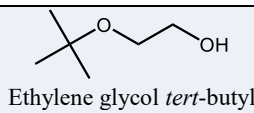
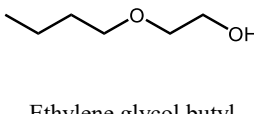
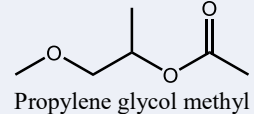
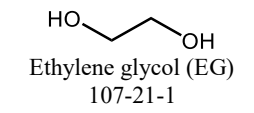
EGME, EGEE, EGiPE, and EGBE are mainly metabolised to their corresponding alkoxyacetic acids (Hutson and Pickering, 1971; Cheever *et al.*, 1984; Miller *et al.*, 1984a; Ghanayem *et al.*, 1987; Sabourin *et al.*, 1992; Sumner *et al.*, 1995), among which methoxyacetic acid and ethoxyacetic acid induce the degeneration of dividing spermatocytes, whereas butoxyacetic acid shows no toxic effects in primary rat testicular cell cultures (Gray *et al.*, 1985). Repeated-dose toxicity studies have shown that EGME has greater testicular toxicity than EGEE (NTP, 1993), while EGiPE, EGtBE, and EGBE have no testicular toxicity (NTP, 1993; MHLW, 2001, 2002).

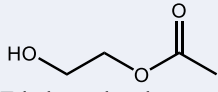
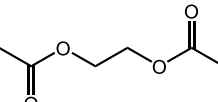
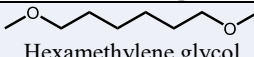
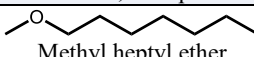
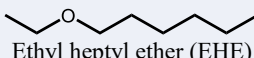
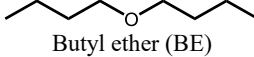
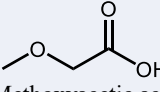
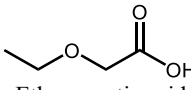
There appears to be a negative correlation between the number of n-alkyl carbon substituents ($n = 1-4$) in ethylene glycol alkyl ethers (EGAEs) and the potency of the testicular toxicity they induce. PGMEA is readily hydrolysed to propylene glycol methyl ether (PGME), which undergoes O-demethylation to form propylene glycol and conjugation with PGME glucuronide and sulphate, with no methoxyacetic acid being detected in rats (Miller *et al.*, 1984b). Furthermore, the repeated administration of PGMEA does not cause any testicular toxicity effects (MHLW, 1998).

Together, this information suggests that EGME and EGEE are similar in terms of their metabolism, mode of action, and testicular toxicity effects. EGiPE, EGtBE, EGBE, and PGMEA are not included in this category due to an absence of the metabolic formation of methoxy- or ethoxyacetic acid and a lack of testicular toxicity effects.

Table 3. A data matrix for ethylene glycol methyl ether (EGME) and EGME-related chemicals that were used to form a category.

Chemical structure Chemical name CAS no. No., group	Key metabolite formed ¹⁾	Testicular toxicity effects ²⁾	Study information ³⁾	Data source
 Ethylene glycol methyl ether (EGME) 109-86-4 No. 1, Group: -	+ Methoxy acetic acid (<i>in vivo</i>)	+ (≥750 ppm)	Fischer 344 rats, 13 weeks (consecutive), drinking water, 0 (water), 750, 1500, 3000, 4500 ppm (NTP guideline, GLP)	NTP, 1993 (retrieved from HESS)
		+ (dose not reported for histopathological changes)	ICR mice, 5 weeks (5 d/w), gavage, 0 (water), 62.5, 125, 250, 500, 1000, 2000 mg/kg/d (non-guideline, non-GLP)	Nagano <i>et al.</i> , 1984
 Ethylene glycol methyl ether acetate (EGMEA) 110-49-6 No. 3, Group: 1	+ EGME (<i>in vitro</i>)	+ (dose not reported for histopathological changes)	ICR mice, 5 weeks (5 d/w), gavage, 0 (water), 62.5, 125, 250, 500, 1000, 2000 mg/kg/d (non-guideline, non-GLP)	Nagano <i>et al.</i> , 1984
 2-Methoxyethyl acrylate 3121-61-7 No. 7, Group: 1	+ EGME (<i>in silico</i>)	+ (≥40 mg/kg/d)	CrI:WI(Han) rats, male: 31–35 days, female: 42–56 days (consecutive) 0 (propylene glycol), 40, 100, 250/150 mg/kg/d (OECD TG422, GLP)	ECHA, 2012
 Ethylene glycol ethyl ether (EGEE) 110-80-5 No. 2, Group: -	+ Ethoxy acetic acid (<i>in vitro</i>) (<i>in vivo</i>)	+ (≥10,000 ppm)	Fischer 344 rats, 13 weeks (consecutive), drinking water 0 (water), 1250, 2500, 5000, 10,000, 20,000 ppm (NTP guideline, GLP)	NTP, 1993 (retrieved from HESS)
		+ (dose not reported for histopathological changes)	ICR mice, 5 weeks (5 d/w), gavage, 0 (water), 500, 1000, 2000 mg/kg/d (non-guideline, non-GLP)	Nagano <i>et al.</i> , 1984
 Ethylene glycol ethyl ether acetate (EGEEA) 111-15-9 No. 4, Group: 1	+ EGEE (<i>in vitro</i>)	+ (dose not reported for histopathological changes)	ICR mice, 5 weeks (5 d/w), gavage, 0 (water), 500, 1000, 2000, 4000 mg/kg/d (non-guideline, non-GLP)	Nagano <i>et al.</i> , 1984
 Ethylene glycol dimethyl ether (EGDME) 110-71-4 No. 11, Group: 2	+ EGME (<i>in silico</i>)	+ (dose not reported for histopathological changes)	ICR mice, 5 weeks (5 d/w), gavage, 0 (water), 250, 500, 1000 mg/kg/d (non-guideline, non-GLP)	Nagano <i>et al.</i> , 1984
 Diethylene glycol	+ EGME (<i>in vitro</i>)	+ (684 mg/kg/d)	Sprague-Dawley rats, 20 days (consecutive), gavage,	Cheever <i>et al.</i> , 1989

 dimethyl ether (DEGDME) 111-96-6 No. 12, Group: 2	(<i>in vivo</i>)		0 (water), 684 mg/kg/d (non-guideline, non-GLP)		
 Diethylene glycol methyl ether (DEGME) 111-77-3 No. 16, Group: 2	+ EGME (<i>in silico</i>) (trace amount)	+	(3600 mg/kg/d)	Rats, 6 weeks, gavage, 0 (water), 900, 1800, 3600 mg/kg/d (non-guideline, non-GLP)	ECHA, 1982
 Ethylene glycol isopropyl ether (EGiPE) 109-59-1 No. 21, Group: -	- Isopropoxy acetic acid (<i>in vivo</i>)	-		Sprague-Dawley rats, 28 days (consecutive) gavage, 0 (water), 30, 125, 500 mg/kg/d (OECD TG 407, GLP)	MHLW, 2002 (retrieved from HESS)
 Ethylene glycol propyl ether (EGPE) 2807-30-9 No. 22, Group: -	- Propoxy acetic acid (<i>in silico</i>)	-		Sprague-Dawley rats 6 weeks (5 d/w), gavage, 0 (water), 3.75, 7.5, 15 mmol/kg/d (non-guideline, non-GLP)	Katz <i>et al.</i> , 1984
		-		ICR mice, 5 weeks (5 d/w), gavage, 0 (water), 250, 500, 1000 mg/kg/d (non-guideline, non-GLP)	Nagano <i>et al.</i> , 1984
 Ethylene glycol <i>tert</i> -butyl ether (EGtBE) 7580-85-0 No. 23, Group: -	- <i>tert</i> -Butoxy acetic acid (<i>in silico</i>)	-		Sprague-Dawley rats, 42 days (consecutive), gavage, 0 (water), 4, 20, 100 mg/kg/d (OECD TG422, GLP)	MHLW, 2001 (retrieved from HESS)
 Ethylene glycol butyl ether (EGBE) 111-76-2 No. 24, Group: -	- Butoxy acetic acid (<i>in vitro</i>) (<i>in vivo</i>)	-		Fischer 344 rats, 13 weeks (consecutive), drinking water, 0, 750, 1500, 3000, 4500, 6000 ppm (NTP guideline, GLP)	NTP, 1993 (retrieved from HESS)
		-		ICR mice, 5 weeks (5 d/w), gavage, 0 (water), 250, 500, 1000 mg/kg/d (non-guideline, non-GLP)	Nagano <i>et al.</i> , 1984
 Propylene glycol methyl ether acetate (PGMEA) 108-65-6 No. 25, Group: -	- (<i>in vivo</i>)	-		Sprague-Dawley rats, 44 days (consecutive), gavage, 0 (water), 100, 300, 1000 mg/kg/d (OECD TG422, GLP)	MHLW, 1998 (retrieved from HESS)
 Ethylene glycol (EG) 107-21-1 No. 26, Group: -	- (<i>in vivo</i>)	-		ICR mice, 5 weeks (5 d/w), gavage, 0 (water), 500, 1000, 2000, 4000 mg/kg/d (non-guideline, non-GLP)	Nagano <i>et al.</i> , 1984

 Ethylene glycol acetate (EGA) 542-59-6 No. 27, Group: -	- (<i>in silico</i>)	-	ICR mice, 5 weeks (5 d/w), gavage, 0 (water), 250, 500, 1000 mg/kg/d (non-guideline, non-GLP)	Nagano <i>et al.</i> , 1984
 Ethylene glycol diacetate (EGDA) 111-55-7 No. 28, Group: -	- (<i>in silico</i>)	-	ICR mice, 5 weeks (5 d/w), gavage, 0 (water), 250, 500, 1000 mg/kg/d (non-guideline, non-GLP)	Nagano <i>et al.</i> , 1984
 Hexamethylene glycol dimethyl ether (HGDE) 13179-98-1 No. 29, Group: -	+ Methoxy acetic acid (<i>in vivo</i>)	+ (200 mg/kg/d)	Sprague-Dawley rats, 4 weeks (5 d/w), gavage, 0 (corn oil), 2, 20, 200 mg/kg/d (non-guideline, non-GLP)	Poon <i>et al.</i> , 2004
 Methyl heptyl ether (MHE) 629-32-3 No. 30, Group: -	- (<i>in vivo</i>)	-	Sprague-Dawley rats, 4 weeks (5 d/w), gavage, 0 (corn oil), 2, 20, 200 mg/kg/d (non-guideline, non-GLP)	Poon <i>et al.</i> , 2005
 Ethyl heptyl ether (EHE) 5756-43-4 No. 31, Group: -	- (<i>in vivo</i>)	-	Sprague-Dawley rats, 4 weeks (5 d/w), gavage, 0 (corn oil), 2, 20, 200 mg/kg/d (non-guideline, non-GLP)	Poon <i>et al.</i> , 2005
 Butyl ether (BE) 142-96-1 No. 32, Group: -	- (<i>in vivo</i>)	-	Sprague-Dawley rats, 4 weeks (5 d/w), gavage, 0 (corn oil), 2, 20, 200 mg/kg/d (non-guideline, non-GLP)	Poon <i>et al.</i> , 2005
 Methoxyacetic acid 625-45-6 No. 33, Group: -	N/A	+ (≥100 mg/kg/d)	Fischer 344 rats, 8 days, gavage, 0 (water), 30, 100, 300 mg/kg/d (non-guideline, non-GLP)	Miller <i>et al.</i> , 1982
 Ethoxyacetic acid 627-03-2 No. 34, Group: -	N/A	+ (577 mg/kg/d)	Sprague-Dawley rats, 11 days, gavage, 0 (water), 577 mg/kg/d (non-guideline, non-GLP)	Foster <i>et al.</i> , 1983

1) +: EGME, EGEE, methoxy, or ethoxyacetic acid.

2) +: Decreased testicular weight and degeneration of the testis; -: no testicular effects up to the highest dose tested.

3) NTP, National Toxicology Program; GLP, good laboratory practice.

To evaluate the proposed category, 19 chemicals were selected from published reports that described repeated-dose toxicity studies of EGME and related chemicals and were not included in the HESS (Table 3). The toxicological profiles for these chemicals are summarised in Annex I.

Five-week oral toxicity studies of ICR mice showed that the administration of 250 mg/kg/d (3.28 mmol/kg/d) or more of EGME and 1000 mg/kg/d (11.09 mmol/kg/d) or more of EGEE led to a decrease in testis weight (Nagano *et al.*, 1984). Ethylene glycol ethyl ether acetate (EGEEA) is similar to ethylene glycol methyl ether acetate (EGMEA) in terms of structure, physicochemical properties, and chemical reactivity, and both chemicals are readily hydrolysed to form EGEE and EGME, respectively, in mice (Stott and McKenna, 1985). Consequently, the toxic potencies of EGMEA and EGEEA on the testis were predicted to be similar to those of EGMA and EGEE, respectively. Supporting this, the literature search revealed that the administration of EGMEA or EGEEA to ICR mice by gavage for 5 weeks resulted in a decreased testis weight at doses of 500 mg/kg/d (4.23 mmol/kg/d) and above for EGMEA and 1000 mg/kg/d (7.57 mmol/kg/d) and above for EGEEA (Nagano *et al.*, 1984). It is thus reasonable to conclude that these chemicals fall into this category.

Although ethylene glycol dimethyl ether (EGDME) and DEGDME are not EGAEs, they have substructures of EGME. No information is publicly available on the metabolism of EGDME. However, evidence strongly suggests that this chemical is metabolised to form EGME (Yokota *et al.*, 2005). In rats, the major metabolic pathway of DEGDME produces 2-(2-methoxyethoxy)ethanol (diethylene glycol methyl ether; DEGME) and the latter pathway generates EGME (Richards *et al.*, 1993). Hence, both chemicals belong to this category. The administration of 5.54 mmol/kg/d or more of EGDME for 5 weeks caused a reduction in testicular weight in mice (Nagano *et al.*, 1984). The administration of 5.10 mmol/kg/d of DEGDME for 20 days led to testicular degeneration and decreased testis weight in rats (Cheever *et al.*, 1989). Furthermore, DEGME is a weak testicular toxicant, with histopathological changes appearing at a high dose (3600 mg/kg/d) after 6 weeks of exposure (ECHA, 1982). Therefore, it is considered that this substance produces low levels of EGME via ether cleavage.

Ethylene glycol propyl ether (EGPE) contains a straight alkyl chain with three carbon atoms. Although no published information was found on the metabolism of EGPE, it is reasonable to presume that it is metabolised to propoxyacetic acid in the same way as EGEE and EGBE (Yamada *et al.*, 2012). Propoxyacetic acid has a very slight effect on primary rat testicular cell cultures at high concentrations (Gray *et al.*, 1985), suggesting that EGPE is not a member of this category. Furthermore, the repeated administration of EGPE does not cause testicular toxicity effects in mice (Nagano *et al.*, 1984) or rats (Katz *et al.*, 1984). These results suggest that there is a negative correlation between chain length and testicular toxicity.

Ethylene glycol (EG) is a minor metabolite of EGME (Sumner *et al.*, 1995). Metabolic information for EG shows that neither methoxy- nor ethoxyacetic acid are formed (Gessner *et al.*, 1961; Lenk *et al.*, 1989), indicating that EG does not fall into this category. Therefore, it is reasonable to presume that ethylene glycol acetate (EGA) and ethylene glycol diacetate (EGDA) do not fall into this category either. None of these chemicals show testicular toxicity effects in mice (Nagano *et al.*, 1984).

Hexamethylene glycol dimethyl ether (HGDE) is structurally related to DEGDME, and methoxyacetic acid has been found at high concentrations in the urine of HGDE-treated rats (Poon *et al.*, 2005; Wade *et al.*, 2006). Therefore, this chemical is also in this category, although its metabolic pathway remains unknown. It has been proposed that HGDE is converted to 1-methoxyhexanol via oxidative demethylation and that the metabolite is further oxidised to 1-methoxyhexanoic acid, which subsequently undergoes beta-oxidation to form methoxyacetic acid (Yamada *et al.*, 2014). In a 4-week gavage study, the

administration of 200 mg/kg/d (1.36 mmol/kg/d) of HGDE to Sprague-Dawley rats induced spermatocyte degeneration in the seminiferous tubules (Poon *et al.*, 2005). It should be noted that HGDE was not included in this category approach as it is not present in the CSCL inventory.

The structural analogues methyl heptyl ether (MHE), ethyl heptyl ether (EHE), and butyl ether (BE) did not produce a significant increase in methoxyacetic acid in the urine of rats dosed at 200 mg/kg/d (1.53 mmol/kg/d). Furthermore, these substances did not cause testicular toxicity effects in 4-week gavage studies, as expected (Poon *et al.*, 2005).

The exposure of rats to methoxyacetic acid on a daily basis over 8 days had similar toxicological effects in the testis as seen with EGME (Miller *et al.*, 1982). Moreover, limited studies that administered equimolar doses of methoxy- and ethoxyacetic acids to their parent compounds (500 mg/kg for 4 or 11 days) gave the same level of testicular damage as the corresponding EGAEs (Foster *et al.*, 1983).

According to the EU harmonised classification data from ECHA, six substances (members 1–4, 11 and 12) have been classified as “Repr. 1B, H360, presumed human reproductive toxicant,” largely based on data from animal studies. A table of the harmonised entries in Annex VI to the classification, labelling, and packaging (CLP) of substances and mixtures is available on the ECHA website (<https://echa.europa.eu/information-on-chemicals/annex-vi-to-clp>). Moreover, scientific evaluation of the CLH report for tetraglyme shows that this substance is proposed to be classified as a reproductive and developmental toxicant in the same way as triglyme, diglyme, and monoglyme under the CLP regulation. The scientific hypothesis for read-across is based on methoxyacetic acid and EGME having the same mode of action (Environment Agency Austria, 2017).

Based on this structural, metabolic, and toxicological information, this category can be finally defined as chemicals that are metabolised to methoxy- or ethoxyacetic acid.

5. Strategy for and integrated conclusion of data gap filling

5.1. Uncertainty

Table 4. Uncertainty of category justification and prediction using read-across.

Factor	Uncertainty (low, medium, high)	Comment
Hypothesis used for the read-across	Low	Ethylene glycol methyl ether (EGME) and ethylene glycol ethyl ether (EGEE) cause testicular toxicity after repeated oral doses in rodents. It is well known that the metabolites of these chemicals, methoxy- and ethoxyacetic acid, are responsible for this effect. Thus, chemicals that are metabolised to methoxy- or ethoxyacetic acid can be assessed for testicular toxicity using grouping and the read-across methodology.
Structural similarity	Low-medium	All of the chemicals in this category contain the same functional group, ether (R ₁ -O-R ₂), where R ₁ is a methyl or ethyl. For Groups 1 and 3, R ₂ commonly contains an ester, while for Group 2, R ₂ is an ether. R ₂ is partially different among members of Group 1; for example, some members have a branched structure. However, the core structural similarities are associated with producing the same active metabolites.
Similarity of physico-chemical properties	Low-medium	The measured or calculated logarithms of the partition coefficients with n-octanol and water (log P _{ows}) were within range of 1.0-1.5 for all of the category members. There is consistency between the measured and model-estimated values. It is expected that all of the category members have high bioavailability.
Toxicokinetic similarity	Low-medium	<i>In vivo</i> and <i>in vitro</i> data for the category members indicate that they have similar metabolic pathways. Group 1: Metabolism data for carboxylate esters with varied structures suggests that category members are mainly hydrolysed to EGME or EGEE. Group 2: Member 12 is oxidatively cleaved by rat liver microsomes to generate 2-(2-methoxyethoxy)ethanol (major) and EGME (minor). The quantitative results for the metabolites obtained <i>in vitro</i> is similar to those <i>in vivo</i> . 2-(2-Methoxyethoxy)ethanol is further metabolised to the corresponding acid as a major product via terminal alcohol oxidation and may be converted to EGME as a minor product via oxidative cleavage. Hence, member 12 generates EGME through two metabolic routes despite the low yield. Likewise, it is reasonable to presume that member 13 generates EGEE as a minor product. Thus, it is likely that members 12 and 13 produce EGME and EGEE, respectively, as minor products, although it is difficult to obtain an exact quantitative estimation of the production of these chemicals. It is also expected that member 11 produces EGME but in this case as a major product due to the absence of other metabolic reaction sites on the molecule. Group 3: Members 14 and 15 are methyl esters of methoxy- or ethoxyacetic acid. It is very likely that both simple esters are readily hydrolysed to form the corresponding alkoxyacetic acids.
Mode of action (MoA) / adverse outcome pathway (AOP)	Medium	This investigation was largely based on the metabolism of the chemicals of interest and a few adverse outcome pathways (AOPs). Other AOPs may play an important role. However, knowledge of the exact mechanisms is not crucial for the grouping and will not affect the conclusions. Evidence of the metabolic formation of methoxy- or ethoxyacetic acid is sufficient.

Number of analogues used for the read-across	Medium	The number of analogues that was used for the read-across was not many but sufficient. Test guideline study data are available for two chemicals that have methoxyacetic acid as an expected metabolite (members 1 and 7) and one chemical that has ethoxyacetic acid as an expected metabolite (member 2).
Quality of the endpoint data used for the read-across	Low	Data gaps were filled using two reliable studies: the good laboratory practice (GLP)-compliant Organisation for Economic Co-operation and Development (OECD) TG422 study (ECHA, 2012) for chemicals that will be metabolised to EGME or methoxyacetic acid (members 3, 5, 6, 9, 11, 12, and 14); and the GLP-compliant National Toxicology Program (NTP) guideline study (NTP, 1993) for chemicals that will be metabolised to EGEE or ethoxyacetic acid (members 4, 8, 10, 13, and 15). In these studies, toxicological effects on organ weight and the histopathology of the testis are well described so the quality of the endpoint data is high.
Similarity of the endpoint data (among source chemicals)	Low	<i>In vivo</i> data are available for seven chemicals in the category (members 1-4, 7, 11, and 12). All available studies have consistently shown that these chemicals have testicular toxicity in rodents. EGME is more toxic than EGEE in terms of testicular toxicity and this feature was also confirmed between members 3 (expected metabolite: EGME) and 4 (expected metabolite: EGEE).
Concordance and weight of evidence for all data used to justify the hypothesis	Low	There is good agreement between the formation of key metabolites based on <i>in vivo</i> , <i>in vitro</i> , or <i>in silico</i> data and testicular toxicity in rodents. It is not fully elucidated that the other carboxylic acid metabolites of target members also produce testicular toxicity. However, the available toxicological information of the structural analogues suggests that this is unlikely.
Overall uncertainty of the read-across	Low	The uncertainty associated with the read-across for testicular toxicity in rodents is judged to be low. All source and target members are expected to have common active metabolites (methoxy- or ethoxyacetic acid) and there are only minor differences among the category members with respect to their core structures, which are not considered to have a significant impact on the toxicity being read-across.

5.2. Integrated conclusion

Metabolism maps are available for members 3, 4, and 12 (EGMEA, EGEEA, and DEGDME, respectively), all of which have been shown to generate EGME or EGEE. Categorisation of the remaining substances (members 5–11 and 13–15) was addressed by predicting the primary metabolic reactions based on *in vivo* and *in vitro* metabolism data for related chemicals in the HESS metabolism database (see Annex III). Data gap filling by read-across was then carried out for ten chemicals (members 5–11, and 13–15) based on the uncertainty analysis shown in Table 4 and using the method described below.

First, the data gaps for the metabolic hydrolysis of members 5 and 6 were filled using the documented metabolism data for carboxylate esters with varied structures. Dimethyl succinate (CAS No. 106-65-0) and bis(2-ethylhexyl) adipate (CAS No. 103-23-1) have been shown to be susceptible to metabolic hydrolysis in both *in vitro* and *in vivo* studies (Takahashi *et al.*, 1981; Ladrière *et al.*, 1999), suggesting that members 5 and 6 are likely to be hydrolysed to form EGME.

Second, data gaps were filled for members 7–10, which have an alpha–beta unsaturated double bond moiety in their molecules. Four metabolism studies in the HESS have demonstrated that the structurally diverse esters ethyl acrylate, butyl acrylate and 2-hydroxyethyl methacrylate (CAS Nos. 140-88-5, 141-32-2 and 868-77-9, respectively) are readily hydrolysed in rats or mice (Gut *et al.*, 1988; Linhart *et al.*, 1994a,b; Durner *et al.*,

2009). Therefore, it is likely that members 7–10 form EGME and EGEE through metabolic hydrolysis.

It has been revealed that member 12 (DEGDME) is oxidatively cleaved by P450 in rat liver microsomes to generate 2-(2-methoxyethoxy)ethanol (major) and EGME (minor) (Richards *et al.*, 1993). Furthermore, rats that were dosed with 5.10 mmol/kg/d of DEGDME for 20 days exhibited testicular degeneration and decreased testis weight (Cheever *et al.*, 1989). It is proposed that 2-(2-methoxyethoxy)ethanol (member 16) generates a low level of EGME via oxidative dealkylation based on the observed weak testicular toxicity of this substance (ECHA, 1982; ECETOC, 2005), which suggests that member 12 produces EGME through two metabolic pathways. Therefore, it is reasonable to presume that member 13 undergoes oxidative cleavage to generate EGEE and it is also expected that member 11 produces EGME.

Members 14 and 15 are methyl esters of methoxy- and ethoxyacetic acid, respectively. Therefore, it is very likely that both simple esters are readily hydrolysed to form the corresponding alkoxyacetic acids.

It was predicted that EGME is a common metabolite for members 5, 6, 7, 9, and 11, and that methoxyacetic acid is formed by member 14. Moreover, it was predicted that EGEE is a metabolite for members 8, 10, and 13, and that ethoxyacetic acid is formed by member 15.

Group 1 members have diverse chemical structures as only core structural similarities are essential. Members 5 and 6 are expected to produce the dicarboxylate metabolites adipic acid and 2-methylenesuccinic acid, respectively, via metabolic hydrolysis, neither of which have been shown to have toxic effects in the reproductive organs (ECHA, 1993; OECD, 2004, see Annex II). Members 7–10 have an alpha–beta unsaturated double bond moiety in their molecules, which may react with nucleophilic groups of macromolecules. However, repeated-dose toxicity studies of acrylate or methacrylate esters in the HESS [2-hydroxyethyl acrylate (CAS No., 818-61-1), dodecyl acrylate (2156-97-0), n-butyl methacrylate (97-88-1), *tert*-butyl methacrylate (585-07-9), and 2-ethylhexyl methacrylate (688-84-6)] have shown that while they have moderate or weak toxic effects in the liver, kidney, spleen or blood, they do not produce any toxic effects in the reproductive organs (see Annex II).

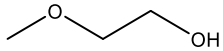
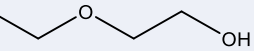
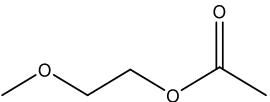
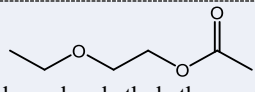
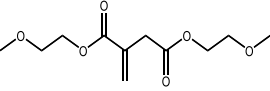
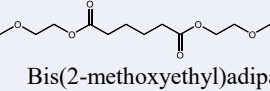
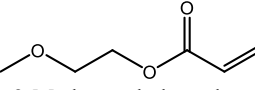
It is reasonable to fill the data gaps for these substances based on animal toxicity data of chemicals that are metabolised to EGME. A lowest-observed-adverse-effect level (LOAEL) of 0.31 mmol/kg/d was obtained for the testicular toxicity of 2-methoxyethyl acrylate (member 7) in a GLP-compliant combined repeated-dose toxicity study using a reproduction/developmental toxicity screening test (OECD TG422) via gavage in CrI:WI(Han) rats (ECHA, 2012). This LOAEL value is lower than that of member 1 (EGME) (0.93 mmol/kg/d). However, it is unlikely that the acrylate moiety is responsible for reproductive toxicity, as discussed above. Therefore, the lower LOAEL of member 7 compared with member 1 likely results from the dose setting in the toxicity studies as the toxicity levels of these two chemicals are considered similar. When performing read-across, a LOAEL value of 0.31 mmol/kg/d was used for the testicular toxicity of these substances as a worst-case scenario.

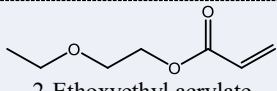
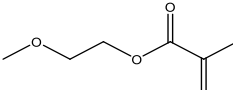
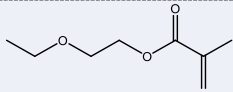
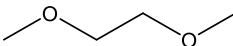
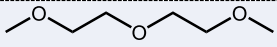
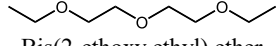
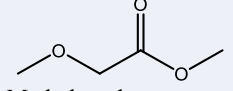
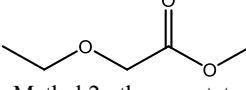
Toxicity data are available for members 3, 4, 11, and 12 (Nagano *et al.*, 1984). However, these data were not used in this assessment because the doses at which testicular pathological changes appeared were not shown. Hence, read-across was applied for

members 3, 11, and 12 using data from the GLP-compliant test guideline study on member 7, and for member 4 using the GLP-compliant NTP study on member 2.

For members 5 and 6, 2 moles of methoxyacetic acid can be formed from 1 mole of the parent compound. Therefore, an additional factor of 2 was applied to estimate the LOAEL value. The read-across approach was applied for members 8, 10, 13, and 15, using a no-observed-adverse-effect level (NOAEL) of 6.35 mmol/kg/d for EGEE based on a GLP-compliant 13-week drinking water study in Fischer 344 rats (NTP, 1993).

Table 5. Summary of the integrated conclusion.

No.	Group	Substance structure Chemical name (CAS No.)	Metabolic information available? (metabolism pathway / metabolite)	Point of Departure		D value (mg/kg/d) ₁
				Experimental result	Prediction by read-across	
1		 Ethylene glycol methyl ether (EGME) (CAS: 109-86-4)	Yes (oxidation / methoxyacetic acid)	LOAEL 0.93 mmol/kg/d (71 mg/kg/d)	/	0.0071
2		 Ethylene glycol ethyl ether (EGEE) (CAS: 110-80-5)	Yes (oxidation / ethoxyacetic acid)	NOAEL 6.35 mmol/kg/d (572 mg/kg)		0.57
3	Group 1	 Ethylene glycol methyl ether acetate (EGMEA) (CAS: 110-49-6)	Yes (hydrolysis / EGME)	(Data not used)	LOAEL 0.31 mmol/kg/d (37 mg/kg/d) (read-across from 2-methoxyethyl acrylate)	0.0037
4		 Ethylene glycol ethyl ether acetate (EGEEA) (CAS: 111-15-9)	Yes (hydrolysis / EGEE)	(Data not used)	NOAEL 6.35 mmol/kg/d (840 mg/kg/d) (read-across from EGEE)	0.84
5		 1,4-Bis(2-methoxyethyl) 2-methylidenebutanedioate (CAS: 51855-82-4)	Prediction (hydrolysis / EGME)	/	LOAEL 0.155 mmol/kg/d (38 mg/kg/d) (read-across from 2-methoxyethyl acrylate)	0.0038
6		 Bis(2-methoxyethyl)adipate (CAS: 106-00-3)	Prediction (hydrolysis / EGME)		LOAEL 0.155 mmol/kg/d (41 mg/kg/d) (read-across from 2-methoxyethyl acrylate)	0.0041
7		 2-Methoxyethyl acrylate (CAS: 3121-61-7)	Prediction (hydrolysis / EGME)		LOAEL 0.31 mmol/kg/d (40 mg/kg/d)	0.0040

8		 2-Ethoxyethyl acrylate (CAS: 106-74-1)	Prediction (hydrolysis / EGEE)		NOAEL 6.35 mmol/kg/d (920 mg/kg/d) (read-across from EGEE)	0.92
9		 2-Methoxyethyl methacrylate (CAS: 6976-93-8)	Prediction (hydrolysis/ EGME)		LOAEL 0.31 mmol/kg/d (45 mg/kg/d) (read-across from 2- methoxyethyl acrylate)	0.0045
10		 2-Ethoxyethyl methacrylate (CAS: 2370-63-0)	Prediction (hydrolysis / EGEE)		NOAEL 6.35 mmol/kg/d (1005 mg/kg/d) (read-across from EGEE)	1.01
11	Group 2	 Ethylene glycol dimethyl ether (EGDME) (CAS: 110-71-4)	Prediction (oxidation / EGME)	(Data not used)	LOAEL 0.31 mmol/kg/d (28 mg/kg/d) (read-across from 2- methoxyethyl acrylate)	0.0028
12		 Diethylene glycol dimethyl ether (DEGDME) (CAS: 111-96-6)	Yes (oxidation / EGME)	(Data not used)	LOAEL 0.31 mmol/kg/d (42 mg/kg/d) (read-across from 2- methoxyethyl acrylate)	0.0042
13		 Bis(2-ethoxy ethyl) ether (CAS: 112-36-7)	Prediction (oxidation / EGEE)		NOAEL 6.35 mmol/kg/d (1030 mg/kg/d) (read-across from EGEE)	1.03
14	Group 3	 Methyl methoxyacetate (CAS: 6290-49-9)	Prediction (hydrolysis / methoxyacetic acid)		LOAEL 0.31 mmol/kg/d (32 mg/kg/d) (read-across from 2- methoxyethyl acrylate)	0.0032
15		 Methyl 2-ethoxyacetate (CAS: 17640-26-5)	Prediction (hydrolysis / ethoxyacetic acid)		NOAEL 6.35 mmol/kg/d (750 mg/kg/d) (read-across from EGEE)	0.75

¹⁾ The D value (hazard assessment value) was calculated as NOAEL (mg/kg/d) / UF1000 or LOAEL (mg/kg/d) / UF10,000.

NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level; UF = uncertainty factor.

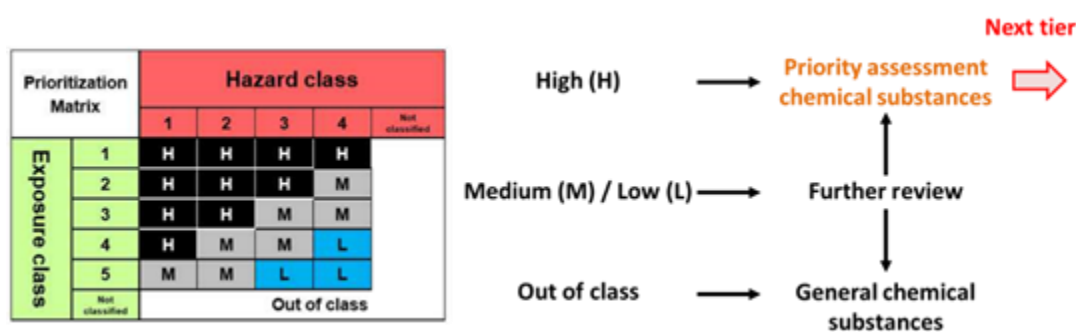
A screening assessment for human health and/or ecological concern is conducted under CSCL to select Priority Assessment Chemical Substances. Human health endpoints include repeated-dose toxicity, developmental and reproductive toxicity, mutagenicity, and carcinogenicity. Hazard classes for human health are assigned based on the hazard assessment value (D value), with a default class (class II) being assigned where no data are available.

The D value for reproductive and developmental toxicity is described by the following equation: $D = \text{NOAEL (mg/kg/d)} / \text{uncertainty factor}$. The uncertainty factor is calculated

by multiplying the species difference between animals and humans (10) by the individual difference between normal and sensitive humans (10), the study factor (10 if the NOAEL is derived from research other than a multi-generation reproductive and developmental study), and the toxicity level (10 if teratogenicity was observed without maternal toxicity). If the NOAEL is not available, the LOAEL is used to calculate the D value with an uncertainty factor of 10. Hazard classification of the toxicity endpoint is based on the following criteria: Class 2, $D \leq 0.005$; Class 3, $0.005 < D \leq 0.05$; Class 4, $0.05 < D \leq 0.5$; Out of Class, $D > 0.5$.

In this specific category approach, D values were derived based on testicular toxicity alone. Developmental toxicity was not assessed due to the limited resources for data gathering and analysis at present. Consequently, D values for the reproductive toxicity of the category members were estimated to be between 0.0028 and 1.03 mg/kg/d (Table 5). The hazard classification for this EGME-related chemical category was provisionally assigned as follows: Class 2 = members 3, 5, 6, 7, 9, 11, 12, and 14; Class 3 = member 1; Out of Class = members 2, 4, 8, 10, 13, and 15.

Figure 4. Workflow for the screening assessment used by the Japanese Chemical Substances of Control Law (CSCL).



In the CSCL screening assessment, the most severe class among the four endpoints (repeated-dose toxicity, developmental and reproductive toxicity, mutagenicity, and carcinogenicity) is applied to the target substance. This is then combined with the results of exposure class assignment to select the substances of high concern as Priority Assessment Chemical Substances, which will be subjected to higher-level risk assessments (Figure 4).

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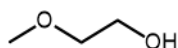
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Annex I. Summary data for toxicity studies on ethylene glycol methyl ether (EGME) and the EGME-related chemicals included in this case study

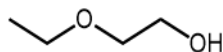
- Member 1: Ethylene glycol methyl ether (EGME) (CAS No. 109-86-4)



Reference	NTP, 1993	
Study type	Repeated dose toxicity study (13-week, NTP)	
Species	Rat (F344/N)	
Route	Oral (drinking water)	
Vehicle	Water	
Dose level	5 doses (750, 1500, 3000, 4500, 6000 ppm; male 71, 165, 324, 715, 806 mg/kg/d; female 70, 135, 297, 546, 785 mg/kg/d)	
Purity	98%	
	Male	Female
NO(A)EL	<750	<750
Death	8/10: 4500 10/10: 6000	5/10: 4500 10/10: 6000
General signs	Tremors: ≥750 Diarrhoea: ≥750 Emaciation: ≥750 Abnormal posture: ≥750 Pallor: ≥750 Tachypnea: ≥750 Comatose state: ≥750	Tremors: ≥750 Diarrhoea: ≥750 Emaciation: ≥750 Abnormal posture: ≥750 Pallor: ≥750 Tachypnea: ≥750 Comatose state: ≥750
Body weight	↓: ≥1500	↓: ≥1500
Food consumption	No effect	No effect
Water consumption	No effect	↓: ≥1500
FOB	No effect	No effect
Urinalysis	No effect	No effect
Haematology	Hct↓: ≥750 Hgb↓: ≥750 Plt↓: ≥750 RBC↓: ≥750 Ret↓: ≥750 WBC↓: ≥1500 Lymph↓: ≥1500 Seg↓: ≥1500	Hct↓: ≥1500 Hgb↓: ≥750 Plt↓: ≥750 RBC↓: ≥3000 Ret↓: 3000 WBC↓: ≥750 Lymph↓: ≥750 Seg↓: ≥3000
Blood chemistry	TP↓: ≥750 Alb↓: ≥750 Bile acid↑: ≥750	TP↓: ≥750 Alb↓: ≥1500 Bile acid↑: ≥1500
Absolute organ weight	Thymus↓: ≥750 Right testis↓: ≥1500	Thymus↓: ≥750
Relative organ weight	Thymus↓: ≥3000 Right testis↓: ≥1500	Thymus↓: ≥750
Necropsy	No effect	No effect
Histopathology	Spleen-Capsular fibrosis: ≥750 Testis-Atrophy: ≥750	Spleen-Capsular fibrosis: ≥1500

	Bone marrow-Cellular depletion: ≥ 4500 Spleen-Atrophy: ≥ 4500 Thymus-Atrophy: ≥ 1500 Prostate-Atrophy: ≥ 4500 Preputial gland-Atrophy: ≥ 3000 Seminal vesicle-Atrophy: ≥ 4500 Bone- metaphysis-Atrophy: ≥ 4500 Liver-Necrosis: ≥ 4500 Salivary glands-Atrophy: ≥ 4500 Stomach, glandular-Erosion: ≥ 4500 Forestomach, glandular-Mineralisation: ≥ 4500 Adrenal gland, cortex-Haemorrhage: ≥ 4500 Lymph node, mandibular-Depletion lymphoid: ≥ 4500 Lymph node, mesenteric-Angiectasis: ≥ 4500 Lymph node, mesenteric-Depletion lymphoid: ≥ 3000 Bone-Metaphysis atrophy: ≥ 4500	Bone marrow-Cellular depletion: ≥ 1500 Spleen-Atrophy: ≥ 1500 Thymus-Atrophy: ≥ 1500 Bone- metaphysis-Atrophy: ≥ 4500 Uterus-Atrophy: ≥ 3000 Ovary-Atrophy: ≥ 3000 Clitoral gland-Atrophy: ≥ 3000 Liver-Necrosis: 6000 Salivary glands-Atrophy: ≥ 4500 Stomach, glandular-Erosion: ≥ 4500 Forestomach, glandular-Mineralisation: 6000 Adrenal gland, cortex-Haemorrhage: ≥ 4500 Lymph node, mandibular-Depletion lymphoid: ≥ 4500 Lymph node, mesenteric-Angiectasis: ≥ 4500 Lymph node, mesenteric-Depletion lymphoid: ≥ 4500 Bone-Metaphysis atrophy: ≥ 4500
Bone marrow cellularity counts	\downarrow : ≥ 3000	\downarrow : ≥ 3000
Reproductive tissue evaluation	Spermatozoal measurements-Motility \downarrow : ≥ 750 Spermatid measurements-Spermatid count \downarrow : ≥ 750 Weights-Left epididymis \downarrow : ≥ 1500 Weights-Left cauda epididymis \downarrow : ≥ 1500 Weights-Left testis \downarrow : ≥ 1500	No effect
Reference	Nagano <i>et al.</i> , 1984	
Study type	Repeated dose toxicity study (5-weeks, 5 days/week)	
Species	Mice (JCL-ICR), males only	
Route	Oral (Gavage)	
Vehicle	Water or Olive oil (not specified)	
Dose level	6 doses (62.5, 125, 250, 500, 1000, 2000 mg/kg/d)	
Purity	-	
	Male	
NO(A)EL	Not reported	
Death	-	
General signs	-	
Body weight	-	
Food consumption	-	
Water consumption	-	
FOB	-	
Urinalysis	-	
Haematology	RBC \downarrow : (Higher dose level) HCT \downarrow : (Higher dose level) HGB \downarrow : (Higher dose level) WBC \downarrow : Not reported	
Blood chemistry	-	
Absolute organ weight	-	
Relative organ weight	Testis \downarrow : ≥ 250	
Necropsy		
Histopathology	(dose-related atrophy of the seminiferous epithelium)	

- Member 2: Ethylene glycol ethyl ether (EGEE) (CAS No. 110-80-5)

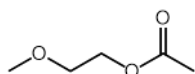


Reference	NTP, 1993	
Study type	Repeated dose toxicity study (13-week, NTP)	
Species	Rat (F344/N)	
Route	Oral (drinking water)	
Vehicle	Water	
Dose level	5 doses (1250, 2500, 5000, 10000, 20000 ppm; male 200, 357, 572, 919, 1582 mg/kg/d; female 192, 360, 526, 824, 1281 mg/kg/d)	
Purity	99%	
	Male	Female
Death	5/10: 20000	7/10: 20000
General signs	Emaciation: ≥ 1250 Diarrhoea: ≥ 1250 Abnormal posture: ≥ 1250 Tremors: ≥ 1250	Emaciation: ≥ 1250 Diarrhoea: ≥ 1250 Abnormal posture: ≥ 1250 Tremors: ≥ 1250
Body weight	\downarrow : ≥ 5000	\downarrow : ≥ 5000
Food consumption	No effect	No effect
Water consumption	\downarrow : ≥ 5000	\downarrow : ≥ 2500
FOB	No effect	No effect
Urinalysis	Volume \downarrow : 10000 pH \downarrow : 10000	No effect
Haematology	Hct \downarrow : ≥ 5000 Hgb \downarrow : ≥ 5000 RBC \downarrow : ≥ 5000 Ret \downarrow : ≥ 5000 Nucleated erythrocytes \uparrow : 10000 MCH \uparrow : ≥ 10000 MCHC \downarrow : ≥ 5000 WBC \uparrow : 10000 Seg \downarrow : ≥ 10000 ·20000 Lymph \downarrow : ≥ 5000	Hct \downarrow : ≥ 5000 Hgb \downarrow : ≥ 5000 RBC \downarrow : ≥ 5000 Ret \downarrow : ≥ 5000 Nucleated erythrocytes \uparrow : 10000 MCH \uparrow : ≥ 10000 MCHC \downarrow : ≥ 10000 WBC \uparrow : 10000 Seg \downarrow : 20000 Lymph \downarrow : ≥ 2500
Blood chemistry	TP \downarrow : ≥ 5000 Alb \downarrow : ≥ 10000 Bile acid \uparrow : ≥ 10000	ALT \uparrow : ≥ 5000 CK \uparrow : ≥ 2500 Bile acid \uparrow : ≥ 5000
Absolute organ weight	Right testis \downarrow : ≥ 10000 Thymus \downarrow : ≥ 2500	Thymus \downarrow : ≥ 10000
Relative organ weight	Right testis \downarrow : 10000 Thymus \downarrow : ≥ 2500	Thymus \downarrow : ≥ 10000
Necropsy	No effect	No effect
Histopathology	Liver-Hematopoietic cell proliferation: ≥ 10000 Liver-Inflammation, chronic, focal: ≥ 10000 Liver-Hepatocyte, centrilobular, degeneration: 20000 Liver-Kupffer cell, pigmentation: ≥ 10000 Salivary glands-Atrophy: 20000 Epididymis-Aspermia: ≥ 10000 Preputial gland-Atrophy: ≥ 10000 Prostate-Atrophy: ≥ 2500 Seminal vesicle-Atrophy: 20000	Liver-Hematopoietic cell proliferation: ≥ 10000 Liver-Hepatocyte, centrilobular, degeneration: 20000 Liver-Kupffer cell, pigmentation: ≥ 10000 Salivary glands-Atrophy: 20000 Epididymis-Aspermia: ≥ 10000 Bone marrow-Atrophy: 20000 Bone marrow-Proliferation, diffuse: ≥ 10000 Lymph node, mandibular-Atrophy: 20000 Lymph node, mesenteric-Atrophy: 20000

	Testis-Atrophy: 20000 Bone marrow-Atrophy: 20000 Bone marrow-Proliferation, diffuse: ≥10000 Lymph node, mandibular-Atrophy: 20000 Lymph node, mesenteric-Atrophy: 20000 Spleen-pigmentation, hemosiderin: 20000 Spleen-Capsule, mineralisation. Focal: 20000 Spleen-Lymphoid follicle, atrophy: 20000 Spleen-Red pulp, hematopoietic cell proliferation, diffuse: ≥5000 Thymus-Atrophy: ≥10000 Kidney-Cortex, mineralisation: 20000	Spleen-pigmentation, hemosiderin: 20000 Spleen-Capsule, mineralisation. Focal: 20000 Spleen-Lymphoid follicle, atrophy: 20000 Spleen-Red pulp, hematopoietic cell proliferation, diffuse: ≥10000 Thymus-Atrophy: ≥10000 Stomach, glandular-Ulcer: 20000 Clitoral gland-Atrophy: ≥10000 Ovary-Atrophy: 20000 Uterus-Atrophy: ≥10000 Vagina-Epithelium, atrophy: ≥10000
Reproductive tissue evaluation	Weights-Left epididymis↓: ≥5000 Weights-Left cauda epididymis↓: ≥5000 Weights-Left testis↓: ≥10000	No effect
Oestrous cycle characterisation	-	Oestrous cycle length↑: ≥10000
Bone marrow cellularity counts	↑: ≥10000	↑: ≥10000

Reference	Nagano <i>et al.</i> , 1984
Study type	Repeated dose toxicity study (5-weeks, 5 days/week)
Species	Mice (JCL-ICR), males only
Route	Oral (Gavage)
Vehicle	Water or Olive oil (not specified)
Dose level	4 doses (500, 1000, 2000, 4000 mg/kg/d)
Purity	-
Male	
NO(A)EL	Not reported
Death	-
General signs	-
Body weight	-
Food consumption	-
Water consumption	-
FOB	-
Urinalysis	-
Haematology	WBC↓: Not reported
Blood chemistry	-
Absolute organ weight	-
Relative organ weight	Testis ↓: ≥2000
Necropsy	-
Histopathology	(dose-related atrophy of the seminiferous epithelium)

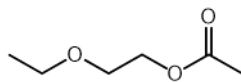
- Member 3: Ethylene glycol methyl ether acetate (EGMEA) (CAS No. 110-49-6)



Reference	Nagano <i>et al.</i> , 1984
Study type	Repeated dose toxicity study (5-weeks, 5 days/week)
Species	Mice (JCL-ICR), males only
Route	Oral (Gavage)

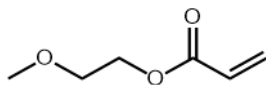
Vehicle	Water or Olive oil (not specified)
Dose level	6 doses (62.5, 125, 250, 500, 1000, 2000 mg/kg/d)
Purity	-
Male	
NO(A)EL	Not reported
Death	-
General signs	-
Body weight	-
Food consumption	-
Water consumption	-
FOB	-
Urinalysis	-
Haematology	RBC↓: (Higher dose level) HCT↓: (Higher dose level) HGB↓: (Higher dose level) WBC↓: Not reported
Blood chemistry	-
Absolute organ weight	-
Relative organ weight	Testis ↓: ≥500
Necropsy	-
Histopathology	(dose-related atrophy of the seminiferous epithelium)

- Member 4: Ethylene glycol ethyl ether acetate (EGEEA) (CAS No. 111-15-9)



Reference	Nagano <i>et al.</i> , 1984
Study type	Repeated dose toxicity study (5-weeks, 5 days/week)
Species	Mice (JCL-ICR), males only
Route	Oral (Gavage)
Vehicle	Water or Olive oil (not specified)
Dose level	4 doses (500, 1000, 2000, 4000 mg/kg/d)
Purity	-
Male	
NO(A)EL	Not reported
Death	-
General signs	-
Body weight	-
Food consumption	-
Water consumption	-
FOB	-
Urinalysis	-
Haematology	RBC↓: (Higher dose level) HCT↓: (Higher dose level) HGB↓: (Higher dose level) WBC↓: Not reported
Blood chemistry	-
Absolute organ weight	-
Relative organ weight	Testis ↓: ≥2000
Necropsy	-
Histopathology	(dose-related atrophy of the seminiferous epithelium)

- Member 9: 2-Methoxyethyl acrylate (CAS No. 3121-61-7)

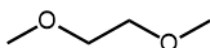


Reference	ECHA, 2012
Study type	Combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test (OECD 422)
Species	Rat (WI(Han))
Route	Oral (Gavage)
Vehicle	Propylene glycol
Dose level	3 doses (40, 100, 250/150 mg/kg/d *) *The dose was reduced from day12 of administration to 150 mg/kg/d due to severe parental toxicity.
Purity	≥99%
Male	
NO(A)EL	< 40
Death	3/10 (died or killed)
General signs	Hunched posture: 250 Salivation: 250
Body weight	↓: 250, Weight gain↓: ≥100
Food consumption	↓: 250
Water consumption	No effect
FOB	-
Urinalysis	No effect
Haematology	HGB↓: 250/150 MCH↓: 250/150 MCHC↓: 250/150 Plt↓: 250/150
Blood chemistry	TP↓: ≥100 Ca↓: ≥100 Cl↑: ≥100
Absolute organ weight	Thymus↓: ≥100 Testis↓: ≥100 Prostate↓: ≥100 Epididymis↓: ≥100 Seminal vesicle↓: ≥100
Relative organ weight	Thymus↓: ≥100 Testis↓: ≥100 Prostate↓: ≥100 Epididymis↓: ≥100 Seminal vesicle↓: ≥100
Necropsy	Testis-Small: ≥100 Epididymis-Small: ≥100 Forestomach-Irregular surface: 250/150 Glandular stomach- Irregular surface: 250/150 Thymus-Small: ≥100
Histopathology	Testis-Degeneration, seminiferous tubular epithelium: ≥ 40 Testis-Oedema: ≥ 100 Testis-Active inflammation, chronic:250/150 Testis-Enlarged cells: 250/150 Epididymis-Sperm degeneration: ≥ 100 Epididymis-Hypospermia: ≥ 100

Epididymis-Atrophy: ≥ 100
 Epididymis-Active inflammation, chronic: ≥ 100
 Spleen-Increased pigment: 250/150
 Liver-Hepatocellular necrosis: 250/150
 Stomach-Hyperplasia, forestomach: ≥ 40
 Stomach-Hyperkeratosis: ≥ 40
 Stomach-Active inflammation, chronic: ≥ 100

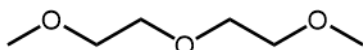
(Data from male rats only were included because female rats have a history of pregnancy and delivery.)

- Member 13: Ethylene glycol dimethyl ether (EGDME) (CAS No. 110-71-4)



Reference	Nagano <i>et al.</i> , 1984
Study type	Repeated dose toxicity study (5-weeks, 5 days/week)
Species	Mice (JCL-ICR)
Route	Oral (Gavage)
Vehicle	Water or Olive oil (not specified)
Dose level	3 doses (250, 500, 1000 mg/kg/d)
Purity	-
Male	
NO(A)EL	Not reported
Death	-
General signs	-
Body weight	-
Food consumption	-
Water consumption	-
FOB	-
Urinalysis	-
Haematology	RBC↓: (Higher dose level) HCT↓: (Higher dose level) HGB↓: (Higher dose level) WBC↓: Not reported
Blood chemistry	-
Absolute organ weight	-
Relative organ weight	Testis ↓: Not determined
Necropsy	-
Histopathology	(dose-related atrophy of the seminiferous epithelium)

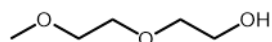
- Member 14: Diethylene glycol dimethyl ether (DEGDME) (CAS No. 111-96-6)



Reference	Cheever <i>et al.</i> , 1989
Study type	Repeated dose toxicity study (20-days)
Species	Rat (CrI: CD(SD)BR outbred), males only
Route	Oral (Gavage)
Vehicle	Water
Dose level	1 dose (684 mg/kg/d)
Purity	99.8%
Male	
NO(A)EL	Not reported

Death	-
General signs	-
Body weight	Gain↓: 684
Food consumption	-
Water consumption	-
FOB	-
Urinalysis	-
Haematology	-
Blood chemistry	No effect
Absolute organ weight	Testis↓: 684 Epididymis↓: 684 Thymus↓: 684
Relative organ weight	Testis↓: 684 Epididymis↓: 684 Thymus↓: 684
Necropsy	-
Histopathology	Testis-Degeneration, germinal cell: 684 Testis-Spermatocyte disappearance, seminiferous tubule: 684 Testis-Sperm bundle disappearance, seminiferous tubule: 684 Testis-Eosinophilic change, seminiferous tubule: 684 Testis-Degeneration, seminiferous tubule: 684 Testis-Spermatidic giant cell: 684 Testis-Oedema, interstitial: 684 Epididymis- Spermatidic giant cell: 684

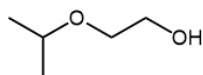
- Member 16: Diethylene glycol methyl Ether (DEGME) (CAS No. 111-77-3)



Reference	ECHA, 1982
Study type	Repeated dose 28-day oral toxicity study in rodents (OECD 407, 6-week, 5 days/week)
Species	Rat (CD), males only
Route	Oral (Gavage)
Vehicle	Water
Dose level	3 doses (900, 1800, 3600 mg/kg/d)
Purity	>99.5%
Male	
NO(A)EL	900
Death	None
General signs	No effect
Body weight	↓: 3600
Food consumption	↓: 3600
Water consumption	No effect
FOB	-
Urinalysis	-
Haematology	No effect
Blood chemistry	BUN↑: 3600
Absolute organ weight	Spleen↑: 3600 Liver↓: ≥1800 Brain↓: 3600
Relative organ weight	Kidney↑: 3600 Heart↑: ≥1800 Testis↓: 3600

Necropsy	No effect
Histopathology	Testis-Atrophy: 3600 Epididymis-Degeneration: 3600 Epididymis-Hypospermia: 3600 Kidney-Proteinaceous, proximal convoluted tubule: 3600

- Member 21: Ethylene glycol isopropyl ether (EGiPE) (CAS No. 109-59-1)



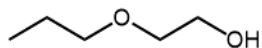
Reference	MHLW, 2002	
Study type	Repeated dose 28-day oral toxicity study in rodents (OECD 407)	
Species	Rat (SD)	
Route	Oral (Gavage)	
Vehicle	Water for injection	
Dose level	3 doses (30, 125, 500 mg/kg/d)	
Purity	99.5%	
	Male	Female
NO(A)EL	< 30	< 30
Death	None	None
General signs	No effect	No effect
Body weight	No effect	No effect
Food consumption	No effect	No effect
Water consumption	No effect	No effect
FOB	-	-
Urinalysis	Bil↑: 500 Occult blood↑: 500	Bil↑: 500 Occult blood↑: 500
Haematology	RBC↓: 500 Hgb↓: 500 MCHC↓: 500 Hct↓: 500 MCV↑: 500 MCH↑: 500 Ret↑: ≥125	RBC↓: ≥125 Hgb↓: ≥125 MCHC↓: ≥125 Hct↓: 500 MCV↑: 500 MCH↑: 500 Ret↑: 500
Blood chemistry	P↑: 500 K↑: 500	No effect
Absolute organ weight	Spleen↑: 500	Spleen↑: 500
Relative organ weight	Liver↑: 500 Kidney↑: 500 Spleen↑: 500	Liver↑: 500 Spleen↑: ≥125
Necropsy	No effect	No effect
Histopathology	Spleen-extramedullary haematopoiesis: ≥125 Bone marrow -haematopoiesis, increased, erythrocyte: ≥125 Spleen-deposit, pigment, brown: 500 Liver-extramedullary haematopoiesis: 500	Spleen-extramedullary haematopoiesis: ≥30 Bone marrow -haematopoiesis, increased, erythrocyte: ≥30 Spleen-deposit, pigment, brown: ≥125 Liver-extramedullary haematopoiesis: 500
Myelogram	Neutrophil↓: ≥30 Eosinophil↓: ≥30 Polychromatic erythroblast↑: ≥30 Myeloid/erythroid↓: ≥30 Promyelocyte↓: 500 Total granulocyte↓: ≥125	Myeloblast↓: ≥30 Polychromatic erythroblast↑: 500 Myeloid/erythroid↓: 500 Basophilic erythroblast↑: 500 Eosinophilic myelocyte↓: ≥125 Normoblast↑: ≥125

Total erythroblast↑: ≥125

Total granulocyte↓: 500

Total erythroblast↑: 500

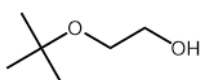
- Member 22: Ethylene glycol propyl ether (EGPE) (CAS No. 2807-30-9)



Reference	Katz <i>et al.</i> , 1984
Study type	Repeated dose toxicity study (6-week, 5 days/week)
Species	Rat (SD), males only
Route	Oral (Gavage)
Vehicle	Water
Dose level	4 doses (1.88, 3.75, 7.5, 15 ppm; 195, 390, 780, 1560 mg/kg/d)
Purity	99.5%
Male	
NO(A)EL	< 195
Death	1/10: 780, 2/10: 1560
General signs	Red coloured urine: ≥195 Weakness: ≥780 Laboured breathing: ≥780 Prostration: ≥780 Rale: ≥780
Body weight	↓: 1560
Food consumption	No effect
Water consumption	-
FOB	-
Urinalysis	Haemoglobinuria: ≥195
Haematology	RGB↓: ≥195 HGB↓: ≥195 MCV↓: ≥780 MCH↓: ≥780
Blood chemistry	No effect
Absolute organ weight	Spleen↑: ≥780
Relative organ weight	Spleen↑: ≥390
Necropsy	Spleen-Enlarged: ≥390
Histopathology	Spleen-Congestion: ≥390 Spleen- Extramedullary haematopoiesis: ≥390 Liver-Hemosiderin: 1560 Kidney-Proteinaceous cast: 1560 Kidney-Hemosiderin, proximal tubule, convoluted: ≥195
Reference	Nagano <i>et al.</i> , 1984
Study type	Repeated dose toxicity study (5-weeks, 5 days/week)
Species	Mice (JCL-ICR), males only
Route	Oral (Gavage)
Vehicle	Water or Olive oil (not specified)
Dose level	3 doses (500, 1000, 2000 mg/kg/d)
Purity	-
Male	
NO(A)EL	2000
Death	-
General signs	-

Body weight	-
Food consumption	-
Water consumption	-
FOB	-
Urinalysis	-
Haematology	No effect
Blood chemistry	-
Absolute organ weight	-
Relative organ weight	No effect
Necropsy	-
Histopathology	No effect

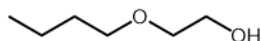
- Member 23: Ethylene glycol *tert*-butyl ether (EGtBE) (CAS No. 7580-85-0)



Reference	MHLW, 2001
Study type	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422)
Species	Rat (SD)
Route	Oral (Gavage)
Vehicle	Purified water
Dose level	3 doses (4, 20, 100 mg/kg/d)
Purity	99.9%
Male	
NO(A)EL	4
Death	None
General signs	No effect
Body weight	No effect
Food consumption	No effect
Water consumption	No effect
FOB	No effect
Urinalysis	No effect
Haematology	Hgb↓: 100 Hct↓: 100 MCV↑: 100 RBC↓: 100 WBC↓: 100 Ret↑: 100 MCHC↓: ≥20 MCH↑: 100
Blood chemistry	Cho↑: 100
Absolute organ weight	Spleen↑: 100
Relative organ weight	Liver↑: 100 Spleen↑: 100
Necropsy	-
Histopathology	Liver-extramedullary haematopoiesis: 100 Liver-hemosiderin deposition, Kupffer cell: ≥20 Kidney-hemosiderin deposition, tubular epithelium: 100 Spleen-extramedullary haematopoiesis, erythrocytic: ≥20 Spleen-hemosiderin deposition: 100 Bone marrow-increase in hematopoietic cell, erythrocytic: 100

(Data from male rats only were included because female rats have a history of pregnancy and delivery.)

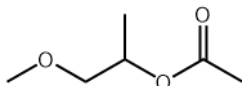
- Member 24: Ethylene glycol butyl ether (EGBE) (CAS No. 111-76-2)



	<i>Male</i>	<i>Female</i>
Reference	NTP, 1993	
Study type	Repeated dose toxicity study (13-week, NTP)	
Species	Rat (F344/N)	
Route	Oral (drinking water)	
Vehicle	Water	
Dose level	5 doses (750, 1500, 3000, 4500, 6000 ppm; male 73, 108, 174, 242, 346 mg/kg/d; female 77, 102, 152, 203, 265 mg/kg/d)	
Purity	99%	
	<i>Male</i>	<i>Female</i>
NO(A)EL	<750	<750
Death	None	None
General signs	Diarrhoea: ≥1500	Diarrhoea: ≥1500
Body weight	↓: ≥4500	↓: ≥3000
Food consumption	No effect	No effect
Water consumption	↓: ≥4500	↓: ≥1500
FOB	-	-
Urinalysis	Volume↓: ≥750 Specific gravity↑: ≥750	Specific gravity↑: ≥1500
Haematology	RBC↓:4500 MCV↑: 6000 MCH↓: 6000 Hgb↓: ≥1500 Ret↑: 6000 Nucleated erythrocytes↑: 6000 MCHC↓: 6000 Plt↓: 6000	Hct↓: ≥750 MCV↑: ≥750 MCH↓: ≥750 Hgb↓: ≥1500 Ret↑: 6000 Nucleated erythrocytes↑: 6000 MCHC↓: ≥4500 Plt↓: ≥3000 WBC↑: ≥4500 Seg↑: ≥4500 Lymph↑: ≥4500
Blood chemistry	BUN↑: ≥750 TP↓: 6000 Alb↓: ≥4500 ALT (GPT) ↑: 6000	BUN↑: 1500 Crn↑: ≥3000 TP↓: ≥1500 Alb↓: ≥4500 Alp↑: 6000 ALT (GPT) ↑: ≥4500
Absolute organ weight	No effect	Thymus↓: 6000
Relative organ weight	Right kidney↑: ≥750	Right kidney↑: ≥750
Necropsy	No effect	No effect
Histopathology	Liver-Cytoplasmic alteration: ≥750 Liver-Degeneration: ≥3000 Liver-Pigmentation: 6000 Bone marrow-Hyperplasia: ≥3000 Spleen-Hematopoietic cell proliferation: ≥4500 Spleen-Pigmentation: ≥1500	Liver-Cytoplasmic alteration: ≥750 Liver-Degeneration: ≥3000 Liver-Pigmentation: 6000 Bone marrow-Hyperplasia: ≥4500 Spleen-Hematopoietic cell proliferation: ≥4500
Reproductive tissue evaluation	Weights-Left epididymis↓: ≥4500	No effect

Bone marrow cellularity counts	No effect	↑: ≥3000
Reference	Nagano <i>et al.</i> , 1984	
Study type	Repeated dose toxicity study (5-weeks, 5 days/week)	
Species	Mice (JCL-ICR), males only	
Route	Oral (Gavage)	
Vehicle	Water or Olive oil (not specified)	
Dose level	3 doses (500, 1000, 2000 mg/kg/d)	
Purity	-	
Male		
NO(A)EL	Not reported	
Death	-	
General signs	-	
Body weight	-	
Food consumption	-	
Water consumption	-	
FOB	-	
Urinalysis	-	
Haematology	RBC↓: ≥500	
Blood chemistry	-	
Absolute organ weight	-	
Relative organ weight	No effect	
Necropsy	-	
Histopathology	No effect	

- Member 25: Propylene glycol methyl ether acetate (PGMEA) (CAS No. 108-65-6)

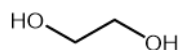


Reference	MHLW, 1998	
Study type	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422)	
Species	Rat (SD)	
Route	Oral (Gavage)	
Vehicle	Purified water	
Dose level	3 doses (100, 300, 1000 mg/kg/d)	
Purity	99.9%	
Male		
NO(A)EL	300	
Death	None	
General signs	No effect	
Body weight	↓: 1000	
Food consumption	↓: 1000	
Water consumption	No effect	
FOB	-	
Urinalysis	No effect	
Haematology	No effect	
Blood chemistry	Glu↓: 1000 P↓: 1000	

Absolute organ weight	No effect
Relative organ weight	No effect
Necropsy	No effect
Histopathology	No effect

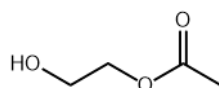
(Data from male rats only were included because female rats have a history of pregnancy and delivery.)

- Member 26: Ethylene glycol (EG) (CAS No. 107-21-1)



Reference	Nagano <i>et al.</i> , 1984
Study type	Repeated dose toxicity study (5-weeks, 5 days/week)
Species	Mice (JCL-ICR), males only
Route	Oral (Gavage)
Vehicle	Water or Olive oil (not specified)
Dose level	4 doses (500, 1000, 2000, 4000 mg/kg/d)
Purity	-
Male	
NO(A)EL	4000
Death	-
General signs	-
Body weight	-
Food consumption	-
Water consumption	-
FOB	-
Urinalysis	-
Hematology	No effect
Blood chemistry	-
Absolute organ weight	-
Relative organ weight	No effect
Necropsy	-
Histopathology	No effect

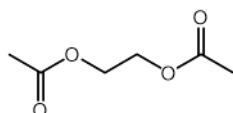
- Member 27: Ethylene glycol acetate (EGA) (CAS No. 542-59-6)



Reference	Nagano <i>et al.</i> , 1984
Study type	Repeated dose toxicity study (5-week, 5 days/week)
Species	Mice (JCL-ICR), males only
Route	Oral (Gavage)
Vehicle	Water or Olive oil (not specified)
Dose level	3 doses (500, 1000, 2000 mg/kg/d)
Purity	-
Male	
NO(A)EL	2000
Death	-
General signs	-
Body weight	-
Food consumption	-
Water consumption	-

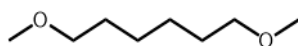
FOB	-
Urinalysis	-
Haematology	No effect
Blood chemistry	-
Absolute organ weight	-
Relative organ weight	No effect
Necropsy	-
Histopathology	No effect

- Member 28: Ethylene glycol diacetate (EGDA) (CAS No. 111-55-7)



Reference	Nagano <i>et al.</i> , 1984
Study type	Repeated dose toxicity study (5-week, 5 days/week)
Species	Mice (JCL-ICR), males only
Route	Oral (Gavage)
Vehicle	Water or Olive oil (not specified)
Dose level	3 doses (500, 1000, 2000 mg/kg/d)
Purity	-
Male	
NO(A)EL	2000
Death	-
General signs	-
Body weight	-
Food consumption	-
Water consumption	-
FOB	-
Urinalysis	-
Haematology	No effect
Blood chemistry	-
Absolute organ weight	-
Relative organ weight	No effect
Necropsy	-
Histopathology	No effect

- Member 29: Hexamethylene glycol dimethyl ether (HGDE) (CAS No. 13179-98-1)



Reference	Poon <i>et al.</i> , 2004
Study type	Repeated dose toxicity study (4-week, 5 days/week)
Species	Rat (SD), males only
Route	Oral (Gavage)
Vehicle	Corn oil
Dose level	3 doses (2, 20, 200 mg/kg/d)
Purity	>99%
Male	
NO(A)EL	< 2
Death	No effect
General signs	No effect

Body weight	No effect
Food consumption	No effect
Water consumption	-
FOB	-
Urinalysis	Ascorbic acid↑: 200
Haematology	No effect
Blood chemistry	PLT↓: 200 LDH↓: 200 Uric acid↓: 200
Absolute organ weight	No effect
Relative organ weight	Testis↓: 200 Thymus↓: 200
Necropsy	No effect
Histopathology	Liver-Vesiculation, nuclei: ≥2 Liver-Homogenisation increase, perivenous: ≥2 Liver-Decreased midzone density: 200 Liver-Basophilic change, peripheral: 200 Liver-Basophilic change, perivenous: 200 Thyroid-Follicle size reduction: ≥20 Thyroid-Colloid density reduction: 200 Testis-Degeneration, seminiferous tubule: 200 Testis-Atrophy: 200 Epididymis-Reduction, sperm: ≥20 Epididymis-Spermatidic giant cell: ≥20 Kidney-Cytoplasmic shedding, tubules outer cortex: ≥2 Kidney-Cytoplasmic inclusion, tubules outer cortex: ≥2 Thymus-Atrophy: 200

* HGDE is in the domain of the category because methoxyacetic acid was found at high concentrations in the urine of HGDE-treated rats (Poon *et al.*, 2005; Wade *et al.*, 2006). However, this chemical was not included in this category approach since it is not present in the Japanese CSCL inventory.

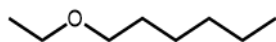
- Member 30: Methyl heptyl ether (MHE) (CAS No. 629-32-3)



Reference	Poon <i>et al.</i> , 2005
Study type	Repeated dose toxicity study (4-week, 5 days/week)
Species	Rat (SD), males only
Route	Oral (Gavage)
Vehicle	Corn oil
Dose level	3 doses (2, 20, 200 mg/kg/d)
Purity	>99%
Male	
NO(A)EL	< 2
Death	None
General signs	No effect
Body weight	No effect
Food consumption	No effect
Water consumption	-
FOB	-
Urinalysis	Ascorbic acid↑: 200
Haematology	No effect
Blood chemistry	No effect

Absolute organ weight	No effect
Relative organ weight	Thymus↓: 200
Necropsy	No effect
Histopathology	Liver-Vesiculation, nuclei: ≥2 Liver-Anisokaryosis, nuclei: ≥2 Liver-Cytoplasmic density increase, portal: ≥2 Liver-Cytoplasmic homogeneity increase, perivenous: ≥2 Thyroid- Follicle size reduction: ≥2 Thyroid-Papillary proliferaton: ≥2 Thyroid-Vacuolation, cytoplasm: ≥20 Thyroid-Vesiculation, nuclei: ≥2 Thyroid-Colloid density reduction: ≥2 Bone marrow-Dyserythopoiesis: ≥2 Bone marrow-Granulocyte increase: ≥2 Bone marrow-Myeloid/erythroid ratio increase: ≥2 Epididymis-Spermatid giant cell, body: ≥2 Epididymis-Sperm density reduction, tail: 200 Epididymis-Spermatid giant cell, tail: ≥2

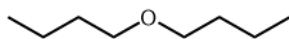
- Member 31: Ethyl hexyl ether (EHE) (CAS No. 5756-43-4)



Reference	Poon <i>et al.</i> , 2005
Study type	Repeated dose toxicity study (4-week, 5 days/week)
Species	Rat (SD), males only
Route	Oral (Gavage)
Vehicle	Corn oil
Dose level	3 doses (2, 20, 200 mg/kg/d)
Purity	>99%
Male	
NO(A)EL	< 2
Death	None
General signs	No effect
Body weight	No effect
Food consumption	No effect
Water consumption	-
FOB	-
Urinalysis	Ascorbic acid↑: 200
Haematology	No effect
Blood chemistry	No effect
Absolute organ weight	No effect
Relative organ weight	No effect
Necropsy	No effect
Histopathology	Liver-Vesiculation, nuclei: ≥2 Liver-Anisokaryosis, nuclei: ≥2 Liver-Cytoplasmic density increase, portal: ≥2 Liver-Cytoplasmic homogeneity increase, perivenous: ≥2 Thyroid- Follicle size reduction: ≥2 Thyroid-Papillary proliferaton: ≥2 Thyroid-Vacuolation, cytoplasm: ≥20 Thyroid-Vesiculation, nuclei: ≥2 Thyroid-Colloid density reduction: ≥2

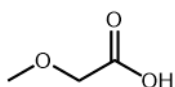
Bone marrow-Dyserythropoiesis: ≥ 2
 Bone marrow-Granulocyte increase: ≥ 2
 Bone marrow-Myeloid/erythroid ratio increase: ≥ 2
 Epididymis-Spermatid giant cell, body: ≥ 20
 Epididymis-Sperm density reduction, tail: ≥ 2
 Epididymis-Spermatid giant cell, tail: ≥ 20

• Member 32: Butyl ether (BE) (CAS No. 142-96-1)



Reference	Poon <i>et al.</i> , 2005
Study type	Repeated dose toxicity study (4-week, 5 days/week)
Species	Rat (SD), males only
Route	Oral (Gavage)
Vehicle	Corn oil
Dose level	3 doses (2, 20, 200 mg/kg/d)
Purity	>99%
Male	
NO(A)EL	< 2
Death	None
General signs	No effect
Body weight	No effect
Food consumption	No effect
Water consumption	-
FOB	-
Urinalysis	Ascorbic acid \uparrow : 200
Haematology	No effect
Blood chemistry	No effect
Absolute organ weight	No effect
Relative organ weight	No effect
Necropsy	No effect
Histopathology	Liver-Vesiculation, nuclei: ≥ 2 Liver-Anisokaryosis, nuclei: ≥ 2 Liver-Cytoplasmic density increase, portal: ≥ 200 Liver-Cytoplasmic homogeneity increase, perivenous: ≥ 2 Thyroid- Follicle size reduction: ≥ 20 Thyroid-Papillary proliferaton: ≥ 20 Thyroid-Vesiculation, nuclei: ≥ 2 Thyroid-Colloid density reduction: ≥ 2 Bone marrow-Dyserythropoiesis: ≥ 2 Bone marrow-Granulocyte increase: ≥ 2 Bone marrow-Myeloid/erythroid ratio increase: ≥ 2 Epididymis-Spermatid giant cell, body: 200 Epididymis-Spermatid giant cell, tail: 200

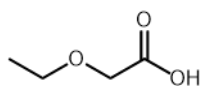
• Member 33: Methoxyacetic acid (MAA) (CAS No. 625-45-6)



Reference	Miller <i>et al.</i> , 1982
Study type	Repeated dose toxicity (10 days (5 days, 2 days off, 3 days))

Species	Rat (F344), males only
Route	Oral (Gavage)
Vehicle	Tap water
Dose level	3 doses (30, 100, 300 mg/kg/d)
Purity	99.3%
Male	
NO(A)EL	Not reported
Death	None
General signs	No effect
Body weight	Weight gain↓: 300
Food consumption	-
Water consumption	-
FOB	-
Urinalysis	-
Haematology	RBC↓: 300 HGB↓: 300 HCT↓: 300 WBC↓: 300
Blood chemistry	-
Absolute organ weight	Spleen↓: 300 Thymus↓: ≥100 Testis↓: 300
Relative organ weight	Spleen↓: 300 Thymus↓: ≥100 Testis↓: 300
Necropsy	Testis-Small size: ≥100 Testis-Flaccid: ≥100 Thymus-Small size: ≥100
Histopathology	Thymus-Depletion, cortical lymphoid elements, diffuse: ≥100 Testis-Degeneration, germinal epithelium, seminiferous tubule: ≥100 Bone marrow-Reduced cellularity: 300

- Member 34: Ethoxyacetic acid (CAS No. 627-03-2)

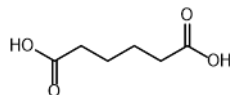


Reference	Foster <i>et al.</i> , 1983
Study type	Repeated dose toxicity (11 days)
Species	Rat (SD), males only
Route	Oral (Gavage)
Vehicle	Tap water
Dose level	1 dose (577 mg/kg/d which is equimolar to 500 mg/kg/d EGEE)
Purity	96.1%
Male	
NO(A)EL	Not reported
Death	-
General signs	-
Body weight	No effect
Food consumption	-
Water consumption	-
FOB	-
Urinalysis	-

<i>Haematology</i>	-
<i>Blood chemistry</i>	-
<i>Absolute organ weight</i>	Testis↓: 577
<i>Relative organ weight</i>	No effect
<i>Necropsy</i>	No effect
<i>Histopathology</i>	Testis-Degeneration, spermatocyte: 577

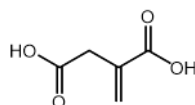
Annex II. Summary data for toxicity studies on carboxylic acids and acrylate or methacrylate esters

- Adipic acid (AA) (CAS No. 124-04-9)



Reference	OECD, 2004.	
Study type	Repeated dose toxicity study (2 years) (1957)	
Species	Rat (Carworth Farm strain)	
Route	Oral (Feed)	
Vehicle	-	
Dose level	4 doses (male; 0.1, 1, 3, 5%, 75, 750, 2250, 3750 mg/kg/d, female; 1%, 750 mg/kg/d)	
Purity	Not specified	
	Male	Female
NO(A)EL	1% (750 mg/kg/d)	1% (750 mg/kg/d)
Death	No effect	No effect
General signs	No effect	No effect
Body weight	↓: ≥3 Gain ↓: ≥3	No effect
Food consumption	↓: 5	No effect
Water consumption	-	-
FOB	-	-
Urinalysis	-	-
Haematology	-	-
Blood chemistry	-	-
Absolute organ weight	No effect	No effect
Relative organ weight	No effect	No effect
Necropsy	No effect	No effect
Histopathology	No effect	No effect

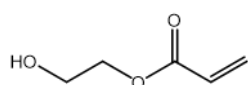
- 2-Methylenesuccinic acid (CAS No. 97-65-4)



Reference	ECHA, 1993	
Study type	Repeated dose 90-day oral toxicity in rodents (OECD 408)	
Species	Rat (SD)	
Route	Oral (Feed)	
Vehicle	-	
Dose level	3 doses (1500, 4700, 14000 ppm; male 106, 341, 1001 mg/kg/d; female 119, 358, 1088 mg/kg/d)	
Purity	-	
	Male	Female
NO(A)EL	1500	14000
Death	None	None
General signs	No effect	No effect

Body weight	No effect	No effect
Food consumption	No effect	No effect
Water consumption	-	-
FOB	-	-
Urinalysis	Specific gravity↑: ≥4700 pH↓: 14000	No effect
Haematology	No effect	No effect
Blood chemistry	No effect	No effect
Absolute organ weight	No effect	No effect
Relative organ weight	No effect	No effect
Necropsy	No effect	No effect
Histopathology	No effect	No effect

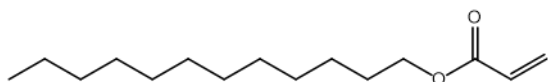
- 2-Hydroxyethyl acrylate (CAS No. 818-61-1)



Reference	MHLW, 2003	
Study type	Repeated dose 90-day oral toxicity study in rodents (OECD 408)	
Species	Rat (F344/DuCrj)	
Route	Drinking water	
Vehicle	-	
Dose level	5 doses (250, 500, 1000, 2000, 4000 ppm; male 17, 31, 58, 107, 190 mg/kg/d; female 24, 49, 73, 136, 228 mg/kg/d)	
Purity	96.4 %	
	Male	Female
NO(A)EL	< 250	< 250
Death	None	
General signs	Soiled muzzle: 4000 Soiled peritoneal region: 4000 Piloerection: 4000	Soiled muzzle: 4000 Piloerection: 4000 Oligo-stool: 500
Body weight	Gain↓: ≥1000	Gain↓: ≥250
Food consumption	↓: ≥250	↓: ≥250
Water consumption	↓: ≥250	↓: ≥250
FOB	-	
Urinalysis	Protein↑: 4000	Protein↑: ≥2000
Haematology	RBC↓: ≥2000 MCV↑: ≥2000 MCH↑: ≥2000 RET↑: 4000 PT↓: 4000	MCV↑: ≥2000 RET↑: 4000
Blood chemistry	TP↓: 4000 A/G↑: ≥2000 T-CHO↑: ≥1000 PL↑: ≥1000 T-BIL↑: 4000 GLUC↓: ≥2000 BUN↑: ≥2000 K↑: ≥2000	TP↓: 4000 ALB↓: 4000 A/G↑: ≥1000 T-CHO↑: ≥2000 TG↑: 1000 PL↑: ≥2000 γ-GTP↑: 4000 BUN↑: 4000

		CRE↓: 4000 Na↓: ≥1000 Kidney↑: ≥1000
Absolute organ weight	Liver↑: 4000	
Relative organ weight	Liver↑: ≥1000 Kidney↑: ≥500	Liver↑: ≥500 Kidney↑: ≥250
Necropsy	No effect	No effect
Histopathology	Kidney-Hyaline cast: 4000 Stomach-Hyperplasia, forestomach: 4000 Salivary gland-Atrophy: 4000	Kidney-Eosinophilic droplet, proximal tubule: ≥500 Salivary gland-Atrophy: 4000

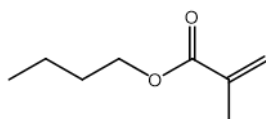
- Dodecyl acrylate (CAS No. 2156-97-0)



Reference	NITE, 2012
Study type	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422)
Species	Rat (SD)
Route	Oral (Gavage)
Vehicle	Corn oil (dissolved)
Dose level	3 doses (100, 300, 1000 mg/kg/d)
Purity	-
Male	
NO(A)EL	300
Death	None
General signs	No effect
Body weight	No effect
Food consumption	No effect
Water consumption	No effect
FOB	-
Urinalysis	No effect
Haematology	No effect
Blood chemistry	No effect
Absolute organ weight	No effect
Relative organ weight	Liver↑: 1000
Necropsy	No effect
Histopathology	No effect

(Data from male rats only were included because female rats have a history of pregnancy and delivery.)

- n-Butyl methacrylate (CAS No. 97-88-1)

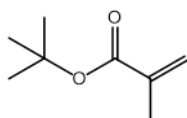


Reference	MHLW, 1992
Study type	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422)
Species	Rat (SD)
Route	Oral (Gavage)

Vehicle	Sesame oil
Dose level	4 doses (30, 100, 300, 1000 mg/kg/d)
Purity	-
Male	
NO(A)EL	30
Death	None
General signs	No effect
Body weight	↓: 1000
Food consumption	↓: 1000
Water consumption	No effect
FOB	-
Urinalysis	Occult blood: 1000
Haematology	No effect
Blood chemistry	No effect
Absolute organ weight	Spleen↓: ≥100
Relative organ weight	Spleen↓: ≥100
Necropsy	No effect
Histopathology	Spleen/red pulp-atrophy: ≥100

(Data from male rats only were included because female rats have a history of pregnancy and delivery.)

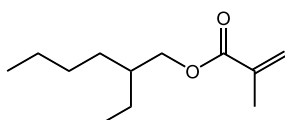
- *tert*-Butyl methacrylate (CAS No. 585-07-9)



Reference	MHLW, 1996	
Study type	Repeated dose 28-day oral toxicity study in rodents (OECD 407)	
Species	Rat (SD)	
Route	Oral (Gavage)	
Vehicle	0.5% CMC-Na added 0.1% Tween 80	
Dose level	3 doses (20, 100, 500 mg/kg/d)	
Purity	-	
	Male	Female
NO(A)EL	20	20
Death	None	None
General signs	Salivation: 500	Salivation: 500
Body weight	No effect	No effect
Food consumption	No effect	No effect
Water consumption	No effect	No effect
FOB	-	-
Urinalysis	Occult blood↑: ≥100 Bil↑: ≥100 Protein↑: 500 RBC↑: 500	Protein↑: 500
Haematology	No effect	No effect
Blood chemistry	Cho↑: ≥100 TP↑: ≥100 Alb↑: 500	Cho↑: ≥100 TP↑: ≥100 Alb↑: ≥100
Absolute organ weight	Liver↑: 500 Kidney↑: 500	Liver↑: 500 Kidney↑: 500
Relative organ weight	Liver↑: ≥100	Liver↑: 500

	Kidney↑: 500	Kidney↑: 500
Necropsy	No effect	No effect
Histopathology	Liver/centrilobular-hypertrophy, hepatocyte: ≥100 Kidney-hyaline droplet, tubular epithelium: ≥100 Kidney/proximal tubular-basophilic change, epithelium: ≥100	Liver/centrilobular-hypertrophy, hepatocyte: 500

- 2-Ethylhexyl methacrylate (CAS No. 688-84-6)

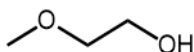


Reference	MHLW, 1996
Study type	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422)
Species	Rat (SD)
Route	Oral (Gavage)
Vehicle	Corn oil
Dose level	4 doses (30, 100, 300, 1000 mg/kg/d)
Purity	-
Male	
NO(A)EL	100
Death	None
General signs	No effect
Body weight	No effect
Food consumption	No effect
Water consumption	No effect
FOB	-
Haematology	Hct↓: 1000 Hgb↓: 1000 RBC↓: 1000 WBC↓:1000 TP↓: 1000
Blood chemistry	BUN↑: 1000 GOT↑(trend): 1000 GPT↑(trend): 1000 Cl↑: 1000
Absolute organ weight	Kidney↑: ≥300 Kidney↑: ≥300
Relative organ weight	Pituitary gland↑: ≥300 Liver ↑: ≥300
Necropsy	No effect
Histopathology	Spleen-decrease, haematopoiesis, extramedullary: 1000 Liver-necrosis, focal: 1000

(Data from male rats only were included because female rats have a history of pregnancy and delivery.)

Annex III. Summary data for metabolism studies included in this study

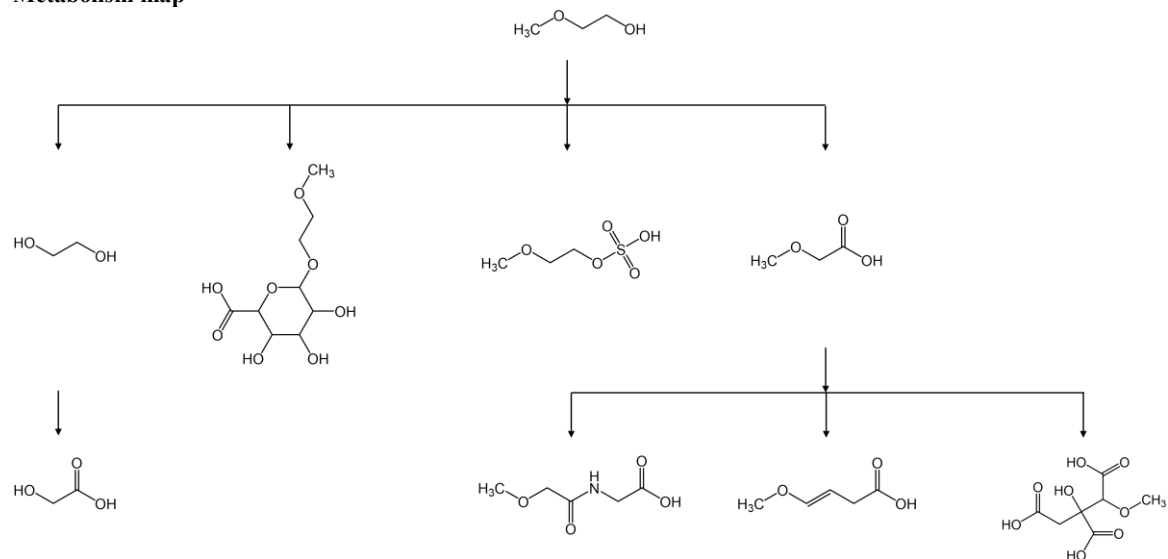
- Member 1: Ethylene glycol methyl ether (EGME) (CAS No. 109-86-4)



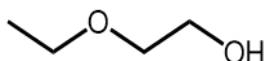
Reference	Sumner <i>et al.</i> , 1995
Subjects	CD-1 mice, female Fischer 344 rats, male
In vivo / in vitro	<i>In vivo</i>
Sampling / analytical	Urine (24 h)
Dose administration	Gavage, 25 and 250 mg/kg

Summary: This study assessed the role of metabolism in the adverse response to ethylene glycol methyl ether (EGME) by examining the metabolites produced in the urine following the administration of low (25 mg/kg) and high (250 mg/kg) doses of [1,2-¹³C]EGME to pregnant CD-1 mice and male Fischer 344 rats using carbon-13 nuclear magnetic resonance (NMR) spectroscopy. The urinary disposition was also examined after dosing pregnant CD-1 mice with a developmentally toxic level of EGME together with serine or acetate (known attenuators of EGME embryotoxicity). Seven novel metabolites were found in rat urine, which were consistent with those assigned in the mice.

Metabolism map



- Member 2: Ethylene glycol ethyl ether (EGEE) (CAS No. 110-80-5)

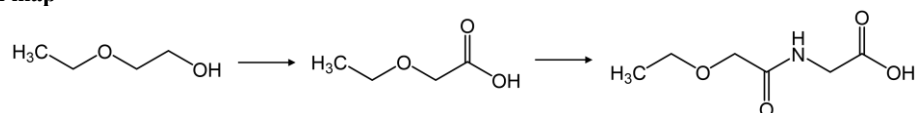


Reference	Cheever <i>et al.</i> , 1984
Subjects	Sprague-Dawley rats, male
In vivo / in vitro	<i>In vivo</i>
Sampling / analytical	Urine (96 h), testis (6 h)
Dose administration	Oral, 230 mg/kg, single dose

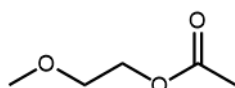
Summary: This study investigated the routes of ¹⁴C excretion following the administration of a single oral dose of 230 mg/kg body weight (bw) of 2-ethoxyethanol [ethanol-1,2-¹⁴C] or 2-ethoxyethanol [ethoxy-1-¹⁴C] to male Sprague-Dawley rats. The elimination of ¹⁴C by the urinary route accounted for 76% to 80% of the dose within 96 h. The main pathway of biotransformation is oxidation to the corresponding acid, with some subsequent conjugation of the acid metabolite with

glycine. The major metabolites, ethoxyacetic acid and N-ethoxy-acetyl glycine, which represented 73% to 76%, respectively, of the administered dose, were eliminated in the urine. The biological half-life was 9.9 ± 1.5 h for the ethoxy-labelled compound and 12.5 ± 1.9 h for the ethanol-labelled compound. Following administration of the ethanol-labelled compound, the only radiolabelled compound that was found in the rat testis was identified as ethoxyacetic acid.

Metabolism map



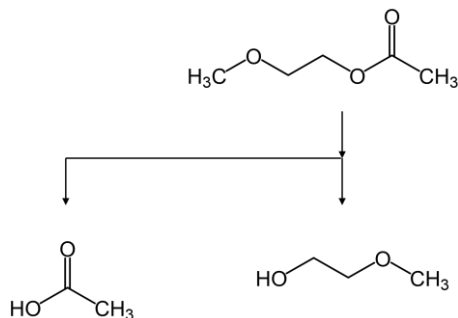
- Member 3: Ethylene glycol methyl ether acetate (EGMEA) (CAS No. 110-49-6)



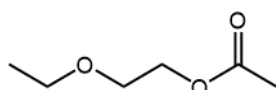
Reference	Stott and McKenna, 1985
Subjects	Fischer 344 rats, male
<i>In vivo / in vitro</i>	<i>In vitro</i>
Sampling / analytical	Incubation media (10 min)
Dose administration	<i>In vitro</i> incubation, 76 mM, single concentration

Summary: This study determined the *in vitro* activity of carboxylesterase recovered from the nasal mucosal tissues of B6C3F1/Cr1BR mice toward several agents that are known to cause olfactory epithelial lesions when inhaled by rodents. Apparent V_{\max} and K_m values were obtained for mouse nasal carboxylesterase using ethylene glycol methyl ether acetate (EGMEA), ethylene glycol ethyl ether acetate (EGEEA), and propylene glycol methyl ether acetate (PGMEA) as substrates. EGMEA and EGEEA appeared to be relatively good substrates for nasal carboxylesterase under enzyme-saturating and subsaturating conditions, as indicated by their high V_{\max} and V_{\max}/K_m values. Using EGMEA as a substrate, no sex differences in nasal carboxylesterase activity were observed in mice or rabbits. The specific activity of nasal carboxylesterase was found to be equivalent to that of the liver and greater than that of the kidney, lung, or blood. Mice and dogs were found to have similar nasal carboxylesterase activities that were slightly higher than that of rats and approximately six-fold higher than that of rabbits.

Metabolism map (in mice)



- Member 4: Ethylene glycol ethyl ether acetate (EGEEA) (CAS No. 111-15-9)

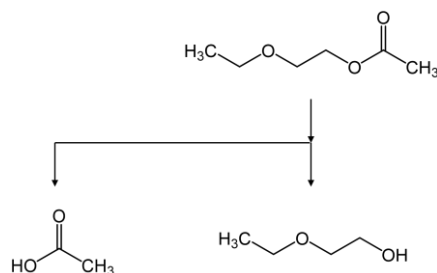


Reference	Stott and McKenna, 1985
Subjects	B6C3F1/Cr1BR mice, Fischer 344 rats, New Zealand albino rabbits, Beagle dogs
<i>In vivo / in vitro</i>	<i>In vitro</i>
Sampling / analytical	Incubation media (10 min)
Dose administration	<i>In vitro</i> incubation, 76 mM, single concentration

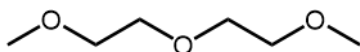
Summary: This study determined the *in vitro* activity of carboxylesterase recovered from the nasal mucosal tissues of

B6C3F1/CrIbR mice toward several agents that are known to cause olfactory epithelial lesions when inhaled by rodents. Apparent V_{max} and K_m values were obtained for mouse nasal carboxylesterase using ethylene glycol methyl ether acetate (EGMEA), ethylene glycol ethyl ether acetate (EGEEA), and propylene glycol methyl ether acetate (PGMEA) as substrates. EGMEA and EGEEA appeared to be relatively good substrates for nasal carboxylesterase under enzyme-saturating and subsaturating conditions, as indicated by their high V_{max} and V_{max}/K_m values. Using EGMEA as a substrate, no sex differences in nasal carboxylesterase activity were observed in mice or rabbits. The specific activity of nasal carboxylesterase was found to be equivalent to that of the liver and greater than that of the kidney, lung, or blood. Mice and dogs were found to have similar nasal carboxylesterase activities that were slightly higher than that of rats and approximately six-fold higher than that of rabbits.

Metabolism map (in mice)



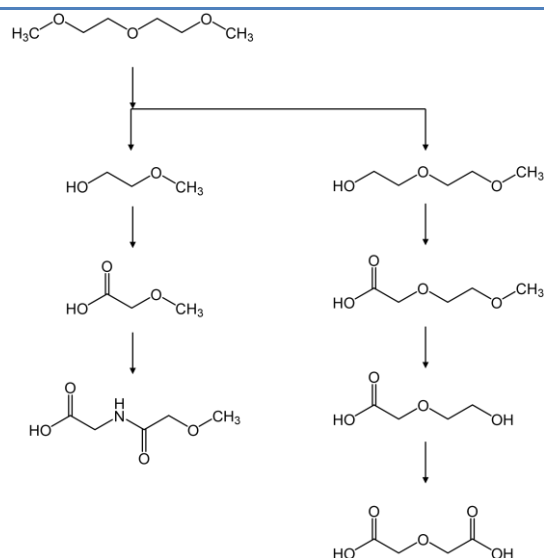
- Member 14: Diethylene glycol dimethyl ether (DEGDME) (CAS No. 111-96-6)



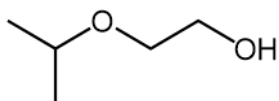
Reference	Richards <i>et al.</i> , 1993
Subjects	Weanling Sprague-Dawley [CrI:CD (SD) BR] outbred rats, male
<i>In vivo</i> / <i>in vitro</i>	<i>In vitro</i> and <i>in vivo</i>
Sampling / analytical	Incubation media (48 h) Urine (96 h)
Dose administration	<i>In vitro</i> incubation (up to 50 μ M) Oral, 5.1 mol/kg (radiolabelled parent), single dose

Summary: In the *in vitro* analysis, hepatocytes that had been isolated through two-step *in situ* collagenase perfusion of the liver were cultured as monolayers and incubated with [14 C]diethylene glycol dimethyl ether (DEGDME, diglyme) at 1, 10, 30, and 50 μ M for up to 48 h. In the *in vivo* analysis, rats were administered single oral doses of DEGDME at 5.1 mmol/kg bw and urine was collected for up to 96 h. The principal metabolite from the hepatocytes and in the urine was (2-methoxyethoxy)acetic acid, which accounted for approximately 36% of the radioactivity in the 48-h culture medium and approximately 67% of the administered dose in the 48-h urine sample. Other prominent metabolites that were common to both systems included 2-(2-methoxyethoxy)ethanol, methoxyacetic acid, 2-methoxyethanol, and diglycolic acid. The DEGDME metabolite profiles from the urine and hepatocytes were qualitatively similar.

Metabolism map



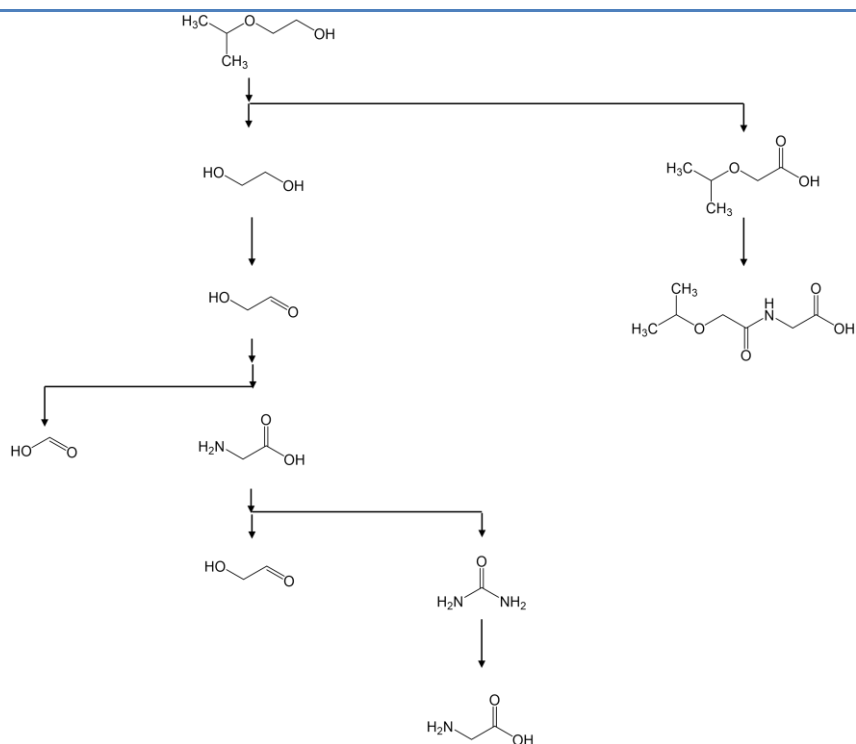
- Member 21: Ethylene glycol isopropyl ether (EGiPE) (CAS No. 109-59-1)



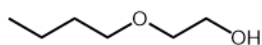
Reference	Hutson and Pickering, 1971
Subjects	Carworth Farm E rats, male and female
<i>In vivo / in vitro</i>	<i>In vivo</i>
Sampling / analytical	Urine (24 h)
Dose administration	Intraperitoneal, 0.993 mg, single dose

Summary: Six rats of each sex received 0.993 mg of [$1,2\text{-}^{14}\text{C}$] ethylene glycol isopropyl ether (EGiPE) (9.55 μCi) in 0.5 ml of 0.9% sterile saline by intraperitoneal injection. Urine and faeces were collected every 24 h for 4 days. The major routes of excretion of radioactivity were the urine (73% of dose) and expired air as [^{14}C]carbon dioxide (14%). The major urinary metabolites were characterised as isopropoxyacetic acid (30% of the urinary radioactivity), *N*-isopropoxyacetyl glycine (46%), and ethanediol (13%).

Metabolism map



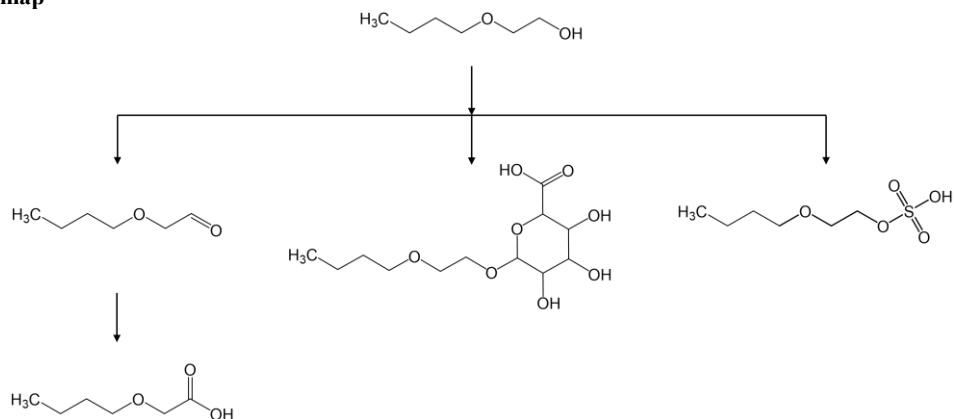
- Member 24: Ethylene glycol butyl ether (EGBE) (CAS No. 111-76-2)



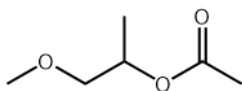
Reference	Ghanayem <i>et al.</i> , 1987
Subjects	Fischer 344 rats, male
<i>In vivo / in vitro</i>	<i>In vivo</i>
Sampling / analytical	Urine (8, 48 h), bile (60 min, 8 h)
Dose administration	Gavage, 500 mg/kg, single dose

Summary: Butoxyacetic acid (BAA) was identified as a urinary metabolite of ethylene glycol butyl ether (EGBE) by a single exposure of rats to EGBE. Treatment of rats with pyrazole (an alcohol dehydrogenase inhibitor) protected rats against EGBE-induced haematotoxicity and inhibited EGBE metabolism to BAA. Pyrazole inhibition of EGBE metabolism to BAA was accompanied by increased EGBE metabolism to EGBE-glucuronide and EGBE-sulphate. Together, these results show that the metabolic activation of EGBE via alcohol and aldehyde dehydrogenases is a prerequisite for the development of EGBE-induced haematotoxicity.

Metabolism map



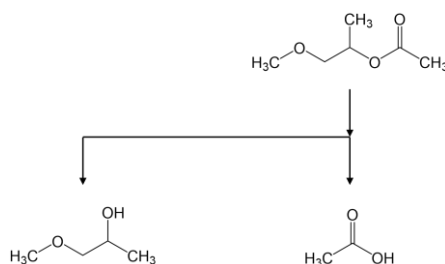
- Member 25: Propylene glycol methyl ether acetate (PGMEA) (CAS No. 108-65-6)



Reference	Domoradzki <i>et al.</i> , 2003
Subjects	Fischer 344 rats, male
<i>In vivo / in vitro</i>	<i>In vivo</i> and <i>in vitro</i>
Sampling / analytical	Blood
Dose administration	Intravenous, 14.7 and 147 mg/kg (<i>in vivo</i>) Incubation, 5 and 50 µg/ml (<i>in vitro</i>)

Summary: This study determined the blood kinetics of rats following intravenous administration of propylene glycol methyl ether (PGME) and Propylene glycol methyl ether acetate (PGMEA) at low [10 and 14.7 mg/kg bw, respectively] or high (100 and 147 mg/kg bw, respectively) equimolar doses. The blood time courses of PGME elimination were identical for both doses of both compounds. The half-lives of PGMEA elimination following the intravenous administration of 14.7 or 147 mg PGMEA/kg bw were calculated to be 1.6 and 2.3 min, respectively. Rat and human *in vitro* hydrolysis rates of PGMEA were determined by incubating 5 or 50 µg PGMEA/ml in whole blood or liver homogenate. The rate of loss of PGMEA was more rapid in rat blood than in human blood, with hydrolysis half-lives of 36 and 34 min in human blood and 16 and 15 min in rat blood for PGMEA concentrations of 5 and 50 µg/ml, respectively. By contrast, the rates of loss of PGMEA were similar in human and rat liver homogenate incubations at 27–30 min and 34 min, respectively.

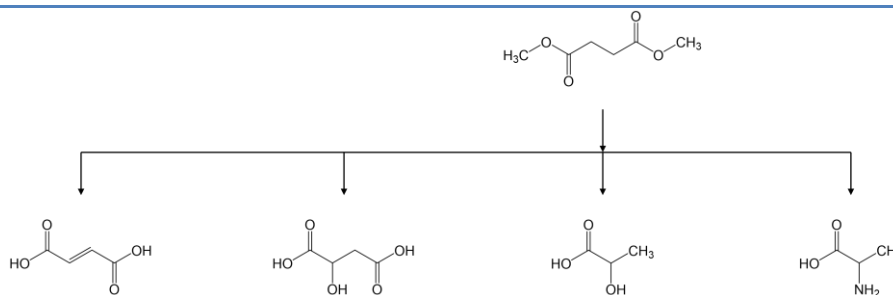
Metabolism map



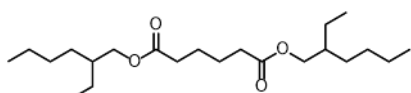
References	Miller <i>et al.</i> , 1984b
Subjects	Fischer 344 rats, male
<i>In vivo / in vitro</i>	<i>In vivo</i>
Sampling / analytical	Urine (12, 24 h)
Dose administration	Oral, 8.7 mmol/kg, single dose Inhalation, 3000 ppm, single concentration

Summary: Male Fischer 344 rats were given a single oral dose of approximately 8.7 mmol/kg of [1-¹⁴C]propylene glycol methyl ether acetate (PGMEA) or were exposed to 3000 ppm of [1-¹⁴C]PGMEA for 6 h. Approximately 64% of the administered ¹⁴C activity was eliminated as ¹⁴CO₂ and approximately 24% was excreted in the urine within 48 h of receiving a single oral dose of radiolabelled PGMEA. Similarly, 53% was eliminated as ¹⁴CO₂ and 26% was excreted in the urine within 48 h of the inhalation exposure. Propylene glycol, propylene glycol methyl ether (PGME), and the sulphate and glucuronide conjugates of PGME were identified as urinary metabolites after both oral dosing and inhalation exposure to PGMEA.

Metabolism map



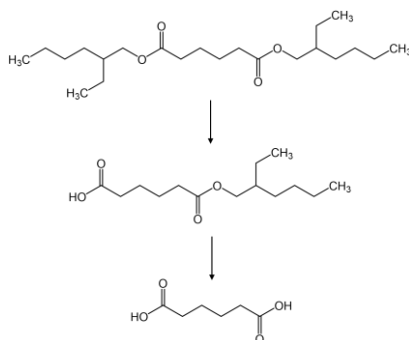
- Bis(2-ethylhexyl)adipate (DEHA) (CAS No. 103-23-1)



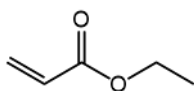
Reference	Takahashi <i>et al.</i> , 1981
Subjects	Wistar rats, male
<i>In vivo / in vitro</i>	<i>In vivo</i> and <i>in vitro</i>
Sampling / analytical	Urine, blood, stomach, liver, intestine (6 h) (<i>in vivo</i>) Incubation media (90 min) (<i>in vitro</i>)
Dose administration	Oral, 100 mg/kg, single dose (<i>in vivo</i>) Incubation, 0.1 mol/L, single dose (<i>in vitro</i>)

Summary: This study examined the excretion, retention, distribution, and metabolism of bis(2-ethylhexyl)adipate (DEHA) in rats. After oral administration of 100 mg/kg of [¹⁴C]DEHA, almost all of the dose was excreted within 48 h, predominantly in the urine and as respiratory carbon dioxide, with only low levels of faecal excretion. There was no evidence of the accumulation of radioactivity in any organs or tissues. Adipic acid (AA) was found to be the main urinary metabolite and was also detected in the digestive tract, blood, and liver. The *in vitro* analysis showed that DEHA was hydrolysed at a significant rate by tissue preparations from the liver, pancreas, and small intestine of rats. These results suggest that orally administered DEHA is rapidly hydrolysed in the body to form AA without any accumulation of mono-(2-ethylhexyl)adipate.

Metabolism map



- Ethyl acrylate (CAS No. 140-88-5)

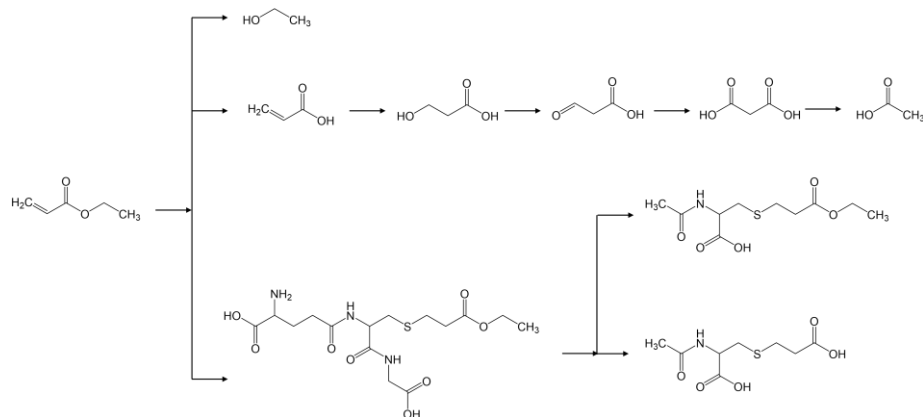


Reference	Linhart <i>et al.</i> , 1994a
Subjects	Wistar rats, female
<i>In vivo / in vitro</i>	<i>In vivo</i>
Sampling / analytical	Urine (24 h)
Dose administration	Intraperitoneal, 0.5, 1, and 2 mmol/kg, single dose

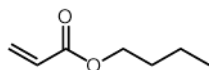
Summary: This study examined the excretion of urinary metabolites in rats dosed intraperitoneally with ethyl acrylate. The

physiological carboxylic acids 3-hydroxypropanoic acid, lactic acid, and acetic acid were detected, which may have been derived from the xenobiotic acrylate. There was a significant increase in the amounts of 3-hydroxypropanoic acid and acetic acid excreted within 24 h in the ethyl acrylate-exposed rats, as well as a slight increase in the excretion of lactic acid ($p < 0.10$). Two mercapturic acids, N-acetyl-S-(2-carboxyethyl)cysteine and the corresponding N-acetyl-S-[(2-alkoxycarbonyl)ethyl]cysteine, were formed from both acrylate esters, with 11% of the administered dose of ethyl acrylate being converted to mercapturic acids.

Metabolism



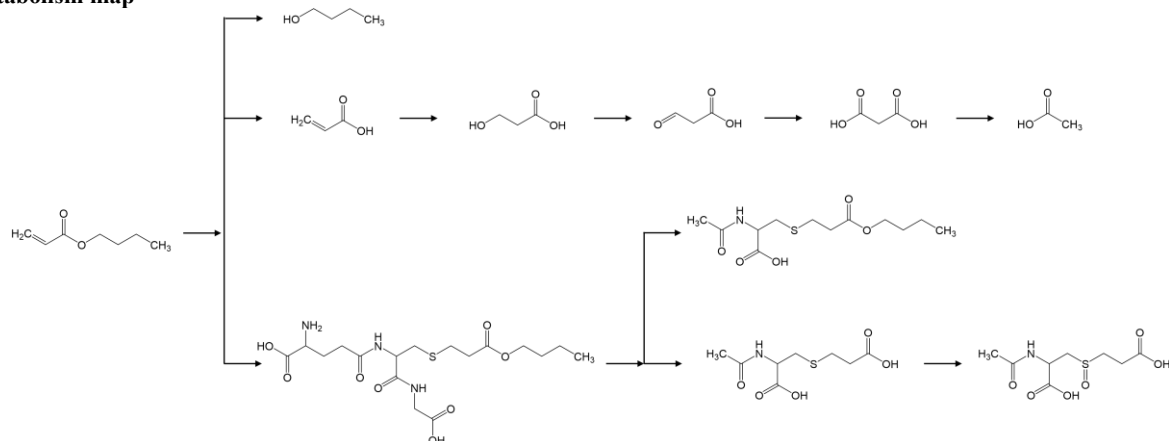
- Butyl acrylate (CAS No. 141-32-2)



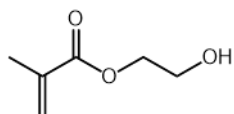
Reference	Linhart <i>et al.</i> , 1994b
Subjects	Wistar rats, female
In vivo / in vitro	<i>In vivo</i>
Sampling / analytical	Urine (20 h)
Dose administration	Intraperitoneal, 1 and 0.5 mmol/kg, single dose

Summary: Rats were administered a single intraperitoneal dose of 1 mmol/kg 1-butyl [$3\text{-}^{13}\text{C}$]acrylate and the urine was analysed in a one-dimensional ^1H -detected and two-dimensional ^1H - ^{13}C shift-correlated heteronuclear multiple-quantum NMR experiment. Three urinary metabolites were identified, namely 3-hydroxypropanoic acid, N-acetyl-S-(2-carboxyethyl)cysteine, and N-acetyl-S-(2-carboxyethyl)cysteine sulfoxide. In a separate experiment, rats were dosed with 0.25 mmol/kg of a carboxylesterase inhibitor, tri-*o*-tolyl phosphate, prior to 0.5 mmol/kg of butyl [$3\text{-}^{13}\text{C}$]acrylate to enhance the minor metabolic pathways. Under these conditions, N-acetyl-S-(2-carboxyethyl)cysteine, N-acetyl-S-[2-(butoxycarbonyl)-ethyl]cysteine, and N-acetyl-S-(2-carboxyethyl)cysteine sulfoxide were found in the urine. These results confirm that the main metabolic pathway for butyl acrylate is carboxylesterase-catalysed hydrolysis.

Metabolism map



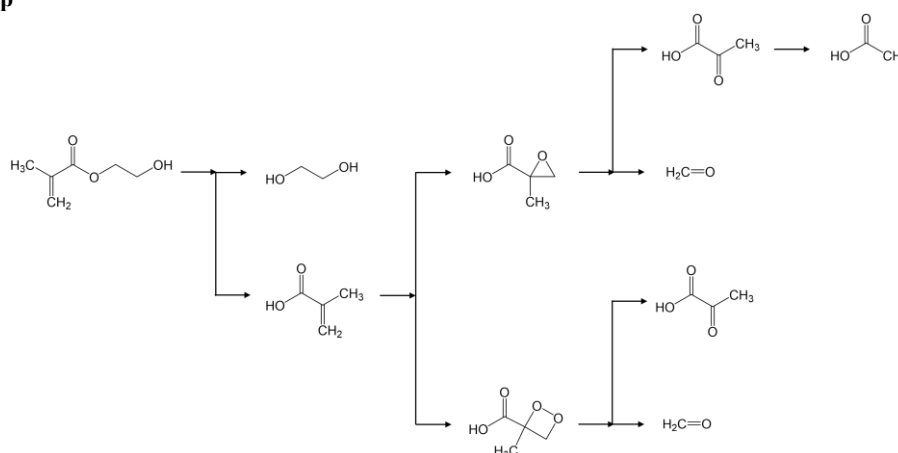
- 2-Hydroxyethyl methacrylate (HEMA) (CAS No. 868-77-9)



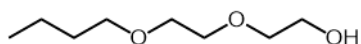
Reference	Durner <i>et al.</i> , 2009
Subjects	CD1 mice, male
<i>In vivo / in vitro</i>	<i>In vivo</i>
Sampling / analytical	Urine (24 h)
Dose administration	Subcutaneous, oral, 20 mol/kg, single dose

Summary: This study examined the uptake, distribution, and excretion of ^{14}C -2-hydroxyethyl methacrylate (HEMA) administered to mice via gastric tube or subcutaneous administration at dose levels that were well above those encountered in dental care to test the hypothesis that HEMA can reach cytotoxic levels in mammalian tissues. ^{14}C -HEMA was taken up rapidly from the stomach and intestines after gastric administration and was widely distributed through the body following administration by both routes. Most ^{14}C was excreted within 1 day as $^{14}\text{CO}_2$ and the peak HEMA levels after 24 h were lower than known toxic levels in all tissues examined. Two metabolic pathways of ^{14}C -HEMA can be described, possibly starting with its hydrolysis to ^{14}C -methacrylic acid and ethane-1,2-diol.

Metabolism map



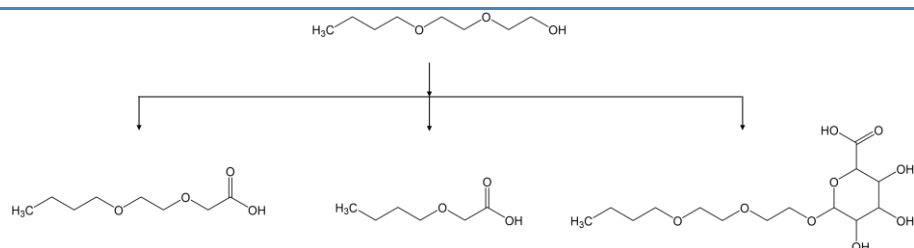
- Diethylene glycol butyl ether (DGBE) (CAS No. 112-34-5)



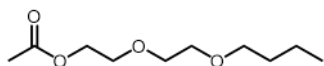
Reference	Boatman <i>et al.</i> , 1993
Subjects	Sprague-Dawley rats, male
<i>In vivo / in vitro</i>	<i>In vivo</i>
Sampling / analytical	Urine (24 h)
Dose administration	Dermal, 200 and 2000 mg/kg

Summary: This study determined the absorption and elimination of dermally applied doses of ^{14}C -diethylene glycol butyl ether (DGBE) and ^{14}C -diethylene glycol butyl ether acetate (DGBA) in Sprague-Dawley rats. The materials were applied under occlusion for 24 h at doses of 200 and 2000 mg/kg (undiluted) and as a 10% aqueous solution (200 mg/kg DGBE). Urinary excretion accounted for the majority of recovered ^{14}C in all studies, with 2-(2-butoxyethoxy)acetate acid being the major urinary metabolite identified. The glucuronide of DGBE represented 5.2% to 8.2% of the urinary ^{14}C . The dermal absorption rates were estimated to be 1.58 (DGBA, male), 1.28 (DGBA, female), 0.73 (DGBE, male), and 1.46 (DGBE, female) $\text{mg cm}^{-2} \text{h}^{-1}$.

Metabolism map



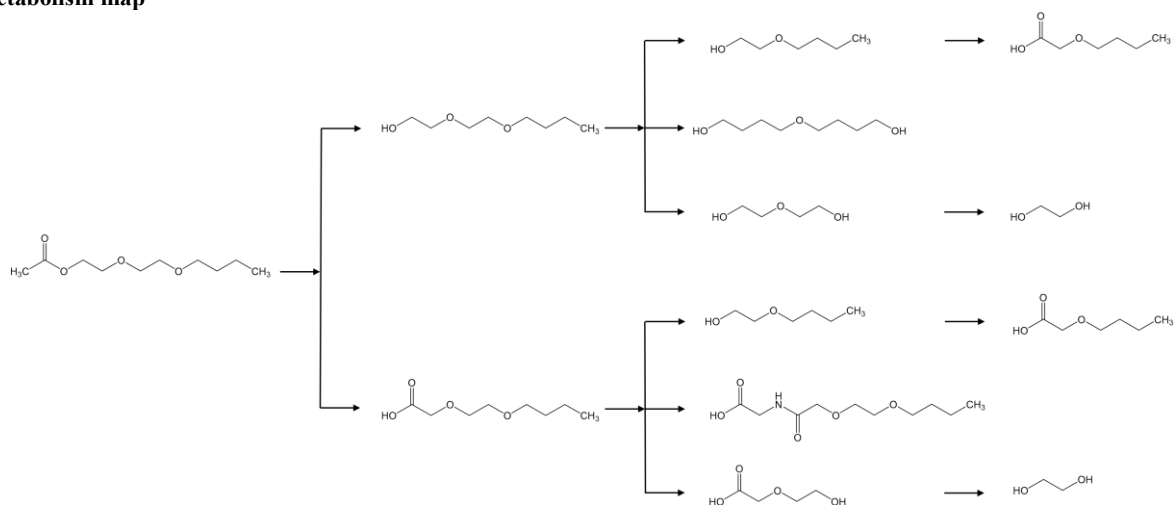
- Diethylene glycol butyl ether acetate (DGBEA) (CAS No. 124-17-4)



Reference	Deisinger <i>et al.</i> , 1989
Subjects	Sprague-Dawley rats, male
<i>In vivo / in vitro</i>	<i>In vivo</i>
Sampling / analytical	Urine (24 h)
Dose administration	Oral, 200 and 2000 mg/kg

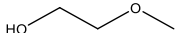
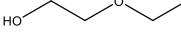
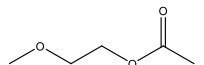
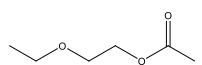
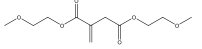
Summary: This study examined the *in vitro* hydrolysis of diethylene glycol butyl ether acetate (DGBEA) in rat blood and its *in vivo* metabolism and disposition in male Sprague-Dawley rats. DGBEA (5 mM) was hydrolysed in rat blood to Diethylene glycol butyl ether (DGBE) with a half-life of <3 min. ¹⁴C- DGBEA was rapidly absorbed from the gastrointestinal tract and eliminated predominantly in the urine within 24 h following the oral administration of 200 or 2000 mg/kg DGBEA. The major urinary metabolite was 2-(2-butoxyethoxy)acetic acid. No unchanged DGBEA or DGBE was detected in rat urine at either dose level. Trace amounts of 2-butoxyacetic acid were also found, which has been shown to exert haematological effects in rats.

Metabolism map



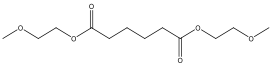
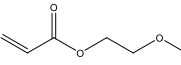
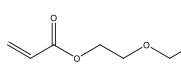
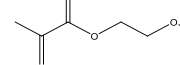
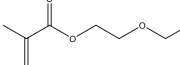
Appendix. Data matrix, IATA for "case study on the use of integrated approaches for testing and assessment for testicular toxicity of ethylene glycol methyl ether (EGME)-related chemicals"

Data matrix, IATA for "case study on the use of integrated approaches for testing and assessment for testicular toxicity of ethylene glycol methyl ether (EGME)-related chemicals"

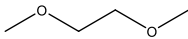
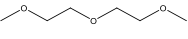
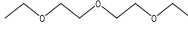
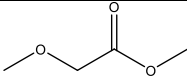
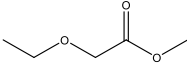
Chemical ID						
		Member 1	Member 2	Member 3	Member 4	Member 5
CAS		109-86-4	110-80-5	110-49-6	111-15-9	51855-82-4
Name		Ethylene glycol methyl ether	Ethylene glycol ethyl ether	Ethylene glycol methyl ether acetate	Ethylene glycol ethyl ether acetate	1,4-Bis(2-methoxyethyl) 2-methylidenebutanedioate
Structure						
Summary of data gap filling						
		Member 1	Member 2	Member 3	Member 4	Member 5
Testicular toxicity	Experimental result	NOAEL: <0.93 mmol/kg/d, LOAEL: 0.93 mmol/kg/d Rat, NTP 13-week drinking water study, Degeneration of germinal epithelium in the seminiferous tubules (NTP, 1993)	NOAEL: 6.35 mmol/kg/d, LOAEL: 10.19 mmol/kg/d Rat, NTP 13-week drinking water study, Degeneration of germinal epithelium in the seminiferous tubules (NTP, 1993)	NOAEL: not reported Mouse, 5-week gavage dose study, Atrophy of the seminiferous epithelium, testicular weight↓ (Nagano et al., 1984)	NOAEL: not reported Mouse, 5-week gavage dose study, Atrophy of the seminiferous epithelium, testicular weight↓ (Nagano et al., 1984)	No data
	Integrated conclusion (eg. read-across)	LOAEL: 0.93 mmol/kg/d (71 mg/kg/d)	NOAEL: 6.35 mmol/kg/d (572 mg/kg/d)	read across from Member 7 LOAEL: 0.31 mmol/kg/d (37 mg/kg/d)	read across from Member 2 NOAEL: 6.35 mmol/kg/d (840 mg/kg/d)	read across from Member 7 LOAEL: 0.155 mmol/kg/d (38 mg/kg/d)
	D value	D=0.0071 mg/kg/d	D=0.57 mg/kg/d	D=0.0037 mg/kg/d	D=0.84 mg/kg/d	D=0.0038 mg/kg/d
	CSCC Hazard Class	Class 3	Out of Class	Class 2	Out of Class	Class 2
Molecular profiling related to the category hypothesis						
Parent chemical	Repeated dose (HESS) ver. 2.8	Ethylene glycol alkyl ether (testicular toxicity) Ethylene glycol alkyl ether (hemolytic anemia)	Ethylene glycol alkyl ether (testicular toxicity) Ethylene glycol alkyl ether (hemolytic anemia)	no alert	no alert	no alert
	Derek (Testicular toxicity in rat)	PLAUSIBLE	PLAUSIBLE	PLAUSIBLE	PLAUSIBLE	PLAUSIBLE
Physical-chemical data						
Molecular weight		76.095	90.122	118.132	132.159	246.259
Melting point [°C] (experimental)		-85	-84.3	-66.3	-61.8	no data
Melting point [°C] (predicted)		-60.6	-45.1	-56.2	-50.4	-4.25
Boiling point [°C] (experimental)		124	135	144	156	no data
Boiling point [°C] (predicted)		122	135	138	154	284
Density		0.957	0.93	0.987	0.972	1.1
logPow (measured value)		-0.77	-0.32	no data	no data	no data
logPow (calculated value)		-0.91	-0.42	0.1	0.59	0.27

Kinetics					
Absorption					
Distribution					
Metabolism (*: measured, **: predicted by HESS rat cellular metabolism simulator)	oxidation/ methoxyacetic acid*	oxidation/ ethoxyacetic acid*	hydrolysis/ EGME*	hydrolysis/ EGEE*	hydrolysis/ EGME** oxidation/ methoxyacetic acid**
Excretion	methoxyacetic acid and the glycine conjugate in urine				

Data matrix, IATA for "case study on the use of integrated approaches for testing and assessment for testicular toxicity of ethylene glycol methyl ether (EGME)-related chemicals"

		Chemical ID				
		Member 6	Member 7	Member 8	Member 9	Member 10
CAS		106-00-3	3121-61-7	106-74-1	6976-93-8	2370-63-0
Name		Bis(2-methoxyethyl) adipate	2-Methoxyethyl acrylate	2-Ethoxyethyl acrylate	2-Methoxyethyl methacrylate	2-Ethoxyethyl methacrylate
Structure						
Summary of data gap filling						
		Member 6	Member 7	Member 8	Member 9	Member 10
Testicular toxicity	Experimental result	No data	NOAEL: <0.31 mmol/kg/d, LOAEL: 0.31 mmol/kg/d Rat, gavage dose study (OECD TG422), Histopathological alterations in testes, Testicular weight↓, (ECHA, 2012)	No data	No data	No data
	Integrated conclusion (eg. read-across)	read across from Member 7 LOAEL: 0.155 mmol/kg/d (41 mg/kg/d)	LOAEL: 0.31 mmol/kg/d (40 mg/kg/d)	read across from Member 2 NOAEL: 6.35 mmol/kg/d (920 mg/kg/d)	read across from Member 7 LOAEL: 0.31 mmol/kg/d (45 mg/kg/d)	read across from Member 2 NOAEL: 6.35 mmol/kg/d (1005 mg/kg/d)
	D value	D=0.0041 mg/kg/d	D=0.0040 mg/kg/d	D=0.92 mg/kg/d	D=0.0045 mg/kg/d	D=1.01 mg/kg/d
	CSCL Hazard Class	Class 2	Class 2	Out of Class	Class 2	Out of Class
Molecular profiling related to the category hypothesis						
Parent chemical	Repeated dose (HESS) ver. 2.8	no alert	no alert	no alert	no alert	no alert
	Derek (Testicular toxicity in rat)	PLAUSIBLE	PLAUSIBLE	PLAUSIBLE	PLAUSIBLE	PLAUSIBLE
Physical-chemical data						
Molecular weight		262.302	130.143	144.17	144.17	158.197
Melting point [°C] (experimental)		no data	-30	-47	no data	no data
Melting point [°C] (predicted)		-15.2	-54.3	-54.7	-38.3	-30.1
Boiling point [°C] (experimental)		no data	162	174	no data	no data
Boiling point [°C] (predicted)		301	155	171	167	182
Density		1.05	0.982	0.966	0.977	0.954
logPow (measured value)		no data	0.56	no data	no data	no data
logPow (calculated value)		0.84	0.45	0.94	1	1.49
Kinetics						
Absorption						
Distribution						
Metabolism (*: measured, **: predicted by HESS rat cellular metabolism simulator)		hydrolysis/ EGME** oxidation/ methoxyacetic acid**	hydrolysis/ EGME** oxidation/ methoxyacetic acid**	hydrolysis/ EGEE** oxidation/ ethoxyacetic acid**	hydrolysis/ EGME** oxidation/ methoxyacetic acid**	hydrolysis/ EGEE** oxidation/ ethoxyacetic acid**
Excretion						

Data matrix, IATA for "case study on the use of integrated approaches for testing and assessment for testicular toxicity of ethylene glycol methyl ether (EGME)-related chemicals"

		Chemical ID				
		Member 11	Member 12	Member 13	Member 14	Member 15
CAS		110-71-4	111-96-6	112-36-7	6290-49-9	17640-26-5
Name		Ethylene glycol dimethyl ether	Diethylene glycol dimethyl ether	Bis (2-ethoxy ethyl) ether	Methyl methoxyacetate	Methyl 2-ethoxyacetate
Structure						
Summary of data gap filling						
		Member 11	Member 12	Member 13	Member 14	Member 15
Testicular toxicity	Experimental result	NOAEL: not reported Mouse, 5-week gavage dose study, Atrophy of the seminiferous epithelium, testicular weight↓ (Nagano et al., 1984)	One dose study Rat, 20-day gavage dose study, Spermatoocyte degeneration, testicular weight↓ (Cheever et al., 1989)	No data	No data	No data
	Integrated conclusion (eg. read-across)	read across from Member 7 LOAEL: 0.31 mmol/kg/d (28 mg/kg/d)	read across from Member 7 LOAEL: 0.31 mmol/kg/d (42 mg/kg/d)	read across from Member 2 NOAEL: 6.35 mmol/kg/d (1030 mg/kg/d)	read across from Member 7 LOAEL: 0.31 mmol/kg/d (32 mg/kg/d)	read across from Member 2 NOAEL: 6.35 mmol/kg/d (750 mg/kg/d)
	D value	D=0.0028 mg/kg/d	D=0.0042 mg/kg/d	D=1.03 mg/kg/d	D=0.0032 mg/kg/d	D=0.75 mg/kg/d
	CSCL Hazard Class	Class 2	Class 2	Out of Class	Class 2	Out of Class
Molecular profiling related to the category hypothesis						
Parent chemical	Repeated dose (HESS) ver. 2.8	no alert	no alert	no alert	no alert	no alert
	Derek (Testicular toxicity in rat)	no alert	no alert	no alert	PLAUSIBLE	PLAUSIBLE
Physical-chemical data						
Molecular weight		90.122	134.175	162.229	104.105	118.132
Melting point [°C] (experimental)		-62.7	-65.6	-44.4	no data	no data
Melting point [°C] (predicted)		-76.4	-53.9	-37.4	-52.6	-55.3
Boiling point [°C] (experimental)		85	162	189	131	no data
Boiling point [°C] (predicted)		82.7	156	191	124	139
Density		0.875	0.924	0.922	1.02	0.99
logPow (measured value)		-0.21	-0.36	0.39	no data	no data
logPow (calculated value)		-0.21	-0.48	0.5	-0.39	0.1
Kinetics						
Absorption						
Distribution						
Metabolism (*: measured, **: predicted by HESS rat cellular metabolism simulator)		hydrolysis/ EGME** oxidation/ methoxyacetic acid**	oxidation/ EGME*	oxidation/ EGEE** oxidation/ ethoxyacetic acid**	hydrolysis/ methoxyacetic acid**	hydrolysis/ ethoxyacetic acid**
Excretion						