Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

12-Sep-2016

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR HEPATOTOXICITY OF ALLYL ESTERS

Series on Testing & Assessment
No. 253

JT03400419

ENV/JM/MONO(2016)51
Unclassified
English - Or. English

OECD Environment, Health and Safety Publications

Series on Testing and Assessment

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CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR HEPATOTOXICITY OF ALLYL ESTERS

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 2016

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FOREWORD

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 Japan for illustrating practical use of IATA in a regulatory context and submitted to the 2015 review cycle of the IATA Case Studies project. This case study was reviewed by the project team and revised to consider the comments from reviewers. The document was endorsed at the 9th Task Force on Hazard Assessment meeting in June 2016.

The following three case studies were also reviewed in the project in 2015 and are published with this case study:

1. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR IN VITRO MUTAGENICITY OF 3,3' DIMETHOXYBENZIDINE (DMOB) BASED DIRECT DYES, ENV/JM/MONO(2016)49, Series on Testing & Assessment No. 251.

2. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR REPEAT DOSE TOXICITY OF SUBSTITUTED DIPHENYLAMINES (SDPA), ENV/JM/MONO(2016)50, Series on Testing & Assessment No. 252.

3. CASE STUDY ON THE USE OF AN INTEGRATED APPROACH FOR TESTING AND ASSESSMENT OF THE BIOACCUMULATION POTENTIAL OF DEGRADATION PRODUCTS OF 4,4'-BIS (CHLOROMETHYL)-1,1'-BIPHENYL, ENV/JM/MONO(2016)52, Series on Testing & Assessment No. 254.

In addition, a considerations document summarizing 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) -First Review Cycle (2015): Case Studies on Grouping Methods as a Part of IATA- ENV/JM/MONO(2016)48, Series on Testing & Assessment No. 250.

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

TABLE OF CONTENTS

INTRODUCTION 8

1. PURPOSE 9

1.1. Purpose of use 9
1.2. Category definition 9
1.3. Endpoint 9

2. HYPOTHESIS FOR THE CATEGORY APPROACH 9

2.1. Read-across hypothesis 9
2.2. Elements for a read-across hypothesis 9

3. CATEGORY MEMBERS 12

3.1. Identification and selection of category members 12
3.2. List of category members 12

4. JUSTIFICATION OF DATA GAP-FILLING 13

4.1. Data collection 13
4.2. Data matrix 14
4.3. Justification 14

5. STRATEGY FOR AND INTEGRATED CONCLUSION OF DATA GAP-FILLING 15

5.1. Integrated conclusion 15
5.2. Uncertainty 19

REFERENCES 21

ANNEX 1 REPEATED-DOSE TOXICITY DATA ON ALLYL ESTERS AND THE METABOLITES USED IN THIS STUDY. 24

ANNEX 2 SUMMARY OF BIOCHEMICAL EVIDENCE FOR ALLYL ESTERS HYDROLYZED IN RATS 31

APPENDIX - DATA MATRIX 32

INTRODUCTION

Repeated-dose toxicity is one of the key regulatory endpoints in the hazard assessment of chemicals. The latest chemical management policies require the toxicological evaluation of marketed but untested chemicals. However, reduced animal testing is desired for economic and animal welfare reasons. In 2014, the Organisation for Economic Co-operation and Development (OECD) published guidance on grouping chemicals for category approach (OECD, 2014). Mechanistic interpretation is necessary for regulatory acceptable toxicological assessment of categorized chemicals as there are examples of structurally similar molecules with different toxicities.

For risk assessment under the Japanese Chemical Substances of Control Law (CSCL), a screening assessment is conducted to select Priority Assessment Chemical Substances. Human health endpoints for the assessment includes four repeated-dose toxicity, reproductive and developmental toxicity, genotoxicity, and carcinogenicity. A hazard class is assigned to at least two endpoints (genotoxicity and repeated-dose toxicity). The hazard class for genotoxicity is assigned based on the test results of Ames and in vitro chromosomal aberration, whereas that for repeated-dose toxicity is determined by the result of the toxicity studies. Category assessment is not currently utilized in the screening assessment but was recommended to be applied to chemical assessment under CSCL (METI et al., 2012).

Allyl esters are mainly used as flavoring agents for food and perfume. Previous toxicity studies revealed that repeated administration of allyl esters caused hepatotoxicity as a critical toxic effect. Allyl esters are hydrolyzed to allyl alcohol, followed by oxidation to a reactive metabolite, acrolein, in the liver. Acrolein appears to be responsible for the hepatotoxicity (Atzori et al., 1989; Ghilarducci and Tjeerdema, 1995). These results suggest that the toxicity of various allyl esters can be evaluated together. Because exposure to various allyl esters is possible, missing toxicity data on particular allyl esters should be filled with available data.

In this case study, an integrated approach to testing and assessment (IATA) was applied to predict the repeated-dose hepatotoxicity of allyl esters. Based on information on the hepatotoxic mechanism of allyl acetate accompanied by toxicity and metabolism information on its structural analogs, the category was defined as esters of single allyl alcohol and saturated aliphatic carboxylic acids. Allyl esters that can be metabolized to allyl alcohol were predicted to cause hepatotoxicity. Based on the results, a hazard class was assigned for each member. This specific case study was developed based on the work published in a recent paper (Yamada et al., 2013) to address how category assessment can be applied to hazard characterization for screening assessment under CSCL.

1. PURPOSE

1.1. Purpose of use

This case study aimed to characterize the hazards of allyl esters by applying an IATA for category evaluation of chemical risk assessment under CSCL. Category assessment is not currently utilized for human health endpoints included in the risk assessment. It was, however, recommended to be incorporated in the chemical assessments (METI *et al*., 2012). This specific case study was developed to address how category assessment can be applied to the screening assessment for the selection of Priority Assessment Chemical Substances in the near future.

1.2. Category definition

The category is defined as esters of single allyl alcohol and saturated aliphatic carboxylic acid.

1.3. Endpoint

Repeated-dose hepatotoxicity characterized as hepatocyte degeneration and necrosis and bile duct hyperplasia

2. HYPOTHESIS FOR THE CATEGORY APPROACH

2.1. Read-across hypothesis

The read-across hypothesis that supports the category approach for allyl esters is based on the following considerations:

Allyl esters that can be predictably metabolized to allyl alcohol are likely to be hepatotoxic.

It is unlikely that saturated aliphatic carboxylic acid metabolites are of toxicological concern.

2.2. Elements for a read-across hypothesis

Absorption, distribution, metabolism, and excretion (ADME) of allyl esters

In rats, allyl esters are hydrolyzed by hydrolytic enzymes to allyl alcohol and carboxylic acids in the intestine, liver, and/or other tissues (Silver and Murphy, 1978). Following hydrolysis, the liberated allyl alcohol is distributed in the liver, and then predominantly oxidized by hepatic alcohol dehydrogenase (ADH) to acrolein, which is further oxidized by aldehyde dehydrogenase (ALDH) to acrylic acid (Patel *et al*., 1980). Acrolein forms a conjugate with glutathione (GSH) in the liver. The conjugate is finally excreted in urine as 3-hydroxypropylmercapturic acid (3-HPM) through metabolic processing (Kaye *et al.*, 1973) (Figure 1).

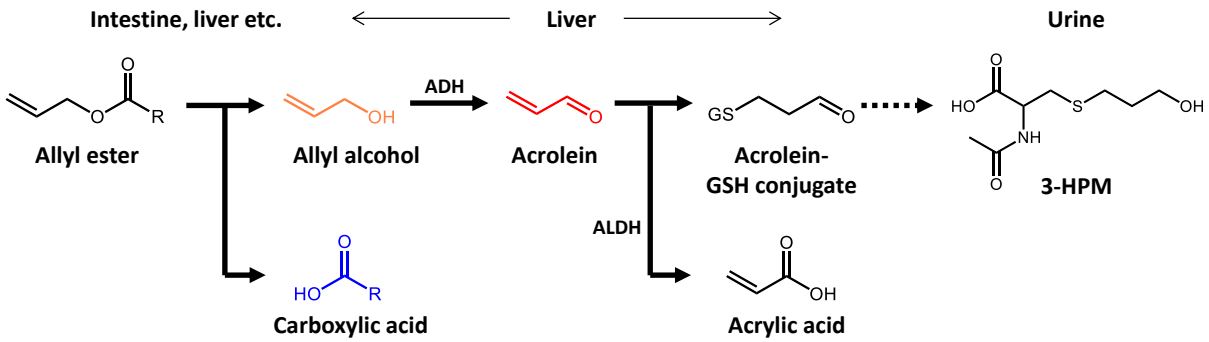


Figure 1 Major metabolic pathway of allyl ester in rats

Mode of action or adverse outcome pathways (MOA/AOP) of allyl ester hepatotoxicity

As more studies on allyl ester-induced hepatotoxicity have been performed with allyl acetate, the MOA/AOP for allyl acetate-induced hepatotoxicity was developed by taking into account the strength, consistency, and specificity of the association with series of sets of experimental evidence, as shown in Figure 2.

Following the hydrolysis of allyl esters, allyl alcohol is readily oxidized to acrolein by ADH in the liver (Patel *et al*., 1980). Acrolein is a highly reactive substance that appears to cause hepatotoxicity (Ghilarducci and Tjeerdema, 1995). It readily forms an acrolein-GSH adduct, leading to GSH depletion, oxygen radical formation, and lipid peroxidation (Ohno *et al*., 1985; Atzori *et al*., 1989; Silva and O'Brien, 1989; Cooper *et al*., 1992; Adams and Klaidman, 1993). Acrolein is also capable of reacting with cellular macromolecules nonenzymatically via Michael additions. Reactions with critical intracellular proteins and subsequent adduct formation are proposed as one component of the cytotoxicity of acrolein (Kaminskas *et al*., 2004). Additionally, it has been proposed that oxidative stress subsequent to the loss of GSH may be related to mitochondrial dysfunction (Arumugam *et al*., 1999; Sun *et al*., 2006). These biochemical events caused by acrolein are believed to be associated with hepatocellular damage and death (Figure 2).

Enzymatic hydrolysis of allyl acetate to allyl alcohol is required for allyl acetate-induced hepatotoxicity, which is supported by the evidence that pre-treatment with carboxylesterase inhibitors significantly reduced allyl acetate-induced hepatotoxicity in rats (Silver and Murphy, 1978). The importance of liver ADH in the toxicity of allyl alcohol has been demonstrated in several studies. Pre-treatment of rats with ADH inhibitors almost completely inhibited allyl acetate-induced hepatotoxicity (Silver and Murphy, 1978). In *in vitro* studies with rat hepatocytes, allyl alcohol-induced cytotoxicity was protected by ADH inhibitors and augmented by ALDH inhibitors (Ohno *et al*., 1985; Rikans *et al*., 1987; Silva and O'Brien, 1989). An ADH-negative deer-mice strain was resistant to allyl alcohol toxicity (Belinski *et al*., 1985).

It is generally considered that saturated aliphatic carboxylic acid metabolites do not cause severe hepatotoxic effects, as they are less reactive and chiefly undergo metabolic breakdown via mitochondrial beta-oxidation processes. However, note that certain carboxylic acid metabolites may produce a toxic response in the liver.

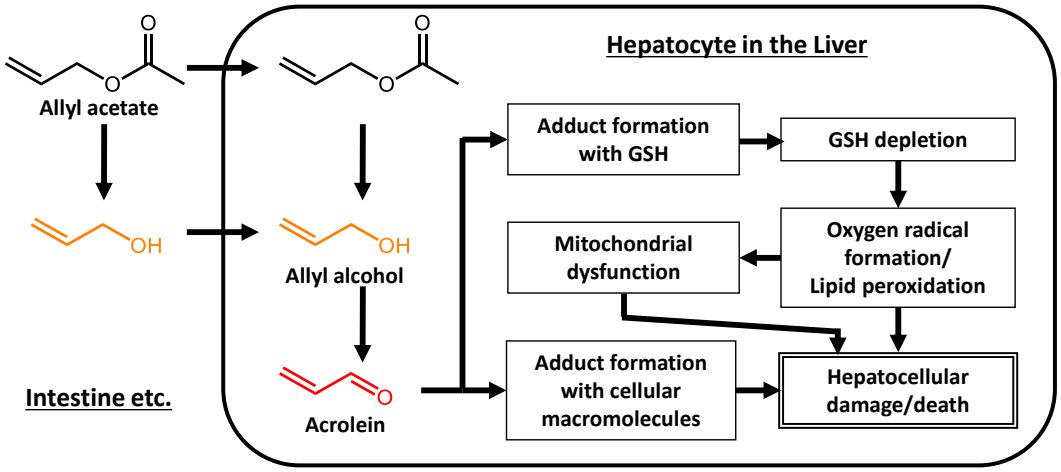


Figure 2 Hepatotoxic pathway induced by allyl acetate

Chemical identity/Physicochemical properties

The category members have a common structure that comprises an allyl ester containing saturated aliphatic chain. Their carboxylic acid moiety permits both linear and branched structures. The category chemicals are liquid at room temperature. Allyl acetate has the lowest logKow of the group; increasing carbon numbers of the alkyl chain contribute to increased hydrophobicity of the entire molecule.

Other toxic endpoints

Repeated-dose toxicity has multiple toxic endpoints. The major toxic response for allyl acetate occurred in the liver and forestomach. Administration of allyl acetate by gavage resulted in forestomach hyperplasia at lower doses at which hepatotoxicity appeared (NTP, 2006). This was, however, beyond the scope of this study, as forestomach irritation is a local point of contact effect only anticipated to be found in gavage studies. Hepatotoxic effects are more important as systemic toxicity and thus more relevant to risk assessment. Oral administration of acrolein resulted in a toxic response in the forestomach but not in the liver. Reactions of acrolein with contents of the gastrointestinal tract most likely reduced the systemic bioavailability to levels low enough to permit effective detoxification in the liver without causing a hepatotoxic response. Because allyl acetate is not as reactive as acrolein, the bioavailability would not have been reduced in the same manner (NTP, 2006).

Route of expected exposure

Allyl esters are approved as food flavoring agents and are used to simulate various fruit flavors in baked goods, candy, ice cream, gelatin, and condiments (Fenaroli, 1971). Many allyl esters are artificial flavors, while some are reported to occur naturally (JECFA, 1991). Furthermore, certain allyl esters are used as raw materials for perfume (Poucher, 1991). As such, oral exposure may be of major concern for this category of chemicals.

Human relevance

There is currently a lack of toxicological information on allyl esters in humans. However, a mechanistic interpretation is valuable for extrapolating hazard evaluation in experimental animals to humans. It is plausible to assume that hydrolytic enzymes, such as lipases and carboxylesterases, in the intestine and other tissues, as well as ADHs in the liver, participate in metabolic activation of allyl esters in humans. It was previously reported that acrolein was detected in the blood, bile, and urine of a man who had accidentally orally ingested allyl alcohol (Toennes *et al*., 2002). Moreover, allyl alcohol and acrolein

showed cytotoxic effects in human hepatocytes (Dvorák *et al*., 2003; Mohammad *et al*., 2012). The results apparently support that metabolic formation and hepatotoxicity of acrolein is relevant to humans.

3. CATEGORY MEMBERS

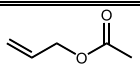
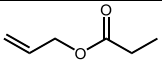
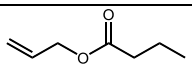
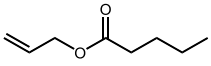
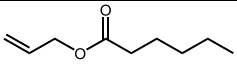
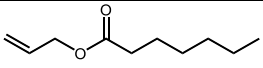
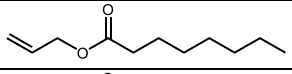
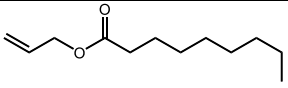
3.1. Identification and selection of category members

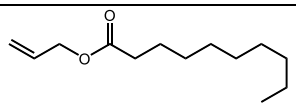
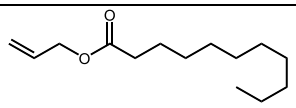
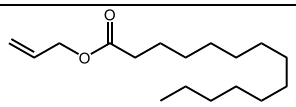
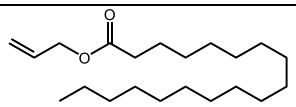
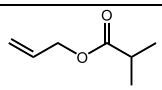
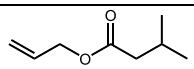
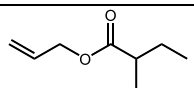
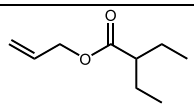
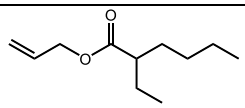
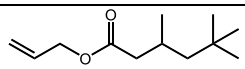
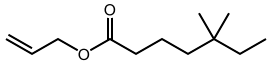
The selection criterion for category members is esters of single allyl alcohol and saturated aliphatic carboxylic acid. The Chemical Risk Information Platform (CHRIP) was utilized to identify category chemicals. CHRIP is a web-based database providing comprehensive information on risk assessments in addition to laws and regulations of chemicals (http://www.safe.nite.go.jp/english/db.html). Chemicals can be searched by their number, name, or structure. The database was searched to obtain chemical structures with an allyl acetate moiety. As a result, 77 substances were found. Of these, the structures of allyl esters consisting of one allyl alcohol and one saturated aliphatic carboxylic acid were then selected by visual judgement, as they met the selection criteria of the category members. However, two allyl esters derived from carboxylic acids having a quaternary carbon atom in the alpha position were excluded because their steric effects may provide inhibitory influence on enzymatic hydrolysis of allyl esters, which is a key factor of category formation. Finally, chemical structures of 19 allyl esters were obtained. They are listed in Table 1.

3.2. List of category members

List of the selected category members are shown in Table 1.

Table 1 List of Allyl Esters for Forming a Category and Read-across

No.	CAS No.	IUPAC name	Structural formula	Molecular formula	Molecular weight
1	591-87-7	Allyl acetate		$C_5H_8O_2$	100.12
2	2408-20-0	Allyl propionate		$C_6H_{10}O_2$	114.14
3	2051-78-7	Allyl butyrate		$C_7H_{12}O_2$	128.17
4	6321-45-5	Allyl pentanoate		$C_8H_{14}O_2$	140.20
5	123-68-2	Allyl hexanoate		$C_9H_{16}O_2$	156.23
6	142-19-8	Allyl heptanoate		$C_{10}H_{18}O_2$	170.25
7	4230-97-1	Allyl octanoate		$C_{11}H_{20}O_2$	184.28
8	7493-72-3	Allyl nonan-1-oate		$C_{12}H_{22}O_2$	198.31

9	57856-81-2	Allyl decanoate		$C_{13}H_{24}O_2$	212.33
10	17308-90-6	Allyl undecanoate		$C_{14}H_{26}O_2$	226.36
11	45236-96-2	Allyl myristate		$C_{17}H_{32}O_2$	268.44
12	6289-31-2	Allyl stearate		$C_{21}H_{40}O_2$	324.55
13	15727-77-2	Allyl isobutyrate		$C_7H_{12}O_2$	128.17
14	2835-39-4	Allyl isovalerate		$C_8H_{14}O_2$	142.20
15	93963-13-4	Allyl 2-methylbutyrate		$C_8H_{14}O_2$	142.20
16	7493-69-8	Allyl 2-ethylbutyrate		$C_9H_{16}O_2$	156.23
17	58105-49-0	Allyl 2-ethylhexanoate		$C_{11}H_{20}O_2$	184.28
18	71500-37-3	Allyl 3,5,5-trimethylhexanoate		$C_{12}H_{22}O_2$	198.31
19	68132-80-9	Allyl trimethylhexanoate		$C_{12}H_{22}O_2$	198.31

4. JUSTIFICATION OF DATA GAP-FILLING

4.1. Data collection

Publicly available repeated-dose toxicity data were collected by toxicity database and literature searches for category members and their possible metabolites. The OECD QSAR Toolbox (http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm) and Hazard Evaluation Support System Integrated Platform (HESS) (http://www.nite.go.jp/en/chem/qsar/hess-e.html) databases were searched with focus on chemical structures and repeated-dose toxicity data. Toolbox version 3.3 and HESS version 3.0 were utilized in this study. Then, PubMed (http://www.ncbi.nlm.nih.gov/pubmed), TOXNET (http://toxnet.nlm.nih.gov/), and Google (https://www.google.co.jp/) were searched with focus on chemical names and repeated-dose toxicity data. As a result, toxicity data of category members (allyl acetate, allyl hexanoate, allyl heptanoate, and allyl isovalerate) were obtained. These four chemicals were

used as source chemicals. Additionally, the metabolite toxicity data of category members (allyl alcohol, acrolein, and two carboxylic acids (2-ethylhexanoic acid and 3,5,5-trimethylhexanoic acid)) were collected by the same method described above for category justification and uncertainty analysis. All of the toxicity profiles and data sources of the studies are summarized in Annex 1.

As measuring or predicting the metabolic formation of allyl alcohol is necessary for categorization and subsequent data gap-filling, empirical metabolism information was gathered for allyl esters. Then, the five references (Kaye, 1973; Butterworth *et al*., 1975; Longland *et al*., 1977; Silver and Murphy, 1978; Auerbach *et al*., 2008) that include *in vitro* and *in vivo* metabolism data on seven allyl esters in rats were obtained. Biochemical evidence for hydrolysis of allyl esters in rats is summarized in Annex 2.

4.2. Data matrix

Chemical structures, data on hepatotoxicity (pathological changes and no-observed-adverse-effect-level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) for the toxicity endpoint), and *in vitro* and *in vivo* metabolism data for the allyl ester category were compiled in a data matrix (Appendix).

4.3. Justification

Allyl acetate (member 1) has been shown to be metabolized to allyl alcohol in rats (Kaye, 1973; Silver and Murphy, 1978). Oral administration of allyl acetate to rats for 14 weeks significantly induced hepatocyte necrosis and bile duct hyperplasia at 50 mg/kg/d (0.50 mmol/kg/d). NOAEL for the hepatotoxicity was 25 mg/kg/d (0.25 mmol/kg/d) (NTP, 2006; see Annex 1). Hydrolysis of allyl isovalerate (member 14) proceeded with rat alimentary tract preparation (Butterworth *et al*., 1975). When allyl isovalerate was given p.o. to rats for 13 weeks, bile duct hyperplasia was found at 125 mg/kg/d (0.88 mmol/kg/d) and focal necrosis and nodular hyperplasia developed in the liver at 250 mg/kg/d (1.76 mmol/kg/d) (NTP, 1983; see Annex 1).

Allyl hexanoate (member 5) has been shown to be readily hydrolyzed to generate allyl alcohol in rat intestinal mucosal preparations and in liver homogenate (Longland *et al*., 1977). When allyl hexanoate was given p.o. to rats for 18 weeks, focal necrosis in the liver and bile duct hyperplasia were observed at 65 mg/kg/d (0.42 mmol/kg/d) (Hagan *et al*., 1967; JECFA, 1991; see Annex 1). NOAEL was 15 mg/kg/d (0.10 mmol/kg/d). Allyl heptanoate (member 6) is quite similar to allyl hexanoate in terms of structure, physicochemical properties, and chemical reactivity. No available metabolism data were found for this chemical. However, it is unlikely that only one methylene group extension of the fatty acid moiety results in a marked decrease in the hydrolytic rate. When allyl heptanoate was administered to rats for 18 weeks in their food at dietary levels of 0, 1000, 2500, and 10,000 ppm (0.40, 1.0, 4.0 mmol/kg/d), perilobular degeneration of liver and bile duct hyperplasia were observed at doses of ≥0.40 mmol/kg/d (Hagan *et al*., 1965; JECFA, 1991; see Annex 1).

It appears that, based on the experimental evidence, hepatotoxicity of these allyl esters is similar to that of allyl alcohol (NTP, 2006; see Annex 1) and carboxylic acid metabolites do not cause severe hepatotoxic effects. Hence, metabolic formation of allyl alcohol is considered relevant. Taken together, it is apparent that allyl esters that are predominantly hydrolyzed to allyl alcohol produce similar hepatotoxic effects.

Measurement or reliable estimation of allyl alcohol formation is critical for categorization. Allyl esters that have experimental data on the metabolic hydrolysis in alimentary tract and/or liver preparations are included in this category. Allyl alcohol is then oxidized to acrolein in the liver, which can be conjugated to GSH. The conjugate is considered a major route of acrolein detoxicfication, as evidenced by the presence of 3-HPM in the urine of rats administered acrolein and allyl alcohol (Auerbach *et al*., 2008).

Hence, 3-HPM is regarded as a metabolic marker for acrolein formation in the liver (Figure 1). Allyl esters that are metabolized to 3-HPM followed by excretion in urine are thus included in this category. Allyl esters that are predicted to be readily hydrolyzed based on the experimental metabolism data on structural analogs also belong here. Structural analogs include esters of allyl alcohol and saturated aliphatic carboxylic acids with different chain lengths and esters of ethanol/propanol and their carboxylic acids.

For allyl esters with linear alkyl chains, the applicability domain is described by alkyl chain carbon numbers ranging from C2 (allyl acetate, member 1) to C18 (allyl stearate, member 12), based on the experimental evidence of metabolic hydrolysis of both compounds (Table 2). For allyl esters with branched alkyl chains, it is difficult to precisely define the applicability domain. The limited number of dataset chemicals appears to be insufficient to define the chemical space of the category. Various branched types of allyl esters may yield allyl alcohol by the action of endogenous hydrolytic enzymes, including lipases and carboxylesterases with broad substrate specificity.

5. STRATEGY FOR AND INTEGRATED CONCLUSION OF DATA GAP-FILLING

5.1. Integrated conclusion

Missing data for the metabolic hydrolysis were predicted by read-across with the documented metabolism data shown in Annex 2. As allyl stearate (member 12) was shown to be susceptible to metabolic hydrolysis, it is logical to predict that allyl esters with straight chains (member 3, 4, and 6–11) are hydrolyzed. In addition, three allyl esters with branched chains (allyl isobutyrate (member 13), allyl isovalerate (member 14), and allyl 2-ethylhexanoate (member 17)) have been reported to be hydrolyzed (Butterworth *et al*., 1975). These results show that the hydrolysis of other allyl esters (members 15 and 16) is likely. Members 18 and 19 have a quaternary carbon atom on the alkyl chain. This bulky structure was not found in the metabolism references. The quaternary carbon atom is located relatively far from the carbonyl carbon that is attacked by a nucleophilic amino acid residue at the active site of the hydrolytic enzymes. Hence, there appears to be little steric influence on enzymatic hydrolysis. Given the notion of the broad substrate specificity of lipases and carboxylesterases, it was assumed that these two allyl esters are within the category. Taken together, it was concluded that all of the untested allyl esters in Table 2 fall into this category. Following hydrolysis of allyl esters, liberated allyl alcohol is predominantly oxidized by ADH in the liver to form acrolein. The reactive substance causes a variety of biochemical events, leading to hepatotoxicity.

For a larger category, several different relationships can be established for a single endpoint. If this is the case, a subcategory should be defined to achieve a more accurate estimation of toxicity levels (OECD, 2014). Both allyl acetate (member 1) and allyl hexanoate (member 5) appear to be highly susceptible to hydrolysis and have similarly severe hepatotoxic effects (Auerbach *et al*., 2008; Longland *et al*., 1977; NTP, 2006; Hegan *et al*., 1967). Thus, members 2–4 can be expected to have the toxicokinetic features and hepatotoxic levels that are similar to those of members 1 and 5. No quantitative metabolism data are available for members 6–12. Similar hydrolytic rates were observed among propyl heptanoate, octanoate, nonanoate, decanoate, and oleate, the structural analogs of members 6–12, respectively, in rat pancreatic preparations (Mattson and Volpenhein, 1969). Hence, it is unlikely that increasing the straight-chain length, ranging from one (member 6) to twelve carbons (member 12), would markedly decrease the rate of enzymatic hydrolysis compared to member 5 (allyl hexanoate). Therefore, members 1–12 (allyl esters with saturated linear alkyl carboxylic acids) can be further grouped into a subcategory (subcategory 1). NOAEL

was estimated to be 0.10 mmol/kg/d for members 2–12 based on the experimental data on member 5, allyl hexanoate (Hagan *et al*., 1967).

Hepatotoxic levels of allyl acetate (member 1) and allyl hexanoate (member 5) were similar despite their different hydrophobicity. Given that allyl hexanoate is likely susceptible to enzymatic hydrolysis in the alimentary tract (Longland *et al*., 1977) and that the hepatotoxicity level of allyl alcohol was also similar to those of the two allyl esters (Annex 1), it is likely that allyl esters with medium or long linear aliphatic chains are largely hydrolyzed in the alimentary tract and that the liberated allyl alcohol is absorbed from the gastrointestinal tract at similar levels.

Members 13–19 have a saturated branched alkyl chain in their structures. The influence of the degree of branching and structural variation on enzymatic hydrolysis is not clear. Hence, these allyl esters are grouped into a different subcategory (subcategory 2). The hepatotoxicity levels are not predictable without additional toxicological and biochemical evidence. Hence, allyl hexanoate is assumed to be the worst case. NOAEL was estimated to be 0.10 mmol/kg/d.

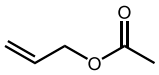
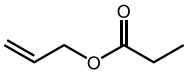
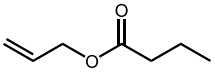
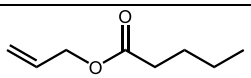
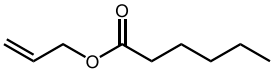
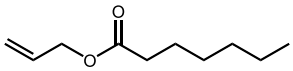
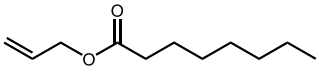
Screening assessment under CSCL is conducted for human health and/or ecological concerns to select priority assessment chemical substances. The human health endpoints include repeated-dose toxicity, reproductive and developmental toxicity, genotoxicity, and carcinogenicity. Hazard classes are assigned based on the hazard assessment value (D value). Then, the most severe class among the endpoints is applied to the target chemical.

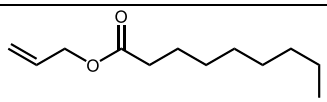
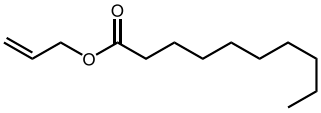
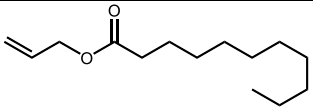
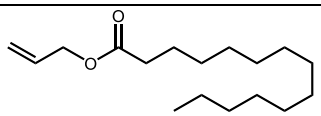
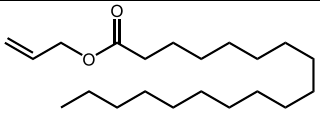
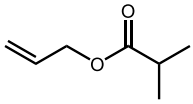
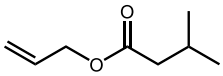
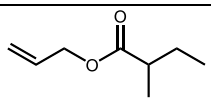
The D value of repeated-dose toxicity is described by the following equation: D = NO(A)EL/uncertainty factor. In this case, the uncertainty factor is 200, which is calculated by multiplying the species difference (10), the individual difference (10), the study duration factor (2: if the NO(A)EL is derived from a 13-week to 1-year study), and severity of toxicity (1: no severe toxicity was observed). Consequently, D values for all of the category members for repeated-dose toxicity were estimated to be 0.06 to 0.31 mg/kg/d (Table 2). 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, $0.05 < D \leq 0.5$; and Out of Class, $D > 0.5$. All of the category member substances were thus assigned to Class 4 based on their allyl alcohol-dependent hepatotoxicity.

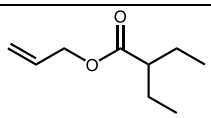
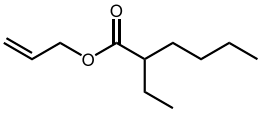
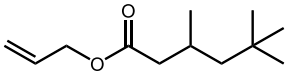
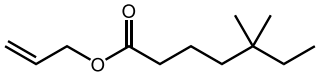
One cannot exclude the possibility that particular carboxylic acid metabolites produce a toxic response in the liver. Toxicity studies revealed that 2-ethylhexanoic acid did not have hepatotoxic effects at 6.3 mmol/kg/d (approximately 900 mg/kg/d) in a 13-week study, but 3,5,5-trimethylhexanoic acid caused fatty infiltration of hepatocyte in the periportal area at 0.32 mmol/kg/d (50 mg/kg/d) in a 4-week study (Annex 1). The hepatic toxicities of the branched carboxylic acids were not clearly evaluated in this case study, although the toxicity does not seem to be serious. Additionally, toxicological profiles other than liver toxicity should be analyzed for the hazard classification of repeated-dose toxicity in the CSCL screening assessment.

This case study indicated that a metabolite-based category approach is useful for endpoint specific IATA assessment. However, to complete IATA assessment for the overall repeated-dose toxicity, more systematic evaluation approaches for every endpoint should be developed in future.

Table 2 Summary of integrated conclusion

No.	Chemical structure Chemical name CAS No.	Metabolic hydrolysis	Repeated-dose toxicity		
			Experimental result (GLP/non-GLP)	Integrated conclusion (read-across)	D value (hazard assessment value)*
1	 Allyl acetate 591-87-7	+ *in vitro* *in vivo*	NOAEL=0.25 mmol/kg/d Hepatocyte necrosis, bile duct hyperplasia F344 rats 5 doses/w for 14 wks, Gavage (NTP, 2006) (GLP)		0.13 mg/kg/d
2	 Allyl propionate 2408-20-0	+ *in vitro* *in vivo*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.06 mg/kg/d
3	 Allyl butyrate 2051-78-7	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.06 mg/kg/d
4	 Allyl pentanoate 6321-45-5	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.07 mg/kg/d
5	 Allyl hexanoate 123-68-2	+ *in vitro*	NOAEL=0.10 mmol/kg/d Hepatic fibrosis, focal necrosis, bile duct proliferation Osborne-Mendel rats 18 wks, Gavage (Hagan *et al*., 1967) (non-GLP)		0.08 mg/kg/d
6	 Allyl heptanoate 142-19-8	+ *in silico*	NOAEL: Not determined LOAEL=0.40 mmol/kg/d Hydropic degeneration of the liver cells in the periportal area, new bile duct formation Osborne-Mendel rats 18 wks, Feeding (Hagan *et al*., 1965) (non-GLP)	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.09 mg/kg/d
7	 Allyl octanoate 4230-97-1	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.09 mg/kg/d

8	 Allyl nonanoate 7493-72-3	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.10 mg/kg/d
9	 Allyl decanoate 57856-81-2	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.11 mg/kg/d
10	 Allyl undecanoate 17308-90-6	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.11 mg/kg/d
11	 Allyl myristate 45236-96-2	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.13 mg/kg/d
12	 Allyl stearate 6289-31-2	+ *in vivo*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.26 mg/kg/d
13	 Allyl isobutyrate 15727-77-2	+ *in vitro*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.06 mg/kg/d
14	 Allyl isovalerate 2835-39-4	+ *in vitro*	NOAEL=0.44 mmol/kg/d Bile duct hyperplasia, hepatic multifocal coagulative necrosis & nodular hyperplasia, cholangiofibrosis F344 rats 5 doses/w for 13 wks, Gavage (NTP, 1983) (GLP)		0.31 mg/kg/d
15	 Allyl 2-methylbutyrate 93963-13-4	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.07 mg/kg/d

16	 Allyl 2-ethylbutyrate 7493-69-8	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.08 mg/kg/d
17	 Allyl 2-ethylhexanoate 58105-49-0	+ *in vitro*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.09 mg/kg/d
18	 Allyl 3,5,5-trimethylhexanoate 71500-37-3	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.10 mg/kg/d
19	 Allyl trimethylhexanoate 68132-80-9	+ *in silico*	No data available	NOAEL=0.10 mmol/kg/d Hepatotoxicity	0.10 mg/kg/d

*D (mg/kg/d) = NO(A)EL (mmol/kg/d) X MW/200 (uncertainty factor)

5.2. Uncertainty

Table 3 Uncertainty of category justification and prediction by read-across

Factor	Uncertainty (low, medium, high)	Comment
MOA/AOP	Medium	It is apparent that allyl esters produce hepatocyte degeneration and necrosis and bile duct hyperplasia through metabolic activation. There are several mechanistic studies on such hepatocyte injury. However, the mechanism of bile duct hyperplasia is not understood, although allyl alcohol formation is apparently linked to the toxic response.
Structural boundary (subcategory 1)	Low	Structural boundary of the linear allyl esters is judged to be clear because it was defined based on the structure and metabolic hydrolysis of allyl acetate (C2) and allyl stearate (C18).
Structural boundary (subcategory 2)	High	No range of ester hydrolysis rate was defined sufficiently to form a category, as there is little data that can be compared directly between chemicals. *In vitro* testing of the hydrolytic rates of these allyl esters may assist in clarifying the structural boundary of subcategory 2.
Prediction by read-across (subcategory 1)	Low	The available data of similar toxicokinetic features and hepatotoxic responses of linear allyl esters suggest that the applicable domain is clear and the toxicity prediction by read-across is reliable.

Prediction by read-across (subcategory 2)	High	It is difficult to define precisely the applicable domain of subcategory 2 owing to the limited number of data set chemicals and the broad substrate specificity of the hydrolytic enzymes that participate in ester hydrolysis. To apply read-across for subcategory 2, allyl hexanoate had to be used as the worst-case analog, as there is an unclear match between their degree of structural complexity and rate of hydrolysis.
Toxicological Similarity (subcategory 1)	Low	It is unlikely that liberated saturated carboxylic acid metabolites cause hepatotoxic effects, which is clearly supported by the similar results of toxicity studies of three allyl esters with linear chains (allyl acetate, allyl hexanoate, and allyl heptanoate). It is logical to presume that hepatotoxicity of the substances is caused by the reactive metabolite acrolein.
Toxicological Similarity (subcategory 2)	High	Uncertainty exists in the hepatotoxicity of branched chain carboxylic acids. 3,5,5-Trimethylhexanoic acid caused vacuolation of hepatocyte in the periportal area at 0.32 mmol/kg/d (50 mg/kg/d). On the contrary, 2-ethylhexanoic acid did not have hepatotoxic effects at 6.3 mmol/kg/d (approximately 900 mg/kg/d). The hepatic toxicities of the other branched carboxylic acids were not clearly evaluated in this case study, although their toxicity does not seem to be serious.
Expansion of the category	Medium	The category could be expanded to other allyl esters that yield allyl alcohol through metabolic hydrolysis. It may include allyl esters of unsaturated carboxylic acid, benzoic acid, and phthalic acid (Yamada *et al*., 2013). On the contrary, allyl esters derived from carboxylic acids having a quaternary carbon atom in the alpha position may not belong to the category since their steric effects may provide an inhibitory influence on the enzymatic hydrolysis of allyl esters.

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ANNEX 1 REPEATED-DOSE TOXICITY DATA ON ALLYL ESTERS AND THE METABOLITES USED IN THIS STUDY.

CAS No. 591-87-7: Allyl acetate

Study type	Repeated dose oral toxicity study (14 weeks)
Species	Rat (F344/N)
Route	Gavage
Solvent	0.5 % Methylcellulose
Dose level	6, 12, 25, 50, 100 mg/kg/d, 10 animals/group/sex
Purity	93.3%
Publication	NTP (2006)
Data source	HESS Repeated Dose Toxicity database
Death	100 (♂: 10/10, ♀: 10/10)
NOEL	<6 mg/kg/d
Clinical observation	-
FOB	-
Body weight	↓: ≧50♂
Food consumption	-
Water consumption	-
Urinalysis	-
Hematology	Hgb↓: 50♀ Plt↑: ≧25♂, 50♀ WBC↑: ≧25♂
Blood Chemistry	TP↓: ≧12♀ Alb↓: ≧12♀ ALT↑: ≧50♂♀ SDH↑: ≧50♂♀ Bile acid↑: ≧25♂, ≧50♀
Absolute organ weight	Liver↑: 50♀
Relative organ weight	Liver↑: 50♀
Necropsy	-
Histopathology	Stomach, forestomach-epithelium, hyperplasia, squamous: ≧6♂♀ Liver-Hemorrhage: ≧50♂ Liver-Pigmentation, hemosiderin: ≧25♂♀ Liver-Bile duct, hyperplasia: ≧50♂♀ Liver-Hepatocyte, periportal, hypertrophy: ≧25♂♀ Liver-Hepatocyte, periportal, mitotic alteration: ≧25♂♀ Liver-Portal, Inflammation, granulomatous: ≧25♂♀ Liver-Hepatocyte, necrosis: ≧50♂♀ Liver-Hepatocyte, periportal, degeneration, hydropic: ≧50♂♀ Liver-Portal, fibrosis: ≧50♂♀ Prostate gland-Inflammation, chronic active: 50♂

CAS No. 123-68-2: Allyl hexanoate

Study type	Repeated Dose Toxicity (18 weeks)
Species	Rat (Osborne-Mendel)
Route	Oral (Gavage)
Solvent	Corn oil
Dose level	15, 65, 100 mg/kg/d
Purity	N/A
Publication	Hagan *et al*. (1967)
Data source	JECFA (1991)
Death	-
NOEL	15 mg/kg/d
Clinical observation	N/A
FOB	-
Body weight	N/A
Food consumption	N/A
Water consumption	-
Urinalysis	-
Hematology	-
Blood Chemistry	-
Absolute organ weight	-
Relative organ weight	-
Necropsy	Liver-Nodular and Wrinkled with granular or rough surface: 100♂♀
Histopathology	Liver-Bile duct proliferation: ≧65♂♀ Liver-Lobular architectural disarrangement: 100♂♀ Liver-Fibrosis: 100♂♀ Liver-Pigment in macrophage: 100♂♀ Liver-Necrotic foci: 100♂♀

CAS No. 142-19-8: Allyl heptanoate

Study type	Repeated Dose Toxicity (18 weeks)
Species	Rat (Osborn-Mandel)
Route	Oral (feeding)
Solvent	-
Dose level	1000, 2500, 10000 ppm
Purity	N/A
Publication	Hagan *et al*. (1965)
Data source	JECFA (1991)
Death	N/A
NOEL	-
Clinical observation	N/A
FOB	-
Body weight	↓: ≧1000♂, 10000♀
Food consumption	↓: 10000♂♀
Water consumption	-
Urinalysis	-
Hematology	-
Blood Chemistry	-
Absolute organ weight	-
Relative organ weight	-
Necropsy	Liver-Enlargement: ≧1000♂♀ Kidney-Enlargement: 10000♂♀ Heart-Enlargement: 10000♂ Testis-Enlargement: ≧2500♂
Histopathology	Liver-Hydropic degeneration, hepatocyte, periportal: ≧1000♂♀ Liver-New bile duct formation: ≧1000♂♀ Liver-Hepatic cell enlargement: ≧1000♂♀

CAS No. 2835-39-4: Allyl isovalerate

Study type	Dose selection study for carcinogenicity (13 weeks)
Species	Rat (F344/N)
Route	Gavage
Solvent	Corn oil
Dose level	15, 31, 62, 125, 250 mg/kg/d, 10 animals/group/sex
Purity	95.6%
Publication	NTP (1983)
Data source	HESS Repeated Dose Toxicity database
Death	250 (♂: 10/10, ♀: 4/10)
NOEL	31 mg/kg/d
Clinical observation	-
FOB	-
Body weight	↓: ≧62♂, 250♀
Food consumption	-

Water consumption	-
Urinalysis	-
Hematology	-
Blood Chemistry	-
Absolute organ weight	-
Relative organ weight	-
Necropsy	-
Histopathology	Liver-Basophilic cytoplasmic change: ≧62♂, ≧125♀ Liver-Multifocal coagulative necrosis: 250♂♀ Liver-Cholangiofibrosis: 250♂♀ Liver-Nodular hyperplasia: 250♂♀ Liver/bile duct- Hyperplasia: ≧125♂♀

CAS No. 107-18-6: Allyl alcohol

Study type	Repeated dose oral toxicity study (14 weeks)
Species	Rat (F344/N)
Route	Gavage
Solvent	0.5% Methylcellulose
Dose level	1.5, 3, 6, 12, 25 mg/kg/d, 10 animals/group/sex
Purity	98.8%
Publication	NTP (2006)
Data source	HESS Repeated Dose Toxicity database
Death	-
NOEL	♂: 3 mg/kg/d, ♀: 1.5 mg/kg/d
Clinical observation	-
FOB	-
Body weight	-
Food consumption	-
Water consumption	-
Urinalysis	-
Hematology	Plt↑: ≧6♂
Blood Chemistry	BUN↑25♂ Bile acid↑: ≧12♂♀
Absolute organ weight	Liver↑: 25♂
Relative organ weight	Liver↑: ≧6♂, 25♀
Necropsy	-
Histopathology	Stomach, forestomach-Epithelium, hyperplasia, squamous: ≧3♀, ≧6♂ Liver-Bile duct, hyperplasia: 25♂♀ Liver-Hepatocyte, periportal, hypertrophy: 25♂♀ Liver-Necrosis: 25♀

CAS No. 107-02-8: Acrolein

Study type	Repeated dose oral toxicity study (14 weeks)
Species	Rat (F344/N)
Route	Gavage
Solvent	0.5% Methylcellulose
Dose level	0.75, 1.25, 2.5, 5.0, 10 mg/kg/d, 10 animals/group/sex
Purity	98.8%
Publication	NTP (2006)
Data source	HESS Repeated Dose Toxicity database
Death	1.25 (♀： 1/10), 2.5 (♂: 2/10, ♀: 2/10), 5 (♂: 2/10, ♀: 1/10), 10 (♂: 8/10, ♀: 8/10)
NOEL	♂: 1.25 mg/kg/d, ♀:<0.75 mg/kg/d
Clinical observation	-
FOB	-
Body weight	↓: 10♂ ♀
Food consumption	-
Water consumption	-
Urinalysis	-
Hematology	Hct↑: ≧5♂ ♀ RBC↑: ≧5♂ ♀ WBC↑: ≧5♀ Hgb↑: ≧5♀ Ret↑: ≧5♂, 10♀ Nucleated erythrocyte↑: 10♂ Plt↑: ≧2.5♂ ♀
Blood Chemistry	BUN↑: ≧0.75♀, ≧2.5♂ TP↓: ≧2.5♂ ♀ Alb↓: ≧2.5♂ ♀ Bile acid↑: 10♂
Absolute organ weight	Liver↑: ≧5♀ Thymus↓: 10♀
Relative organ weight	Liver↑: ≧2.5♂, ≧5♀ Thymus↓: 10♀
Necropsy	-
Histopathology	Liver-Inflammation, chronic/Necrosis: 10♂ Stomach, forestomach-Hemorrhage: 10♂ ♀ Stomach, forestomach-Inflammation, chronic active: 10♂ ♀ Stomach, forestomach-Epithelium, necrosis: 10♂ ♀ Stomach, forestomach-Epithelium, hyperplasia, squamous: ≧1.25♀, ≧2.5♂ Stomach, forestomach-Foreign body: 10♀ Stomach, forestomach-Epithelium, inflammation, chronic active: 10♀ Stomach, glandular-Hemorrhage: ≧5♂, 10♀ Bone marrow-Hyperplasia: 10♂ ♀

	Spleen-Lymphoid follicle, depletion cellular: 10♂♀ Spleen-Red pulp, hematopoietic cell proliferation: 10♂ Spleen-Lymphoid follicle, necrosis: 10♀, 2.5♂ Thymus-Thymocyte, atrophy: 10♂♀ Thymus-Thymocyte, necrosis: ≧2.5♂♀ Nose-Inflammation, acute: 10♂♀

CAS No. 149-57-5: 2-Ethylhexanoic acid

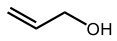
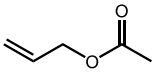
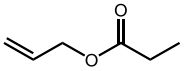
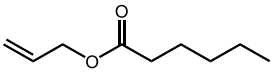
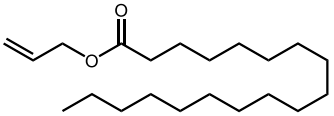
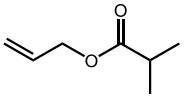
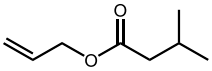
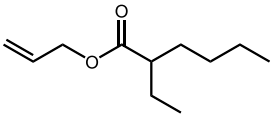
Study type	Repeated dose oral toxicity study (13 weeks)
Species	Rat (F344/CrJBR)
Route	Oral (feeding)
Solvent	-
Dose level	3 doses (0.1, 0.5, 1.5%), 10 animals/sex/group
Purity	99.9±0.05%
Publication	Juberg *et al*., (1998)
Data source	HESS Repeated Dose Toxicity database
Death	None
NOEL	<0.1% (♂: 61, ♀: <71 mg/kg)
Clinical observation	N/A
FOB	-
Body weight	↓: 1.5♂♀
Food consumption	↓: 1.5♂♀
Water consumption	-
Urinalysis	N/A
Hematology	-
Blood Chemistry	Cho↑: >0.1♂ Alb↑: 1.5♂
Absolute organ weight	Liver↑: 1.5♂♀
Relative organ weight	Liver↑: >0.5♂♀, Kidney↑: 1.5♂♀, Testis↑: 1.5♂ Recovery: Liver↑, Testis↑
Necropsy	-
Histopathology	Liver-Hepatocyte hypertrophy: >0.5♂, 1.5♀

CAS No. 3302-10-1: 3,5,5-Trimethylhexanoic acid

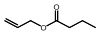
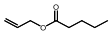
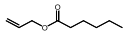
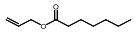
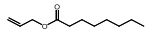
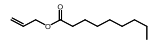
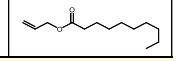
Study type	Repeated-dose oral toxicity study (4 weeks)
Species	Rat (Wistar)
Route	Gavage
Solvent	0.1% Cremophore
Dose level	10, 50, 200 mg/kg/d, 10 animals/group/sex
Purity	94.9%
Publication	Unpublished
Data source	RepDose Tox Fraunhofer ITEM in OECD QSAR Toolbox
Death	-
NOAEL	50 mg/kg/d

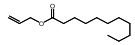
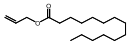
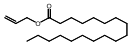
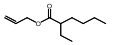
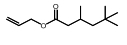
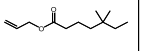
Clinical observation	Motor activity↓: 200♀
FOB	-
Body weight	-
Food consumption	-
Water consumption	-
Urinalysis	Volume↑: ≧50♂, 200♀ Specific gravity↓: ≧50♂, 200♀ Blood↑: ≧50♂ Renal tubular cells, squameous epithelial cells, granular casts, epithelial casts↑: ≧50♂ Transitional epithelial cells↑: ≧10♂
Hematology	-
Blood Chemistry	Alb↓: 200♂ TP↓: 200♀ Glb↓: ≧200♀ ALT↑: 200♀
Absolute organ weight	Liver↑: 200♂ Kidney↑: 200♂
Relative organ weight	Liver↑: 200♂♀ Kidney↑: 50♂♀
Histopathology	Liver-fatty infiltration, periportal hepatocyte: 200♂, ≧50♀ Kidney-α2u-globulin accumulation, epithelia of proximal tubules↑: ≧10♂ Kidney-tubular dilatation: ≧50♂

ANNEX 2 SUMMARY OF BIOCHEMICAL EVIDENCE FOR ALLYL ESTERS HYDROLYZED IN RATS

Chemical structure Chemical name CAS No.	Detection	Enzyme source /animal, dosage	Reference
 Allyl alcohol 107-18-6	3-HPM in urine, 1, 45 day(s) post-dosing	F344/N male rats 25 mg/kg/d, p.o., 1, 45 day(s)	Auerbach *et al*., 2008
	3-HPM in urine, 24 h post-dosing	CFE male rats 1 % (v/v), 1 ml, s.c.	Kaye, 1973
 Allyl acetate 591-87-7	3-HPM in urine, 1, 45 day(s) post-dosing	F344/N male rats 25 mg/kg/d, p.o., 1, 45 day(s)	Auerbach *et al*., 2008
	3-HPM in urine, 24 h post-dosing	CFE male rats 1 % (v/v), 1 ml, s.c.	Kaye, 1973
	Hydrolytic reaction	Rat alimentary tract preparation	Butterworth *et al*., 1975
	Hydrolytic reaction	Rat liver preparation	Silver and Murphy, 1978
 Allyl propionate 2408-20-0	3-HPM in urine, 24 h post-dosing	CFE male rats 1 % (v/v), 1 ml, s.c.	Kaye, 1973
	Hydrolytic reaction	Rat alimentary tract preparation	Butterworth *et al*., 1975
 Allyl hexanoate 123-68-2	Hydrolytic reaction	Rat alimentary tract preparation	Butterworth *et al*., 1975
	Hydrolytic reaction	Rat liver and small intestinal mucosa preparations	Longland *et al*., 1977
 Allyl stearate 6289-31-2	3-HPM in urine, 24 h post-dosing	CFE male rats 0.060 g, s.c.	Kaye, 1973
 Allyl isobutyrate 15727-77-2	Hydrolytic reaction	Rat alimentary tract preparation	Butterworth *et al*., 1975
 Allyl isovalerate 2835-39-4	Hydrolytic reaction	Rat alimentary tract preparation	Butterworth *et al*., 1975
 Allyl 2-ethylhexanoate 58105-49-0	Hydrolytic reaction	Rat alimentary tract preparation	Butterworth *et al*., 1975

APPENDIX - DATA MATRIX

Chemical ID										
		Member 1	Member 2	Member 3	Member 4	Member 5	Member 6	Member 7	Member 8	Member 9
CAS		591-87-7	2408-20-0	2051-78-7	6321-45-5	123-68-2	142-19-8	4230-97-1	7493-72-3	57856-81-2
Name		Allyl acetate	Allyl propionate	Allyl butyrate	Allyl pentanoate	Allyl hexanoate	Allyl heptanoate	Allyl octanoate	Allyl nonanoate	Allyl decanoate
Structure										
Summary of data gap filling										
		Subcategory 1								
		Member 1	Member 2	Member 3	Member 4	Member 5	Member 6	Member 7	Member 8	Member 9
Repeated Dose Toxicity	Experimental result (GLP)	NOAEL=0.25 mmol/kg/d Hepatocyte necrosis, bile duct hyperplasia F344 rats 5 doses/w for 14 wks, Gavage (NTP, 2006)	No data	No data	No data	No data	No data	No data	No data	No data
	Experimental result (non-GLP)	No data	No data	No data	No data	NOAEL=0.10 mmol/kg/d Hepatic fibrosis, focal necrosis, bile duct proliferation Osborne-Mendel rats 18 wks, Gavage (Hagan et al., 1967)	NOAEL: Not determined LOAEL=0.40 mmol/kg/d Hydropic degeneration of the liver cells in the periportal area, new bile duct formation Osborne-Mendel rats 18 wks, Feeding (Hagan et al., 1965)	No data	No data	No data
	Integrated conclusion (read-across)		NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity		NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity
	D value	D=0.13 mg/kg/d	D=0.06 mg/kg/d	D=0.06 mg/kg/d	D=0.07 mg/kg/d	D=0.08 mg/kg/d	D=0.09 mg/kg/d	D=0.09 mg/kg/d	D=0.10 mg/kg/d	D=0.11 mg/kg/d
Molecular profiling related to the category hypothesis										
Parent chemical	Toxicological category (HESS ver. 3.0)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)
	Chain length of carboxylic acid	2	3	4	5	6	7	8	9	10
	Substructure of carboxylic acid	Linear alkyl	Linear alkyl	Linear alkyl	Linear alkyl	Linear alkyl	Linear alkyl	Linear alkyl	Linear alkyl	Linear alkyl
	Subcategory	Subcategory 1	Subcategory 1	Subcategory 1	Subcategory 1	Subcategory 1	Subcategory 1	Subcategory 1	Subcategory 1	Subcategory 1
Physical-chemical data										
Melting point [K]		186.18	197.45	208.72	219.99	231.26	242.53	253.8	265.07	276.34
Boiling point [K]		368.9	391.78	414.66	437.54	460.42	483.3	506.18	529.06	551.94
logKow (measured value)		0.97	No data	No data	No data	No data	No data	No data	No data	No data
logKow (calculated value)		1.22	1.71	2.2	2.69	3.18	3.67	4.17	4.93	5.15
MW		100.12	114.14	128.17	140.2	156.23	170.25	184.28	198.31	212.33
Kinetics										
Absorption		No data	No data	No data	No data	No data	No data	No data	No data	No data
Distribution		No data	No data	No data	No data	No data	No data	No data	No data	No data
Metabolism		Ester hydrolysis in liver and alimentary tract and liver preparations (Butterworth et al., 1975; Silver and Murphy, 1978)	Ester hydrolysis in alimentary tract preparation (Butterworth et al., 1975)	No data	No data	Ester hydrolysis in alimentary tract and liver preparations (Butterworth et al., 1975; Longland et al., 1977)	No data	No data	No data	No data
Excretion		3-HPM in urine (Auerbach et al., 2008; Kaye, 1973)	3-HPM in urine (Kaye, 1973)	No data	No data	No data	No data	No data	No data	No data
Supporting data related to the target endpoint(s)										
		Member 1	Member 2	Member 3	Member 4	Member 5	Member 6	Member 7	Member 8	Member 9
In silico	Metabolic hydrolysis of allyl esters (read-across)			Positive	Positive		Positive	Positive	Positive	Positive

Chemical ID											
		Member 10	Member 11	Member 12	Member 13	Member 14	Member 15	Member 16	Member 17	Member 18	Member 19
CAS		17308-90-6	45236-96-2	6289-31-2	15727-77-2	2835-39-4	93963-13-4	7493-69-8	58105-49-0	71500-37-3	68132-80-9
Name		Allyl undecanoate	Allyl myristate	Allyl stearate	Allyl isobutyrate	Allyl isovalerate	Allyl 2-methylbutyrate	Allyl 2-ethylbutyrate	Allyl 2-ethylhexanoate	Allyl 3,5,5-trimethylhexanoate	Allyl trimethylhexanoate
Structure											
Summary of data gap filling											
		Member 10	Member 11	Member 12	Member 13	Member 14	Member 15	Member 16	Member 17	Member 18	Member 19
Repeated Dose Toxicity	Experimental result (GLP)	No data	No data	No data	No data	NOAEL=0.44 mmol/kg/d Bile duct hyperplasia, hepatic multifocal coagulative necrosis & nodular hyperplasia, cholangiofibrosis F344 rats 5 doses/w for 13 wks, Gavage (NTP, 1983)	No data	No data	No data	No data	No data
	Experimental result (non-GLP)	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
	Integrated conclusion (read-across)	NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity		NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity	NOAEL=0.10 mmol/kg/d Hepatotoxicity
	D value	D=0.11 mg/kg/d	D=0.13 mg/kg/d	D=0.16 mg/kg/d	D=0.06 mg/kg/d	D=0.31 mg/kg/d	D=0.07 mg/kg/d	D=0.08 mg/kg/d	D=0.09 mg/kg/d	D=0.10 mg/kg/d	D=0.10 mg/kg/d
Molecular profiling related to the category hypothesis											
Parent chemical	Toxicological category (HESS ver. 3.0)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)
	Chain length of carboxylic acid	11	14	18	3	4	4	4	6	6	7
	Substructure of carboxylic acid	Linear alkyl	Linear alkyl	Linear alkyl	Branched alkyl	Branched alkyl	Branched alkyl	Branched alkyl	Branched alkyl	Branched alkyl	Branched alkyl
	Subcategory	Subcategory 1	Subcategory 1	Subcategory 1	Subcategory 2	Subcategory 2	Subcategory 2	Subcategory 2	Subcategory 2	Subcategory 2	Subcategory 2
Physical-chemical data											
Melting point [K]		287.61	321.42	366.5	193.72	204.99	204.99	216.26	238.8	252.49	267.49
Boiling point [K]		574.82	643.46	734.98	414.22	437.1	437.1	459.98	505.74	525.39	525.83
logKow (measured value)		No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
logKow (calculated value)		5.64	7.11	9.08	2.13	2.62	2.62	3.11	4.09	4.47	4.55
MW		226.36	268.44	324.55	128.17	142.2	142.2	156.23	184.28	198.31	198.31
Kinetics											
Absorption		No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
Distribution		No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
Metabolism		No data	No data	No data	Ester hydrolysis in alimentary tract preparation (Butterworth et al., 1975)	Ester hydrolysis in alimentary tract preparation (Butterworth et al., 1975)	No data	No data	Ester hydrolysis in alimentary tract preparation (Butterworth et al., 1975)	No data	No data
Excretion		No data	No data	3-HPM in urine (Kaye, 1973)	No data	No data	No data	No data	No data	No data	No data
Supporting data related to the target endpoint(s)											
		Member 10	Member 11	Member 12	Member 13	Member 14	Member 15	Member 16	Member 17	Member 17	Member 18
In silico	Metabolic hydrolysis of allyl esters (read-across)	Positive	Positive				Positive	Positive		Positive	Positive