Category Justification

Acrylic acid and Lower Alkyl Acrylates

Last Updated 10/2/19

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Executive Summary

Data on physico-chemical properties, environmental fate, ecotoxicity, and human health effects have been collected for the following substances: acrylic acid (CAS No. 79-10-7), methyl acrylate (CAS No. 96-33-3), ethyl acrylate (CAS No. 140-88-5), n-butyl acrylate (CAS No. 141-32-2), isobutyl acrylate (CAS No. 106-63-8), tert-butyl acrylate (CAS No. 1663-39-4), and 2-ethylhexyl acrylate (CAS No. 103-11-7). Because these substances exhibit similarity in their physico-chemical properties and toxicological properties in mammals, and because the acrylate esters have been shown to be metabolised in the mammalian body in minutes to acrylic acid and the corresponding alcohol, they can be considered to constitute a chemical category. Data gaps for mammalian toxicity can be addressed by read-across between category members. In addition, because these substances exhibit similarity in their physicochemical, environmental fate and eco-toxicological properties, data gaps in physico-chemical properties and ecotoxicity can be addressed by read-across between category members. The read-across strategy for the endpoints with data-gap was based on the following scenarios in accordance with the RAAF (ECHA, 2017).

Endpoint	**RAAF scenario**	**Read-across hypothesis based on**
Skin sensitisation	4	Different compounds have qualitatively similar properties
Repeated dose toxicity	3	(Bio) transformation to common compound(s)
In vito genetic toxicity	4	Different compounds have qualitatively similar properties
Carcinogenicity	3	(Bio) transformation to common compound(s)
Toxicity of reproduction	3	(Bio) transformation to common compound(s)
Long-term toxicity testing on invertebrates	6	Different compounds have quantitatively similar properties
Short-term toxicity testing on invertebrates (marine)*	6	Different compounds have quantitatively similar properties
Short-term toxicity testing on fish (marine)*	6	Different compounds have quantitatively similar properties
* Read-across of information on endpoints is technically not required to address any REACH endpoint		

1. Introduction

Article 13(1) of legislation EC 1907/2006 (REACH) states that "Information on intrinsic properties of substances may be generated by means other than tests, provided that the conditions set out in Annex XI are met. In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, *in vitro* methods or qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across)."

In the ECHA Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals, a chemical category is defined as "a group of chemicals whose physico-chemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (or other similarity characteristic)." The guidance then provides a list of characteristic properties upon which structural similarity may be based.

The acrylic acid and lower alkyl acrylate esters category is defined as a structurally related group of seven substances including acrylic acid (AA; CAS No. 79-10-7) and its six esters; methyl acrylate (MA; CAS No. 96-33-3), ethyl acrylate (EA; CAS No. 140-88-5), n-butyl acrylate (nBA; CAS No. 141-32-2), isobutyl acrylate (iBA; CAS No. 106-63-8), tert-butyl acrylate (tBA; CAS No. 1663-39-4), and 2-ethylhexyl acrylate (2EHA; CAS No. 103-11-7). The short-chain acrylate esters in this category are classed as alpha, beta-unsaturated esters with having potential Michael acceptors capable of electrophilic attack of protein and other cellular macromolecules. AA is a common major metabolite in the category that is considered to be the most relevant compound for systemic toxicity for the category.

The principle of toxicological read-across within the lower alkyl acrylate esters category is that the acrylates with the common chemical reactivity and common primary metabolic pathway to acrylic acid have similar toxicological properties. These aspects can be either qualitatively categorised as "same type of effect" (i.e. scenario 4 according to RAAF (ECHA, 2017); chemical reactivity of the category members) or as "(Bio) transformation to common compound(s)" (i.e. scenario 3 according to RAAF; common primary metabolic pathway). Moreover, these data serve as the basis for the category assessment in many endpoints in this category document. The read-across within the category that is presented in this report is supported by the similarity among the lower acrylates in the category on toxicokinetics and toxicodynamics behaviour. **The key read-across hypothesis that supports the category approach for short-chain acrylate esters (C1-C8) is that the acrylate esters are rapidly metabolised via two pathways: esterase hydrolysis to acrylic acid and alcohols and glutathione conjugation, hence they have similar toxicological properties (i.e. a lack of systemic toxicity).** Overall, the uncertainties associated with the read-across, based on prediction of systemic toxicity of the acrylate esters within the category, are considered to be minimal. The alcohol metabolites of acrylate esters are not expected to make a significant contribution to the systemic toxicity profiles of acrylate esters due to local toxicity limiting the dose that can be applied.

Read across within the category for ecotoxicity and physicochemical properties is established quantitatively on the basis of the "same type of effect" corresponding to scenario 6 and 4 of the RAAF, respectively where clear trends or patterns are observed amongst other members of the category.

Read-across from the studies on the source substances are considered to be an appropriate adaptation to the standard information requirements of Annex VII, VIII, IX and X of the REACH Regulation for the target substances, in accordance with the provisions of Annex XI, 1.5 of the REACH Regulation. The justification of the proposed read-across approach is elaborated in the next chapters. The endpoint specific "scientific assessment option" of the read across is "acceptable with high/medium confidence" for the all category substances.

2. Hypothesis for the category approach

The read-across hypothesis that supports the category approach for acrylic acid and lower alkyl acrylate esters is based on the following considerations.

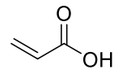
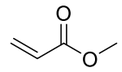
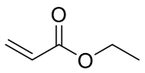
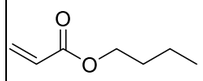
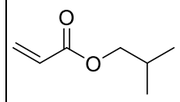
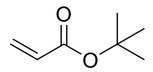
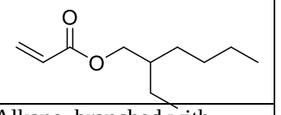
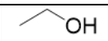
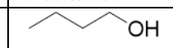
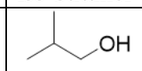
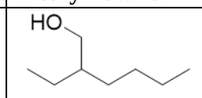
- After uptake of the lower alkyl acrylate esters, they are metabolised by carboxyl-esterase catalysed ester hydrolysis, conjugation with glutathione and binding to protein (Frederick *et al.*, 1992).
- The potential toxicity from the remaining parental acrylate esters is considered minimal as evidenced in the available toxicology information.
    - They have fast metabolism with short half-lives
    - The various alcohols do not make a significant contribution to the systemic toxicity profiles of acrylate esters

The short-chain acrylate esters in this category are classed as alpha, beta-unsaturated esters that are potential Michael acceptors capable of electrophilic attack of protein and other cellular macromolecules. Therefore, the different acrylate esters are considered to cause toxicity through similar mechanisms. Acrylate esters are rapidly metabolised to AA and the corresponding alcohols by carboxylesterases which are widely distributed throughout the body. The steric hindrance of the tertiary structure of the side chain (tBA in particular) has been shown to supress the rate of ester hydrolysis *in vitro*. Acrylate esters have also been demonstrated to undergo conjugation with GSH to form thioesters, leading to GSH depletion, with a trend for decreasing capacity to cause GSH depletion level with increasing chain length. The data indicate that, although there may be potential for differences in the extent of metabolism due to structure, there are no obvious differences in toxicity.

3. Category Members

The category members along with common chemical identifiers (i.e., substance name, CAS number, EC number, SMILES notation and chemical structure) are presented in Table 1 along with the EU CLP classification listed in the CLP inventory, typical concentration and concentration range for the constituents and all identified impurities. The structurally related alcohols and their substance identities are also presented in the table.

Table 1 - Members of the acrylic acid and lower alkyl acrylate esters category

Category Members	Acrylic acid (AA)	Methyl acrylate (MA)	Ethyl acrylate (EA)	n-Butyl acrylate (nBA)	Isobutyl acrylate (iBA)	tert-Butyl acrylate (tBA)	2-Ethylhexyl acrylate (2EHA)
Structure							
Organic functional groups	Alkene; Carboxylic acid; Acrylic acids	Alkene; Carboxylic acid ester; Acrylate	Alkene; Carboxylic acid ester; Acrylate	Alkene; Carboxylic acid ester; Acrylate	Alkane, branched with tertiary carbon; Alkene; Carboxylic acid ester; Acrylate; Isopropyl	Alkane, branched with tertiary carbon; Alkene; Carboxylic acid ester; Acrylate; tert-Butyl	Alkane, branched with tertiary carbon; Alkene; Carboxylic acid ester; Acrylate
SMILE	OC(=O)C=C	COC(=O)C=C	CCOC(=O)C=C	CCCCOC(=O)C=C	CC(C)COC(=O)C=C	CC(C)(C)OC(=O)C=C	CCCCC(CC)COC(=O)C=C
Molecular formula	C3H4O2	C4H6O2	C5H8O2	C7H12O2	C7H12O2	C7H12O2	C11H20O2
Molecular weight (g/mol)	72.1	86.1	100.1	128.2	128.2	128.2	184.3
REACH Annex	X	X	X	X	IX	Confidential	X
CAS number	79-10-7	96-33-3	140-88-5	141-32-2	106-63-8	1663-39-4	103-11-7
EC number	201-177-9	202-500-6	205-438-8	205-480-7	203-417-8	216-678-7	203-080-7
EU CLP	(Harmonised classification) Flam. Liq. 3 (H226) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Corr. 1A (H314) STOT SE 3 (H335) Aquatic Acute 1 (H400)	(Harmonised classification) Flam. Liq. 2 (H225) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) STOT SE 3 (H335) (Self classification) Acute Tox. 3 (H331) Aquatic Chronic 3 (H412)	(Harmonised classification) Flam. Liq. 2 (H225) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) STOT SE 3 (H335) (Self classification) Acute Tox. 3 (H331) Aquatic Chronic 3 (H412)	(Harmonised classification) Flam. Liq. 3 (H226) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) STOT SE 3 (H335) (Self classification) Acute Tox. 4 (H332) Aquatic Chronic 3 (H412)	(Harmonised classification) Flam. Liq. 3 (H226) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Skin Sens. 1 (H317) (Self classification) STOT SE 3 (H335) Aquatic Chronic 3 (H412)	(Harmonised classification) Flam. Liq. 2 (H225) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Skin Sens. 1 (H317) STOT SE 3 (H335) Aquatic Chronic 2 (H411) (Self classification) Acute Tox. 3 (H331)	(Harmonised classification) Skin Irrit. 2 (H315) Skin Sens. 1 (H317) STOT SE 3 (H335) (Self-classification) Aquatic Chronic 3 (H412)
Purity*	> 95 - < 100 % (w/w)	>=99 - <=100 % (w/w)	>=99 - <=100 % (w/w)	>=98 - <=100 % (w/w)	> 98 - <=100 % (w/w)	>= 98.5 - < 100 % (w/w)	>=99.6 % (w/w)
Structurally related alcohols	-	**Methanol**	**Ethanol**	**n-butanol**	**iso-butanol**	**tert-butanol**	**2-ethylhexanol**
Structure	-						
Molecular weight (g/mol)	-	32	46	74	74	74	130
CAS number	-	67-56-1	64-17-5	71-36-3	78-83-1	75-65-0	104-76-7
EC number	-	200-659-6	200-578-6	200-751-6	201-148-0	200-889-7	203-234-3
EU CLP	-	(Harmonised classification) Flam. Liq. 2 (H225) Acute Tox. 3 (H301) Acute Tox. 3 (H311) Acute Tox. 3 (H331) STOT SE 1	(Harmonised classification) Flam. Liq. 2	(Harmonised classification) Flam. Liq. 3 (H226) Acute Tox. 4 (H302) Skin Irrit. 2 (H315) Eye Dam. 1 (H318) STOT SE 3 (H335) STOT SE 3 (H336)	(Harmonised classification) Flam. Liq. 3 (H226) Skin Irrit. 2 (H315) Eye Dam. 1 (H318) STOT SE 3 (H335) STOT SE 3 (H336)	(Harmonised classification) Flam. Liq. 2 (H225) Acute Tox. 4 (H332) Eye Irrit. 2 (H319) STOT SE 3 (H335)	(Self classification) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) STOT SE 3 (H335)

*There are no impurities or stabilizers which influence the classification.

4. Read-across strategy

Within this category approach read-across is applied to the following (eco)toxicological endpoints (see Table 2) and is based on experimental data that are available from protocols equivalent to or similar to OECD test guidelines for one or more category members for each endpoint.

Toxicological endpoints:

- Skin sensitisation
- Sub-chronic toxicity (oral, inhalation)
- *In vitro* mutagenicity in mammalian cells
- Carcinogenicity
- Reproductive toxicity
- Developmental toxicity (rat, rabbit)

Ecotoxicological endpoints:

- Long-term toxicity testing on invertebrates
- Short-term toxicity testing on invertebrates (marine)*
- Short-term toxicity testing on fish (marine)*

* Read-across of information on endpoints is technically not required to address any REACH endpoint.

5. Justification of data gap filling

The data matrices for physico-chemical properties, environmental fate properties, ecotoxicological and toxicological data are presented in Annex 1.

5.1 Structural Similarities

The category members consist of acrylic acid and its lower acrylate esters (Table 1). The acrylates are esters of a short chain length alcohol and AA. The acid part is always the same, therefore all have one C=C-double bond as common functional group. AA has a carboxylic acid functional group and the acrylates have a carboxylic acid ester group. AA has a carbonyl group but additionally an alcohol component which differs within the category in the chain length (C1-C6) and/or the configuration.

The QSAR Toolbox (v4.3)[1] has been used to assess the similarity of acrylate esters in the category (Annex 2) with respect to their potential reactivity. For a comparison, the profiling of AA was also presented. Overall, the outcomes of profiles support similarity in the reactivities associated with the acrylate esters based on Michael addition to alpha, beta-unsaturated acids and esters. The structural alerts indicate a low level of toxicity predicted based on the assignment of Cramer Class I for all category members. There is no indication of potential receptor binder reactivity or a different carcinogenic mechanism within the category. No alerts were flagged for the protein binding for the alcohol metabolites of acrylate esters (Annex 3).

[1] Provided by Organisation for Economic Co-operation and Development and European Chemicals Agency.

5.2 Comparison of physico-chemical Properties

The physico-chemical properties of the category members are summarised in Table 2 as well as in

Annex . The data are derived from studies of an appropriate duration and quality to warrant a high degree of reliability and accordingly have Klimisch ratings of 1 or 2. There are data available on all members of the category, for all of the required physico-chemical endpoints, with the exception of viscosity data (tBA).

In RAAF nomenclature, the read-across approach for this endpoint is described in scenario 4 (different compounds have qualitatively similar properties) and governed by AE 4.3 (common underlying mechanism, quantitative aspects). Here, a clear trend of increasing viscosity with increasing molecular weight and chain length is observed. Read-across is applied with a high level of certainty.

Discussion

Trends can be observed in physico-chemical properties of the category members. With increasing chain length and molecular weight the autoflammability, vapour pressure and water solubility decreases while the boiling point, flashpoint, viscosity and partition coefficient increases. All members of the category are colourless liquids with freezing points between -90 °C (2EHA) and -61 °C (iBA) and relative densities between 0.87 (tBA) and 0.95 (MA). None of the category members are surface active or oxidising and none have explosive properties. All of the substances are flammable (iBA, nBA) or highly flammable (MA, EA, tBA), except for 2EHA which is considered a combustible liquid under GHS. Due to the overall similarity of the physico-chemical findings, and in consideration of recognisable trends in the results, read-across – to address the viscosity of tBA – is justified. The viscosity of tBA is considered to be higher than that of MA and EA and can be predicted to be similar to that of iBA and nBA due to the analogy of the molecular weights of the butyl acrylates. The read-across is applied with a high level of confidence.

Conclusions

The data serve to demonstrate that there are clear trends in the physico-chemical properties of the members of the category, related to molecular weight, molecular size and hydrophilicity and supports the hypothesis that properties can be read-across between category members in predictable manner. The available physico-chemical results support the broader use of the category approach to (eco)toxicity endpoints.

Table 2 - Summary of key physico-chemical properties

Property	AA	MA	EA	tBA	iBA	nBA	2EHA
Physical state at 20 °C and 101.3 kPa	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Freezing point [°C]	13	-76.5	-71.2	-69	-61	-64.6	-90
Boiling point [°C]	141	80.1	99.8	119.2	132	147	215
Relative density	1.05	0.95	0.92	0.87	0.89	0.90	0.88
Vapour pressure [hPa]	5.29 (25 °C)	90 (20 °C)	40 (21 °C)	20 (23 °C)	10 (25 °C)	5 (22 °C)	0.24 (25 °C)
Water solubility [g/L]	1 000 (25 °C)	60 (25 °C)	20 (20 °C)	2 (20 °C)	1.8 (25 °C)	1.7 (20 °C)	0.01 (25 °C)
Partition coefficient n-octanol/water (Log value)	0.46	0.74	1.18	2.32	2.38	2.38	4.00
Surface tension	Not surface active	Not surface active	Not surface active	Not surface active	Not surface active	Not surface active	Not surface active
Flammability	Flammable	Highly flammable	Highly flammable	Highly flammable	Flammable	Flammable	Not-flammable (Combustible liquid – GHS)
Autoflammability / self-ignition temperature [° C]	438	468	372	400	350	275	252
Flashpoint [°C]	48.5	-2.8	9	14	30	37	86
Explosiveness	Non explosive	Non explosive	Non explosive	Non explosive	Non explosive	Non explosive	Non explosive
Oxidising properties	Not oxidising	Not oxidising	Not oxidising	Not oxidising	Not oxidising	Not oxidising	Not oxidising
Dissociation constant (pKa)	4.26 (25 °)	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Viscosity [mPa.s]	1.149 (25 °C)	0.472 (25 °C)	0.5351 (25 °C)	0.9 (20 °C)	0.82 (20 °C)	0.88 (20 °C)	1.75 (20 °C)

5.3 Comparison of toxicokinetics

5.3.1 Absorption

All members of the category are expected to be readily absorbed by oral, inhalation and dermal routes based on the experimental data and predictions from physico-chemical properties. The available repeated dose toxicity studies for oral, inhalation and dermal routes show the acrylate esters, either as parents and/or their metabolites, are absorbed based on the systemic effects observed.

The acrylate esters have relatively small molecular size ranging 86.1 to 184.3 g/mol. The partition coefficients range between 0.74 (MA) and 4.00 (2EHA) which is considered in the favourable range for absorption via oral, dermal and inhalation routes. Based on these partition coefficients, there is no concern for bioaccumulation (ECHA guidance R7c. section R.7.12.). The majority of the acrylate esters have very high water solubilities ranging from 1.7 g/L (nBA) to 60 g/L (MA). The water solubility of 2EHA is considerably lower (0.01 g/L) at three orders of magnitude compared to the rest of the acrylate esters. However, overall all the category members are water soluble and readily absorbed. All substances are liquids, which favours dermal absorption. The volatile nature of acrylate esters would limit the rate of dermal absorption due to the loss of materials via evaporation. This may occur however to a lower extent for 2EHA with its vapour pressure of 0.24 hPa at 25 °C, which is considerably lower than the rest of the category members (the range of the vapour pressure is from 5 to 90 hPa at 25°C). All acrylate esters in the category are weak skin sensitisers, which indicates the molecules are likely to be absorbed through the skin. The irritant nature of the acrylate esters may enhance the penetration through the skin.

Dermal absorption

Overall, the small molecular weights with a combination of the moderate to high water solubility and a moderate log P_{OW} range suggests dermal absorption will occur for all the acrylate esters in the category.

Dermwin (EPISuite) calculates a trend of increasing dermal absorption of the parent ester C1-C8 with increasing ester chain length.

Table 3 -Results of the Dermwin

	CAS No.	Molecular Weight (g/mol)	Log P_{OW} (at 25 °C)	Dermwin Kp est. [cm/hr]
MA	96-33-3	86.1	0.74	0.00175
EA	140-88-5	100.1	1.18	0.00324
nBA	141-32-2	128.2	2.38	0.0111
iBA	106-63-8	128.2	2.38	0.00893
tBA	1663-39-4	128.2	2.32	0.00732
2EHA	103-11-7	184.3	4	0.0759
DK-EPA heuristics are used to classify ranges of Kp values: Classifications: <0.001 Very Low; >= 0.001-<0.005 Low; >=0.005-<0.05 Moderate; >= 0.05 High				

5.3.2 Metabolism

This category is based on the hypothesis that the acrylate esters have similar toxicological properties and they have a common rapid metabolism pathway described by two primary routes: carboxylesterase mediated hydrolysis of the ester linkage to acrylic acid and the corresponding alcohol; and conjugation of AA-ester with glutathione. The primary hydrolysis products for the acrylate esters are summarised in Table 4.

Table 4 - Primary hydrolysis products

Acrylates	Primary Hydrolysis Products
Methyl acrylate (MA)	Acrylic acid and methanol
Ethyl acrylate (EA)	Acrylic acid and ethanol
n-Butyl acrylate (nBA)	Acrylic acid and n-butanol
Isobutyl acrylate (iBA)	Acrylic acid and iso-butanol
tert-Butyl acrylate (tBA)	Acrylic acid and tert-butanol
2-Ethylhexyl acrylate (2EHA)	Acrylic acid and 2-ethylhexanol

The alcohols associated with the esters being formed after hydrolysis are methanol (CAS No. 67-56-1), ethanol (CAS No. 64-17-5), n-butanol (CAS No. 71-36-3), iso-butanol (CAS No. 78-83-1), tert-butanol (CAS No. 75-65-0), and 2-ethylhexanol (CAS No. 104-76-7). Except for 2-ethylhexanol harmonised classifications exist for all of the alcohols. None of the alcohols are classified for sensitisation, mutagenicity, carcinogenicity or reproductive toxicity. The local toxicity of the acrylates is limiting the uptake of the alcohol; therefore, these alcohols are not considered to impact on the read-across approach within the category. Due to the rapid metabolism of the acrylate esters as demonstrated in the *in vitro* assays, the systemic toxicity exerted from the parental acrylate esters is considered to be of minimal relevance. However, the available toxicological studies of the category members for systemic toxicity endpoints suggest the similarity in toxicological properties. Therefore, any potential variation in toxicity associated with differences in the ester chain length and/or the presence of the tertiary structure is considered to be negligible. It is therefore concluded that AA is the common product of metabolism that is partly responsible for systemic toxicity for all substances within the category.

The major route of metabolism of acrylate esters has been shown to involve the rapid cleavage of the ester bond by carboxylic esterases (Figure 1; ECETOC, 1998; WHO, 1997), resulting in internal exposure to AA. Following carboxylesterase-catalysed hydrolysis to AA and the corresponding alcohol, a subsequent metabolic pathway involves metabolism of AA to carbon dioxide (CO_2) via the propionate degradation pathway. The respective alcohols are metabolised via either a catalase peroxidative pathway or the alcohol dehydrogenase pathway. Acrylate esters are also expected to undergo conjugation with GSH to form thioesters (Frederick *et al*., 1992), with the main urinary conjugate identified as N-acetyl-S-(2-carboxyethyl)cysteine. Inhibition of the hydrolytic pathway with a carboxylase inhibitor results in increased metabolism via the GSH conjugation route. There is no evidence to suggest that the vinyl moiety undergoes epoxidation. Based on a recent *in vitro* investigation for the hydrolysis and glutathione conjugation rates of the acrylate esters, all substances apart from tBA were metabolised by rat liver microsomes in the presence or absence of β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH) to form AA (ARTF, 2018). It was reported that the hydrolysis of the acrylate esters in rat liver microsomes is mainly mediated by esterases which do not require NADPH.

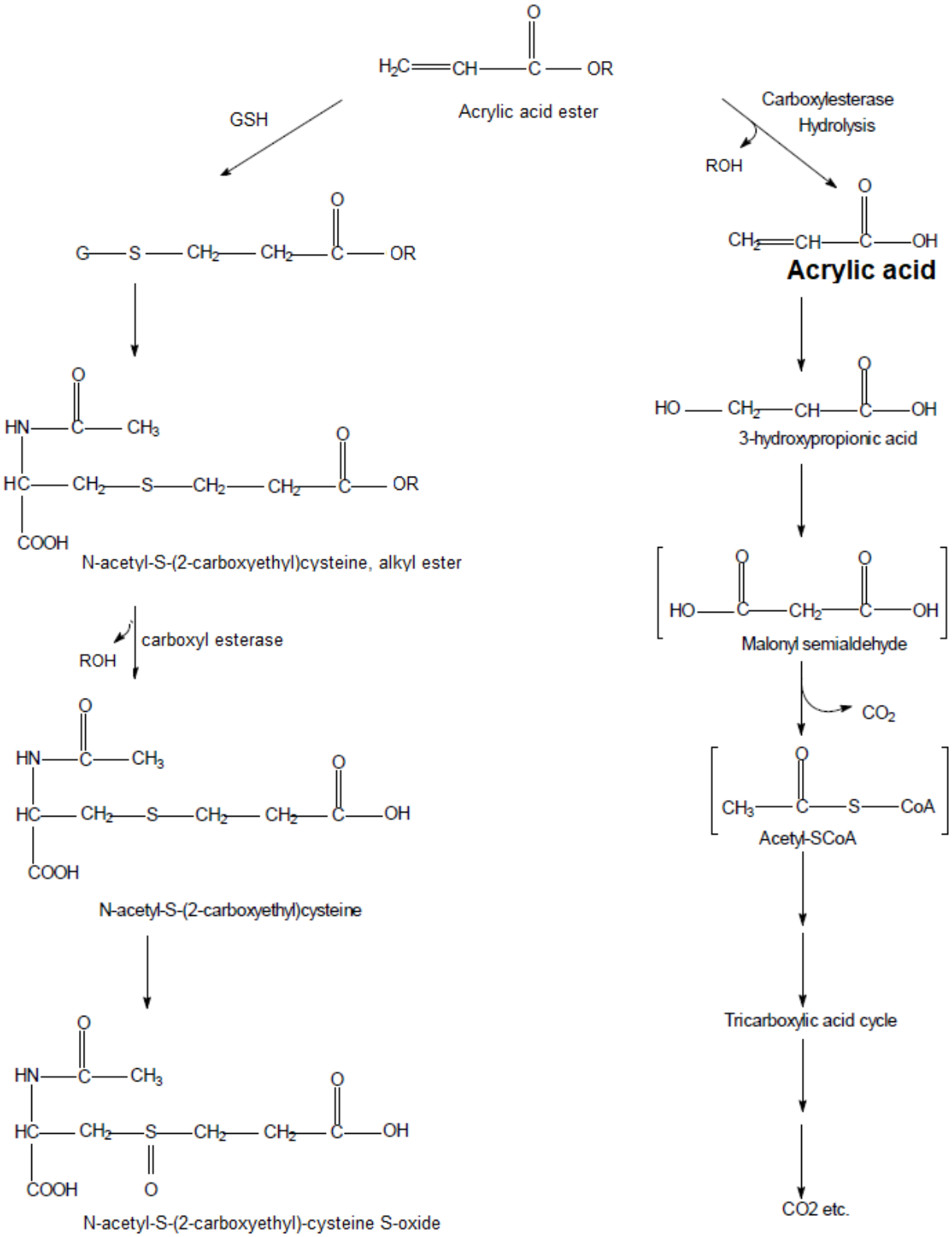


Figure 1. Proposed metabolic pathway for acrylate esters in rats (ECETOC, 1998; WHO, 1997)

Selected *in vivo* study results demonstrating comparable metabolism in mammals:

AA (CAS No. 79-10-7): C3H mice and Fischer 344 rats, respectively, were treated by gavage (40 or 150 mg/kg bw) with [1-^{14}C]-acrylic acid. Mice rapidly absorbed and metabolised orally administered acrylic acid (AA), with about 80% of the dose exhaled as $^{14}CO_2$ within 24 h. Excretion in urine and faeces accounted for approximately 3% and 1% of the dose, respectively. Elimination of the ^{14}C radiolabel from plasma, liver and kidney was rapid but it was slower from fat. The disposition of orally administered acrylic acid in rats was similar to the results obtained from mice. High-performance liquid chromatography (HPLC) analysis of rat urine and rat and mouse tissues indicated that absorbed AA was rapidly metabolised by the ß-oxidation pathway of propionate catabolism. No unchanged AA was detected 1 h after oral administration; however, several metabolites that were more polar than AA were measured, including 3-hydroxypropionate. Neither AA nor its metabolites were detected at later times after oral administration (Black *et al*., 1995).

MA (CAS No. 96-33-3): Methyl acrylate is rapidly absorbed by the oral and inhalation routes and distributed throughout the body. After oral or intraperitoneal administration, greater than 90% is excreted within 72 hours, primarily via the lungs as CO_2 (> 50%), and kidneys as products of glutathione conjugation reactions (10-50%) (Delbressine 1981, Sapota 1988 & 1990, Seutter 1981). The predominant pathway of metabolism of methyl acrylate, by many tissues (including lung, liver, kidney and plasma) appears to be hydrolysis to **acrylic acid** and **methanol**, which is catalysed by carboxyl esterase enzymes. Thus, under normal circumstances, a relatively small amount of the intact ester is absorbed into the blood through the lungs. The subsequent metabolism will follow that for acrylic acid, and involves metabolism to CO_2 via the propionate degradation pathway (**acrylic acid → 3-hydroxypropionic acid → malonyl semialdehyde → acetyl S CoA → tricarboxylic acid cycle → CO_2**). Metabolism of methanol proceeds via a catalase peroxidative pathway or alcohol dehydrogenase pathway. Intact methyl acrylate, which reaches the blood, is detoxified by hydrolysis, as well as by conjugation (by Michael addition) with glutathione (GSH) to form thioethers. The conjugates are then converted to mercapturic acids and excreted in the urine. The main conjugate has been identified as **N-acetyl-S-(2-carboxyethyl)cysteine**. Inhibition of the hydrolytic pathway with carboxylase inhibitor results in increased metabolism via the GSH conjugation route (Silver & Murphy 1981a, Miller 1981a).

EA (CAS No. 140-88-5): Toxicokinetic and metabolic studies on rats show that ethyl acrylate is rapidly absorbed after oral and inhalation uptake. The substance is rapidly hydrolysed to **acrylic acid** and **ethanol** by unspecific carboxylesterases which e.g. were detected in the liver, kidney, lung, plasma, nasal mucous membrane and stomach (Silver & Murphy, 1981a; Stott & McKenna, 1984; 1985; De Bethizy *et al*., 1987; Ghanayem *et al*., 1987; Vodicka *et al*., 1990). The half-life of ethyl acrylate in rat blood is less than 15 minutes (Miller *et al.*, 1981b). After further metabolism, the substance is mostly exhaled as **CO_2** (about 70% of the applied dosage within 24 h) or is eliminated with the urine as **3-hydroxypropionic acid** (De Bethizy *et al*., 1987; Ghanayem *et al*., 1987). After uptake, ethyl acrylate is conjugated with non-protein-bound sulfhydryl groups (glutathione) and, following further reaction, is excreted with the urine and faeces in the form of **mercapturic acid derivatives** (De Bethizy *et al*., 1987).

nBA (CAS No. 141-32-2): After oral administration by gavage, butyl [2,3-^{14}C]-acrylate was rapidly absorbed and metabolised in male Fischer 344 rats, 75% of the initial dose was eliminated as CO_2, approximately 10% via urine and 2% via faeces). The major portion of n-butyl acrylate (nBA) was hydrolysed by carboxy esterase to **acrylic acid** and **n-butanol** and then eliminated as CO_2. A smaller portion was conjugated with endogenous GSH to be subsequently excreted as mercapturic acids in

the urine (Sanders, 1988). After i. v. administration, the labelled nBA was rapidly absorbed and metabolised. The acrylate moiety was metabolised primarily to **CO_2**, accounting for elimination of up to 45% of the administered radiolabel. The second major route of elimination was in urine, with only trace amounts in faeces and as volatiles (Sanders, 1988). No parent compound was detected in any of the urine, bile, or tissue extract samples by HPLC analysis. The two major metabolites in urine after both oral and intravenous routes of exposure were identified as **N-acetyl-S-(2-carboxyethyl)cysteine** and **N-acetyl-S-(2-carboxyethyl)cysteine-S-oxide** (Sanders, 1988). Thus, after oral and i. v. administration, nBA is rapidly absorbed and metabolised in male rats. The major portion of nBA was hydrolysed by carboxy esterase to **acrylic acid** and **n-butanol**. The subsequent metabolism follows that for acrylic acid and involves metabolism to CO_2 via the propionate degradation pathway (**acrylic acid → 3-hydroxypropionic acid → malonyl semialdehyde → acetyl S CoA → tricarboxylic acid cycle → CO_2**). Metabolism of **n-butanol** proceeds via the **alcohol** and **aldehyde dehydrogenase** pathway. A smaller portion of the administered nBA was conjugated with endogenous GSH to be subsequently excreted as **mercapturic acid derivatives** in the urine.

2EHA (CAS No. 103-11-7): The substance is rapidly and extensively absorbed, distributed and eliminated after oral administration. Studies on rats have indicated that short-chain acrylate esters such as 2EHA undergo carboxylesterase-catalysed hydrolysis to **acrylic acid** and **2-ethylhexanol**. The acrylic acid is decarboxylated and degraded to **carbon dioxide** (EC, 2005; OECD, 2003). In a recent *in vivo* comparative toxicokinetic study (ARTF, 2017f), **no detectable ^{14}C-2EHA levels were found in any C_{max} (0.17hr), $_{1/2}C_{max}$ (1 hr) or $_{1/5}C_{max}$ (12 hr) blood samples. ^{14}C-2EH was the only major metabolite observed in all C_{max} or $_{1/2}$Cmax blood samples.** In this study, a group of three male F344/DuCrl rats were administered ^{14}C-ladiolabelled 2EHA and its expected metabolite 2-ethylhexanol (2EH) at a single dose level of 100 or 70.6 mg/kg bw in propylene glycol, respectively, via gavage. Blood sample was collected from animals at 0.08, 0.17, 0.25, 0.5, 1, 2, 3, 6, 12, and 24-hour as well as every 24 hours post-dosing and thereafter up to the study termination (seven days post-dosing). Based on the blood time courses of each exposure group, C_{max}, ½C_{max} and 1/5C_{max} time points were selected at 0.17h, 1h and 12h, respectively. Following the determination of the respective C_{max}, additional groups of male rats were administered 2EHA and 2EH at a single dose level of 100 or 70.6 mg/kg bw in propylene glycol, respectively, and blood samples were collected at the determined C_{max}, $_{1/2}C_{max}$ and $_{1/5}C_{max}$ and processed for clinical chemistry analysis. The area under the curve (AUC) were identified as 249.47 µg h/g (C_{max}=18.67 ug/g, t_{max}=0.28 hour) for 2EHA and 151.67 µg h/g (C_{max}=27.14 ug/g, t_{max}=0.25 hour) for 2EH. Blood concentrations of ^{14}C-2EHA or ^{14}C-2EH equivalents were detectable over the entire study collection interval of 0.08 to 168 hours post-dosing. Blood time courses from both 2EHA and 2EH were similar, with enterohepatic recirculation being observed for both substances. This indicates that 2EHA and 2EH have similar pharmacokinetic profiles in rats after a single oral gavage dose. Half-life time ($t_{1/2}$) for absorption and elimination were also similar (

Table 5), but T_{max} and C_{max} showed variability amongst animals at earlier time points, especially for 2EHA. As the ^{14}C-labelled position in 2EHA was in the 2-ethylhexyl group (the same position as in ^{14}C-labelled 2EH), the similarity of pharmacokinetic parameters from both 2EHA and 2EH indicated that 2EHA was quickly hydrolysed to 2EH in rats after oral gavage dosage and resulted in showing a similar pharmacokinetic profile to 2EH.

Table 5 - Kinetic parameters of 2EHA and 2EH equivalents in rat blood following a single oral dose of ^{14}C-2EHA or ^{14}C-2EH (ARTF, 2017f)

Test Substance	2EHA		2EH	
Pharmacokinetic Parameters (Based on measured 2EH labelled radioactivity)	**Mean**	**S.D.**	**Mean**	**S.D.**
t_{max} (h) estimated from 1st peak	0.28	0.21	0.25	0.22
C_{max} (µg/g) estimated from 1st peak	18.67	24.04	27.14	8.92
t_{max} (h) estimated from 2nd peak[#1]	6.00	0.00	6.00	0.00
C_{max} (µg/g) estimated from 2nd peak[#1]	6.42	0.57	4.47	0.32
Absorption $t_{½}$ (h)	2.83	0.37	2.56	1.55
Elimination $t_{½}\alpha$ (h)	6.23	0.65	5.53	0.39
Elimination $t_{½}\beta$ (h)	65.08	2.81	64.47	3.05
AUC_{0-168} (µg h g^{-1})	249.47	10.79	151.67	14.06

[#1] Peak and Cmax resulted from enterohepatic circulation.

Similar mass balance (urine, faeces and CO_2), and metabolite profiles from C_{max} (0.17 hr), $_{1/2}C_{max}$ (1 hr) or $_{1/5}C_{max}$ (12 hr) blood samples from rats administered ^{14}C2EHA or ^{14}C2EH were observed for EHA and 2EH. The total average recoveries from both 2EHA and 2EH are similar (94% for 2EHA and 96% for 2EH), with the corresponding recoveries from urine, faeces and CO_2 trapping solution at each collection time point are very similar for both substances; the mean recovery of 2EHA in urine, faeces and CO_2 was 59.37%, 20.94% and 12.59%, respectively; and that for 2EH was 65.71%, 17.28% and 10.59%, respectively. Especially, majority of radioactivity of CO_2 which was recovered in the first 24 hours post-dosing are similar for both 2EHA and 2EH, supporting this hypothesis that 2EHA was hydrolysed to form 2EH, which was further metabolised to radioactive CO_2. No detectable ^{14}C-2EHA levels were found in any C_{max} (0.17hr), $_{1/2}C_{max}$ (1 hr) or $_{1/5}C_{max}$ (12 hr) blood samples. ^{14}C-2EH was the only major metabolite observed in all C_{max} or $_{1/2}$Cmax blood samples. These study results support a common metabolic pathway with 2EH after oral gavage administration of 2EHA or 2EH in rats.

In a recent *in vivo* comparative study in male F344/DuCrl rats, MA, EA, nBA and 2EHA were dosed at the level equivalent to 0.2 mmol/kg bw in corn oil by gavage (ARTF, 2017e). Approximately 3-hours after the administration of test materials, a timepoint optimised from previous studies with EA, the forestomach was excised at necropsy and the concentrations of glutathione (GSH) and glutathione disulphide (GSSG) were determined. The control animals received corn oil only. The results showed a treatment-related GSH-depleting potency in rat forestomach in the following order: MA > EA > 2EHA > nBA, showing a trend for a decreased GSH depletion level with increasing chain length (**Error! Reference source not found.**). The GSSG concentration was also decreased in the same order as per GSH depletion. The depletion of GSH in the forestomach following a single administration of EA was also reported in male F344 rats at 50 mg/kg bw resulting in 63-84% reduction (Udinsky and Frederick, 1994).

Table 6- GSH reactivity of acrylate esters (ARTF, 2017e)

Substance	Dose level		No. of animals	GSH		GSSG		
	mg/kg	mmol/kg		ug/g tissue	% reduction	ug/g tissue	% reduction	GSH:GSSG
Control	0	0	5	527.5 ± 108.6	-	9.4 ± 1.6	-	56.3 ± 5.5
MA	17.2	0.2	5	221.9 ± 12.7	57.9	5.8 ± 1.0	38.1	39.3 ± 7.7
EA	20.0	0.2	5	295.4 ± 114.8	42.9	6.9 ± 2.1	26.0	42.5 ± 4.6
nBA	25.6	0.2	5	535.6 ± 136.7	1.5	8.7 ± 1.8	7.5	61.6 ± 5.3
2EHA	36.8	0.2	5	494.2 ± 9.8	6.3	8.3 ± 1.0	11.7	60.3 ± 7.2

The depletion of GSH in the forestomach following a single administration of EA, at the dose levels of 0, 20, 50 and 100 mg/kg bw, was also reported in male C57BL/6 mice (ARTF, 2017d). Based on the analysis that was conducted approximately three hours after the dosing via oral gavage, the levels of GSH and GSSG were substantially decreased in a dose-dependent manner. The GSH depletion, relative to the concurrent vehicle control, was 52.7, 63.6 and 71.7% at 20, 50 and 100 mg/kg groups, respectively. The GSSG reduction, relative to the control, was 64.8, 76.8 and 81.3% at 20, 50 and 100 mg/kg groups, respectively. As per the finding in male F344/DuCrl rats (ARTF, 2017e), the GSH:GSSG ratio was not appreciably changed in the treated animals.

In vitro **hydrolysis studies**

The acrylate esters were found to disappear rapidly in rat whole blood *in vitro;* the $t_{½}$ was 3.6, 4.6, and 7.1 minutes for disappearance of methyl, ethyl, and butyl acrylate, respectively (Miller *et al.*, 1979). Subsequent studies demonstrated that AA was quite stable in rat blood as well as in rat liver, kidney and lung homogenates *in vitro (*Miller *et al*., 1981a). EA disappeared in tissue homogenates *in vitro*; the rate of hydrolysis was ~20 times greater in liver homogenates than in kidney or lung homogenates. Similar results were obtained for MA.

The ester hydrolysis was examined *in vitro* in rat liver S9 and rat plasma for the lower acrylate esters, showing a fast hydrolysis especially for the linear alkyl acrylate, but to a lesser extent to the tertiary structure (BASF SE, 2017b; Roos, 2015). The *in vitro* metabolism of acrylate esters showed a fast esterase cleavage within the first 10 minutes of incubation, with a parallel increase of acrylic acid after incubation with S9 fraction of rat liver for MA, EA, nBA, iBA and 2EHA (Table 7 -). The $t_{½}$ was 0.84 min for nBA and 1.4 min for EA. For tBA, the acid formation was so low in the culture that the decrease of the test substances stagnated after 10 to 30 minutes of incubation time. The metabolic turn-over of tBA however is slower compared to other acrylate esters, which is probably due to the steric hindrance caused by the tertiary structure of the side chain (BASF SE, 2017b). In plasma, the disappearance of acrylate esters was a factor of 10 slower compared to that in S9 fraction and much lower concentrations or no AA was produced in the plasma during the degradation process of acrylate esters. No half-lives could be determined for EA and tBA in the plasma. The acid formation was so low in the culture that the decrease of the test substances stagnated after 10 to 30 minutes of incubation time. The tertiary compounds showed only a small conversion in plasma, as in the S9 fraction. Overall, a clear association was observed between the stability of substrates for hydrolysis and the presence of the tertiary structure of the side chain. The degradation pattern of nBA and iBA was similar. The amount of acrylate esters decreased steadily and were completely diminished within five minutes in the rat S9 fraction while the formation of AA increased and its concentration exceeded that of acrylate esters within 1 – 2 minutes. In the plasma, acrylate esters decreased steadily and completely diminished within 30 minutes while the formation of AA

increased, and its concentration reached the level of acrylate esters within 10 minutes. Furthermore, hydrolysis of selected acrylate esters was also investigated in a recent *in vitro* assay (ARTF, 2018). A group of acrylate esters (i.e., MA, EA, nBA, iBA, tBA and 2EHA) was chosen for initial experimental determination of metabolism rates in rat liver microsomes and whole rat blood at a single substrate concentration of 500 μM. The incubation was performed in combination with the presence or absence of microsomes and NADPH. All acrylate esters except tBA were metabolised by rat liver microsomes in the presence or absence of NADPH to form AA (Table 8). Without microsomes, all acrylate esters were relatively stable under the incubation conditions, indicating the hydrolysis of acrylate esters was mainly catalysed by the enzymes contained in rat liver microsomes. The concentrations of the remaining acrylate esters, both measured concentrations and the back-calculated concentrations from the formation of AA, support the similarity between the microsomal incubations regardless of the presence of NADPH. This suggests that the hydrolysis of acrylate esters in rat liver microsomes is mainly due to the esterases which do not require NADPH for the enzymatic hydrolysis of acrylate esters. The tested acrylate esters (MA, EA, nBA, iBA, and 2EHA) have a half-life of less than 8.5 minutes (0.77-8.2 min) in the rat liver microsomes, indicating that metabolism is rapid. tBA was relatively stable under the same microsomal incubation conditions, probably due to the presence of steric hindrance due to its tertiary structure. The time-course of the remaining acrylate esters, both measured and back-calculated values, showed a rapid metabolism of the acrylate esters with almost complete consumption of the acrylate esters within the culture. However, the concentrations of the formed AA were significantly lower in the rat blood compared to the microsomal culture. The half-lives for all acrylate esters, based on the measured concentrations of the remaining parent acrylate esters, were less than 12 minutes in rat blood, ranging 0.99 – 11.2 minutes. Overall, the rate of hydrolysis of the acrylate esters increased in the order butyl > ethyl > methyl. Both studies showed slower hydrolysis rates of acrylate esters in whole rat blood than in the rat liver microsomes.

Table 7 - *In vitro* half-life degradation data (Roos, 2015)

	CAS No.	S9 Rat ($t_{1/2}$ min.)	Plasma ($t_{1/2}$ min.)
MA	96-33-3	-	34.62
EA	140-88-5	1.40	-
nBA	141-32-2	0.84	8.45
iBA	106-63-8	0.74	8.15
tBA	1663-39-4	-	-
2EHA	103-11-7	1.15	6.48

Initial degradation half-life in rat S9 calculated based on AA formation

Table 8 - Kinetic values of acrylate esters in rat liver microsomes and in rat whole blood (ARTF, 2018)

Substance	Rat Liver Microsomal Incubation		Rat Blood Incubation		Hydrolysis Rate[1]		
	Half-Life (min)		Half-Life (min)		V_{max}[2]	V_{max} (nmol/min/mg)	Km (µM)[2]
	Parent	Based on AA formation	Parent	Based on AA formation			
MA	8.20	5.73	1.49	-	512[3]	216[3]	2002[3]
EA	1.87	1.80	2.29	-	410	410	1157
nBA	0.77	0.71	2.33	2.68	788	788	731
iBA	0.8	0.888	2.47	2.77	1188	1188	1293
tBA	-	-	7.37	-	-	-	-
2EHA	2.26	2.83	3.85	3.87	602	602	503

[1] In rat liver microsomes.
[2] Vmax and Km were calculated by GraphPad Prism based on the Michaelis-Menten kinetic model.
[3] Averaged values from both microsomal protein concentrations (0.1 and 0.5 mg/mL).

The *in vitro* evidence consistently suggests a rapid metabolism of acrylate esters, catalysed by hepatic enzymes for the linear alkyl acrylate but to a lesser extent to the tertiary structure, with both ways forming AA as the common primary metabolite responsible for the systemic effects of these acrylate esters. In rat liver, the enzymatic hydrolysis of acrylate esters did not appear to involve NADPH. The acrylate esters were metabolised by rat liver microsomes in the presence or absence of NADPH. The exception was tBA where the metabolism was slow due to the presence of steric hindrance.

5.3.2.1 Glutathione conjugation

The C=C double bond of acrylate esters makes these chemicals potential Michael acceptors capable of electrophilic attack of protein and other cellular macromolecules. This is the mode of action through which a wide range of toxicities including allergic contact dermatitis is thought to be mediated. This reactivity also means that acrylate esters are capable of conjugating with cellular GSH, as evidenced in the *in vitro* studies (ARTF, 2017d; ARTF, 2017e; ARTF, 2018; Udinsky and Frederick, 1994). The structural alerts generated using QSAR Toolbox v4.3 show the similarity in electrophilic reactivity for all the category members (Annex), with experimental confirmation in the ARTF, 2018 study. Following a rapid metabolism of acrylate esters to AA, electrophilic reactivity is no longer apparent. Some minor impact is exerted by the positive inductive effect of the alcohol sub-group, but the incremental impact on electrophilicity rapidly decreases with increasing alcohol chain length. Therefore, for direct electrophilic reactions the alcohol group will only have a minor, rather monotonic influence with increasing chain length. McCarthy *et al.* (1994) reported that increased alcohol chain length moderately affected the apparent second-order rate constant for the spontaneous reaction of acrylate esters with GSH in the *in vitro* study but did not affect potency relative to cellular GSH depletion. There is no structural alert for Michael addition reactivity for the alcohol metabolites (**Error! Reference source not found.**).

In a recent *in vitro* assay, a group of acrylate esters (MA, EA, BA, iBA, tBA and 2EHA) was individually incubated with tritiated- and non-tritiated glutathione in the presence or absence of GST at pH 7.4 for 60 minutes (ARTF, 2018). All acrylate esters can react with GSH in the presence of GST to form one major peak (Acrylate-SG adduct) in addition to the GSH peak. Under the same conditions, AA did not form GSH adduct. Overall, the rates of formation of the GSH conjugates in the presence of GST for all the acrylate esters were similar (ranging from 1.20 to 3.94 nmol/mg protein/min), suggesting the involvement of GST to conjugate GSH with the acrylate esters in the category.

Overall the *in vivo* and *in vitro* data provide convincing evidence which shows that the acrylate esters undergo GST-mediated conjugation with GSH in rodents. There may be a slight trend towards decreasing conjugation rates with increasing chain length or with a tertiary structure of the side chain, indicating some variation in the metabolism rate within the category.

5.3.2.2 Protein binding reactivity

Supporting information for the glutathione conjugation potential of the acrylate esters is available from the Direct Peptide Reactivity Activation Assay (DPRA, OECD TG 442c). In this assay, peptide depletion was demonstrated to be significant with all the tested acrylate esters (Table 10). While still being significant, the peptide depletion levels observed for 2EHA were lower than the other category members, again suggesting that side chain length and the tertiary structure has a modifying impact on the metabolism of the acrylate esters.

Table 9 - Peptide depletion in the DPRA

Substance	CAS no.	Mean Peptide Depletion	Cysteine Peptide Depletion	Lysine Peptide Depletion
MA	96-33-3	93 %	100 %	90.70 %
EA	140-88-5	95 %	100 %	90.20 %
nBA	141-32-2	95.80 %	100 %	91.70 %
tBA	1663-39-4	90.82 %	100 %	81.64 %
2EHA	103-11-7	60.50 %	100 %	20.90 %

Overall, the available toxicokinetic data for the category members show that the short-chain acrylate esters in the category are rapidly absorbed and metabolised to AA and the structurally corresponding alcohols by carboxylesterases or are eliminated by conjugation with GSH to form thioesters. With high local doses or under *in vitro* conditions, the glutathione has been demonstrated to result in GSH depletion. Significant GSH depletion was reported to be associated with the toxic response only at the site of gavage dosing of EA (Frederick *et al*., 1992), suggesting that the rapid detoxification of acrylate esters would prevent toxic responses occurring in tissues remote from the dosing site. In conclusion, all category members are readily absorbed and rapidly eliminated from the body. The available data for the category members clearly indicate that AA is the common metabolite of the ester hydrolysis of all category acrylate esters, and GSH depletion is the common mode of action through which site of contact toxicity may occur. There is therefore a strong justification for read-across between the category members in order to address relevant toxicological end-points.

5.4. Read Across

In chapter 5.3 the similarity of the Acrylates in the metabolism was justified (scenario 3). For all Category members the phys-chem- and environmental fate data are available which show similarity or a similar trend depending on the length and the branching of the side chain for the Acrylates. Additionally it can be shown that the different Category members have qualitatively similar properties (please see the spreadsheet in Annex I). For a lot of endpoints, for all members of the Category study, results are available (e.g. acute oral, acute inhalation, acute dermal, skin irritation, eye irritation, Ames test, algae and acute fish). Also, those anchor points show similarity or a similar trend depending on the length and the branching of the side chain for the Acrylates. Therefore, the read-across strategy for the endpoints with data-gap was based additionally to scenario 3 to scenario 4 - variations in the properties observed among source substances. Prediction based on a regular pattern or on a worst-case approach - or 6 – No relevant variations in properties observed among source substances and the same strength predicted for the target substance (please see executive summary on page 4) in accordance with the RAAF (ECHA, 2017). In the next chapters the read-across for each different toxicological and ecotoxicological endpoint is justified in detail.

Table 10 - Endpoints for which read-across is applied

	Endpoint	Exposure route	MA	EA	nBA	iBA	tBA	2EHA
Toxicological information	Sensitisation	Dermal			Source substance	Target substance with data-gap		
					Source substance		Target substance with data-gap	
	Repeated dose toxicity	Oral			Source substance	Target substance with data-gap		
		Inhalation			Source substance	Target substance with data-gap		
	In vitro mutagenicity in mammalian cells	N/A			Source substance	Target substance with data-gap		
	Reproductive toxicity	Oral			Source substance		Target substance with data-gap	
					Source substance			Target substance with data-gap
		Inhalation	Source substance	Target substance with data-gap				
			Source substance		Target substance with data-gap			
			Source substance		Source substance	Target substance with data-gap		
			Source substance				Target substance with data-gap	Source substance
			Source substance					Target substance with data-gap
	Developmental toxicity (rat)	Oral			Source substance	Target substance with data-gap		
					Source substance		Target substance with data-gap	
		Inhalation			Source substance	Target substance with data-gap		
				Source substance	Source substance		Target substance with data-gap	Source substance
	Developmental toxicity (rabbit)	Oral		Target substance with data-gap	Source substance			
					Source substance	Target substance with data-gap		
					Source substance		Target substance with data-gap	
					Source substance			Target substance with data-gap
		Inhalation	Source substance	Target substance with data-gap				
			Source substance		Target substance with data-gap			
			Source substance			Target substance with data-gap		
			Source substance				Target substance with data-gap	
			Source substance					Target substance with data-gap
Ecotoxicological information	Long-term toxicity testing on invertebrates	N/A	Target substance with data-gap	Source substance	Source substance			
				Source substance	Source substance	Target substance with data-gap		
				Source substance	Source substance		Target substance with data-gap	
				Source substance	Source substance			Target substance with data-gap
	*Short-term toxicity testing on invertebrates (marine)**	N/A	Source substance	Target substance with data-gap				
			Source substance		Target substance with data-gap			
			Source substance			Target substance with data-gap		
			Source substance				Target substance with data-gap	
			Source substance					Target substance with data-gap
	*Short-term toxicity testing on fish (marine)**	N/A	Source substance	Source substance	Source substance	Target substance with data-gap		
			Source substance	Source substance	Source substance		Target substance with data-gap	
			Source substance	Source substance	Source substance			Target substance with data-gap

* Read-across of information on endpoints written in italics is technically not required to address any REACH endpoint.
AA is not presented in the table since no read-across was applied for this substance. AA was not used as a source substance for any of the read-across applied within the category.

Target substance with data-gap
Source substance

5.4.1 Read-across justification for toxicological information

5.4.1.1 Skin sensitisation

Table 11 - Summary of skin sensitisation data

Substance	Method	Results	Reference
AA	Modified Maguire Method (guinea pig)	Negative	Rao *et al.*, 1981
	Freund's complete adjuvant test (guinea pig)	Negative	Van der Walle *et al.*, 1982
	Modified Freund's complete adjuvant test (guinea pig)	Negative	Waegemaekers *et al*. 1984
MA	OECD 429 LLNA (mouse)	Positive 19.6 %	Syngenta CTL, 2006a
EA	OECD 429 LLNA (mouse)	Positive 36.8 %	Syngenta CTL, 2006b
tBA	**Read across from nBA**	-	-
	Magnusson & Kligman Maximisation test (guinea pig)*	Positive	Van der Walle *et al.*, 1982
	Freund's complete adjuvant test (guinea pig)*	Positive	Van der Walle *et al.*, 1982
	OECD 442C (DPRA)*	High reactivity in the DPRA	BASF SE, 2017a
iBA	**Read across from nBA**	-	-
nBA	OECD 429 LLNA (mouse)	Positive 11.2 %	Syngenta CTL, 2006c
2EHA	OECD 429 LLNA (mouse)	Positive 18.96 %	Dow, 2017

* data utilised as supporting information only

Discussion

iBA and tBA

Due to a very similar physico-chemical properties between nBA, iBA and tBA (liquid, having the molecular weights of 128.2 g/mol with a similar log Pow ranging from 2.32 to 2.38, with water solubilities ranging from 1.7 g/L to 2.0 g/L), all three acrylate esters are expected to be absorbed via dermal route. They are all skin irritants hence dermal penetration may be enhanced due to the irritancy to some extent, although the substances are expected to be evaporated fast due to the high vapour pressures ranging from 5 to 20 hPa. A sign of dermal absorption is evidenced by the systemic effects observed in the acute dermal toxicity studies. The only structural difference between iBA/tBA and nBA is the presence of tertiary structure of the side chain.

All acrylate esters within the category have the EU harmonised classification for skin sensitisation Category 1. nBA has shown a skin sensitising potency in animals. In a mouse local lymph node assay (LLNA, conducted in 2006), an EC_3 value of 11.2% was derived based on the stimulation index of 0.8, 1.3, 1.4, 2.5 and 8.7 at 1, 2.5, 5, 10 and 25% w/v in acetone in olive oil (4:1). The study was conducted in accordance with the OECD test guideline 429 with the Klimisch score of 1. nBA is classified for skin sensitisation category 1B (self-classification) in the REACH registration dossier. nBA also showed positive reactions in the other supporting *in vivo* studies (Klimisch 2) including a mouse ear swelling test, guinea pig maximisation tests and Freund's complete adjuvant test. nBA was positive in a battery of *in chemico/in vitro* skin sensitisation assays. It was positive in the dendritic cell line activation assay myeloid U937 Skin Sensitization Test (MUSST; Klimisch 2, 2011), LuSens Assay (Klimisch 2, 2013) and in h-CLAT (Klimisch 2, 2013). The results of DPRA show a high protein binding reactivity for both nBA and iBA with the mean peptide depletion level of 95.8% and 90.82%, respectively (Table 10).

The similarity in the protein binding reactivity for the acrylate esters within the category are supported by the QSAR Toolbox v4.3 output (Annex), indicating that they have the structural alert for Michael addition on conjugated systems with electron withdrawing group, which is considered to be the molecular initiating event triggering the skin sensitisation reaction as seen for the acrylate esters within the category.

In the *in vitro* hydrolysis assay, nBA and iBA have shown a very similar half-life in both rat liver microsome and whole blood incubation systems (ARTF, 2018; BASF SE, 2017b; Roos, 2015). The assays also showed the similar metabolism patterns between nBA and iBA, whereas tBA was relatively stable under the same microsomal incubation conditions, probably due to the presence of steric hindrance. The half-life of tBA in the rat whole blood incubation system was longer than that of nBA and iBA, suggesting the differences in the metabolism rate in the *in vitro* systems. However, the variation in the metabolic rate between the target and source substances is not considered to give a significant impact on the prediction for skin sensitisation. This is because all the acrylate esters within the category are potent haptens linked to their structure (a double bond and carboxylic acid ester) without autoxidation or biotransformation.

Due to the similarity in the expected skin absorption and the structural reactivity, a positive skin sensitisation potency is also expected for iBA and tBA. The available evidence does not indicate the tertiary structure of the side chain of the acrylate esters would have a significant impact on the read-across approach for skin sensitisation. Indeed, nBA and iBA were categorised for hazard assessment in the OECD SIDS (OECD, 2002) and weight of evidence approach was applied to assess their toxicological properties.

Conclusion

The variable part of the category approach is the length or configuration of the side chain of the parent ester and the alcohol metabolite, as well as their impacts on physico-chemical properties and subsequent properties. Despite these variations, the available data support the similarity in skin sensitisation potency for all the acrylate esters within the category. MA, EA, nBA and 2EHA are skin sensitisers based on the LLNA, whereas AA is a non-sensitiser. Skin sensitisation potency of acrylate esters involves reaction with tissue nucleophiles via Michael addition on the electrophilic C of the a,β-unsaturated carboxyl group (Freidig *et al*., 1999; Greim *et al*., 1995; McCarthy *et al*., 1994; cited in Borak *et al*., 2011). The prototype for such reactions is conjugation with GSH, which occurs spontaneously and enzymatically, leading to formation of thioethers and mercapturic acids. Increased urinary excretion of thioethers and depletion of hepatocyte GSH have been documented following *in vivo* and *in vitro* exposures to acrylate esters (Delbressine *et al*., 1981; Elovaara *et al*., 1983; cited in Borak *et al*., 2011). The electrophilic reactivity of low-molecular-weight molecules, as reflected by their interactions with GSH and other nucleophiles, is an important aspect of their ability to act as sensitizers (Enoch *et al*., 2008, 2009, 2010; Roberts *et al*., 2007, 2008; Smith and Hotchkiss, 2001; cited in Borak *et al*., 2001). In skin sensitisation studies, a key early step in the process leading to sensitisation is the formation of covalent adducts with a carrier protein, thereby forming an antigenic hapten-protein complex (Natsch and Emter, 2008; Roberts *et al*., 2008; Roberts and Aptula, 2008; Smith and Hotchkiss, 2001; cited in Borak *et al*., 2011). The difference in the skin sensitisation potency between the acrylate esters and AA are due to the presence or absence of C=C double bond in their structures. Indeed, all the acrylate esters within the category are classified for skin sensitisation Category 1 (EU harmonised classification). A comparison of the LLNA results for acrylate esters within the category does not suggest a clear correlation between the side chain length and the level of skin sensitisation potency. However, the EC_3 values are all within the range to warrant the skin sensitisation Category 1B (weak sensitisers). There is a data gap for skin sensitisation for iBA and tBA, which is assessed by a category based read across from a reliable Local Lymph Node Assay of nBA (LLNA; OECD Guideline 429; 2006). Overall, the read across approach is applied with a high level of confidence.

In RAAF nomenclature, the read-across approach for this endpoint is described in scenario 4 (different compounds have qualitatively similar properties) and governed by AE 4.2 and 4.3 (common underlying mechanism, qualitative and quantitative aspects). Here, a common underlying mechanism is a direct electrophilic reaction of the intact ester.

5.4.2.2 Repeated dose toxicity

Table 12 - Summary of repeated dose toxicity studies via the oral route

Substance	Study design	Results	Reference
AA	90 day (rat) gavage	LOAEL 150 mg/kg bw/day (nominal) No NOAEL derived	BASF AG, 1987b
	90 day (rat) drinking water	LOAEL 250 mg/kg bw/day (nominal) NOAEL 83 mg/kg bw/day (nominal)	Bushy Run Research Center, 1980a
	1 year (rat) drinking water (equivalent to OECD 452)	LOAEL 100 mg/kg bw/day (male) (nominal) NOAEL 40 mg/kg bw/day (male), 375 mg/kg bw/day (female) (nominal)	BASF AG, 1987a
MA	90 day (rat) drinking water (equivalent to OECD 408)	LOAEL 20 mg/kg bw/day (nominal) NOAEL 5 mg/kg bw/day (nominal)	Dow Chemical, 1981a
EA	90 day (rat - male) gavage (equivalent to OECD 408)	LOAEL 20 mg/kg bw/day (nominal)	Rohm and Haas Company, 1987a
	90 day (rat) gavage (equivalent to OECD 408)	LOAEL 110 mg/kg bw/day (nominal) NOAEL 55 mg/kg bw/day (nominal)	NTP, 1986a
nBA	90 day (rat) drinking water (equivalent to OECD 408)	NOAEL 84 mg/kg bw/day (male), 111 mg/kg be/day (female) (nominal)	Dow Chemical, 1980a
iBA	No data	-	-
tBA	No data	-	-
2EHA	OECD 422 (rat) gavage	-in progress	-ARTF

Inhalation is the most appropriate route of administration based on exposure considerations.

Table 13 - Summary of repeated dose toxicity studies via the dermal route

Substance	Study Design	Results	Reference
AA	90 day (mouse) 3 days/week	No NOAEL identified; Skin irritation (4 % acrylic acid); No skin irritation (1 % acrylic acid)	BAMM, 1987
MA	No data		
EA	No data		
nBA	No data		
iBA	No data		
tBA	No data		
2EHA	90 day (mouse - male) 3 days/week	NOAEL 170 mg/kg bw/day (local) (nominal) Skin irritation (more severe in C3H than NMRI mice)	BASF AG, 1986

Inhalation is the most appropriate route of administration based on exposure considerations.

Table 14 - Summary of repeated dose toxicity studies via the inhalation route

Substance	Study Design	Results	Reference
AA	90 day (rat) whole body (vapour) 6 hours/day; 5 days/week (equivalent to OECD 413)	NOAEC 0.074 mg/L air (local), 0.221 mg/L air (systemic) (analytical)	Dow Chemical Company, 1979a
	90 day (mouse) whole body (vapour) 6 hours/day; 5 days/week (equivalent to OECD 413)	NOAEC: 0.221 mg/L air (male), 0.015 mg/L air (female) (systemic) (analytical) LOAEC 0.015 mg/L air (male/female) (local) (analytical)	Dow Chemical Company, 1979a
MA	90 day (rat) whole body (vapour) 6 hours/day; 5 days/week (equivalent to OECD 413)	LOAEC 0.44 mg/L air NOAEC 0.082 mg/L air	BASF AG, 1978a and 1980c
EA	6 month (rat) whole body (vapour) 6 hours/day; 5 days/week (equivalent to OECD 413)	LOAEC 0.31 mg/L air (systemic) NOAEC 0.1 mg/L air (local and systemic) (nominal)	Dow Chemical USA (1983a)
	24 month (rat) whole body (vapour) 6 hours/day; 5 days/week (equivalent to OECD 453)	NOAEC 0.02 mg/L air (local) (nominal)	Dow Chemical USA (1983b)
	24 month (mouse) whole body (vapour) 6 hours/day; 5 days/week (equivalent to OECD 453)	NOAEC 0.02 mg/L air (local) (nominal)	Dow Chemical USA (1983b)
	27 month (rat) whole body (vapour) 6 hours/day; 5 days/week (equivalent to OECD 453)	LOAEC 0.31 mg/L air (systemic), 0.1 mg/L air (local) NOAEC 0.1 mg/L air (systemic) (nominal)	Dow Chemical USA (1983a)
	27 month (mouse) whole body (vapour) 6 hours/day; 5 days/week (equivalent to OECD 453)	LOAEC 0.31 mg/L air (systemic), 0.1 mg/L air (local) NOAEC 0.1 mg/L air (systemic) (nominal)	Dow Chemical USA (1983c)
nBA	90 day (rat) (vapour) 6 hours/day; 5 days/week (equivalent to OECD 413)	LOAEC 1.11 mg/L air (systemic), 0.57 mg/L air (local) (analytical) NOAEC 0.57 mg/L air (systemic), 0.11 mg/L air (local) (analytical)	BASF AG, 1979b
iBA	ND		
tBA	90 day (rat) (vapour) 6 hours/day; 5 days/week combined OECD 413 and 422)	NOAEC: 0.319 mg/L air (nominal)	BASF AG, 2004a
2EHA	90 day (rat) whole body (vapour) 6 hours/day; 5 days/week (equivalent to OECD 413)	LOAEC 0.753 mg/L air (systemic), 0.226 mg/L air (local) (nominal) NOAEC 0.226 mg/L air (systemic), 0.075 mg/L air (local) (nominal)	BASF AG, 1989b
	90 day (rat) whole body (vapour) 6 hours/day; 5 days/week (equivalent to OECD 413)	NOAEC: > 0.75 mg/L air (nominal)	BASF AG, 1989c

Discussion

Repeated dose toxicity studies are available for all the category members in rats and/or mice for oral, inhalation or dermal routes except for iBA. The primary adverse effects observed in these studies are irritating effects to nasal and respiratory mucosa. No systemic effects were observed at non-irritating concentrations/doses. Repeated oral and inhalation exposure of AA to rats and mice resulted in dose related local effects. Gavage administration over 90 days revealed dose-dependent mortality, irritation and ulceration of the stomach, and renal tubular necrosis in rats (LOAEL 150 mg/kg bw/d). No specific toxic effects were noted in subchronic and chronic drinking water studies. Reduced palatability (decreased water consumption) and unspecific signs of toxicity (decreased food consumption, body weight gain) at dosages >2000 ppm (100 mg/kg bw/d in male rats, 150 mg/kg bw/d in females) were observed. In a 90-day inhalation study, AA induced degenerative lesions on the olfactory mucosa in mice at 5 ppm (0.015 mg/L) and in rats at 75 ppm (0.221 mg/L) (OECD, 2001). In repeated-dose studies for MA, the main effects observed following inhalation exposure were irritation of the respiratory tract and mucous membranes. Systemic effects were mainly associated with changes in body weights and organ weights (OECD, 2003). Repeated-dose studies of EA confirm the irritant properties of ethyl acrylate with localised irritation, often severe, occurring at the site of contact for oral dosing, including forestomach tumors following chronic gavage dosing, and metaplasia or atrophy of the olfactory epithelium following inhalation exposure at concentrations greater than 5 ppm (0.02 mg/L). Repeated dose studies indicate that systemic toxicity, manifested primarily as body weight reduction, from oral or inhalation exposure to ethyl acrylate for periods up to 2 years, is minimal. No systemic toxicity was observed in oral (gavage or drinking water) studies below approximately 100 mg/kg/day for 90 days or 2 years (OECD, 2004). After repeated inhalation exposure to nBA, irritating effects to nasal and respiratory mucosa and the eyes predominate. No other primary systemic toxicity was observed in inhalation or oral studies (OECD, 2002). In a combined 90-day repeated dose toxicity and reproductive toxicity study in rats, the inhalation of 180 ppm tBA vapours (equivalent to 0.956 mg/L) caused slight irritation of the eyes and upper respiratory tract, retarded body weight development, mild impairment of renal function, a reduced general health status and two deaths during gestation (ECHA, 2019b). In a subchronic inhalation toxicity study, of 2EHA a NOAEC of 0.075 mg/L (10 ppm) was determined in rats for local effects (degeneration of the olfactory epithelial layer in the cranial part of the nasal cavity) (ECHA, 2019d).

iBA

iBA has not been tested in repeated dose studies. Therefore, a category-based approach to read-across is applied to fill the data-gap. The prediction of toxicity is made based on the 90-day sub-chronic study in rats via drinking water and 90-day inhalation study in rats, both of which were conducted for a close analogue nBA. The primary adverse effects observed in these studies are irritating effects to nasal and respiratory mucosa. No systemic effects were observed at non-irritating concentrations/doses. A lack of systemic toxicity is demonstrated for iBA in the acute studies. Following acute exposure, both iBA and nBA exhibit low toxicity (OECD, 2002). iBA has an oral LD_{50} of 4895 mg/kg bw (rat), an inhalation LC_{50} (4-hour, rat) of 10.5 mg/L and a dermal LD_{50} of 793 mg/kg bw (rabbit, occlusive). nBA has an oral LD_{50}s of 3143 mg/kg bw (rats) and 9050 mg/kg bw (male rats), an inhalation LC_{50} (4-hour, rat) of 10.3 mg/L and a dermal LD_{50} (rabbit) of 2000 to 3024 mg/kg.

Due to the similarity in physico-chemical properties between iBA and nBA (liquid, having the molecular weights of 128.2 g/mol with log Pow 2.38 at 25°C, with water solubilities ranging from 1.7 g/L to 1.8 g/L 25°C and vapour pressures ranging from 5 – 10 hPa at 25°C), their toxicokinetic profiles are expected to be similar. The only structural difference between iBA and nBA is the presence of iso structure of the side chain of iBA. The *in vitro* hydrolysis assay showed very similar half-lives for iBA and nBA in both rat liver microsome and whole blood incubation systems (ARTF, 2018; BASF SE, 2017b; Roos, 2015). The assays also showed

similar metabolism patterns, they are metabolised by rat liver microsomes in the presence or absence of NADPH to form acrylic acid. Without microsomes, both iBA and nBA were stable under the incubation conditions, indicating the hydrolysis of acrylate esters was mainly catalysed by the enzymes contained in rat liver microsomes.

All the acrylate esters in this category including nBA and iBA are highly similar regarding *in silico* toxicodynamics. The structural alerts using the QSAR Toolbox showed a similarity in protein binding reactivity (Michael addition), with a low level of toxicity with Cramer Class I. None of the category substances, including AA, are flagged for potential receptor binders (Annex).

The similar toxicological properties between nBA and iBA is supported by the structural similarity and associated alerts, a lack of systemic toxicity and the similarity in metabolism. There are no indications that the iso structure of the side chain of iBA would have a significant impact on the read-across strategy, evidenced by a very similar hydrolysis and GSH depletion rates. This supports the read-across between nBA and iBA from the toxicokinetic and toxicodynamic perspectives.

There are no signs of adverse systemic toxicity in the repeated dosing regimens for the alcohol metabolites of iBA (iso-butanol). In a 90-day drinking water study, no significant differences were seen in the dosed rats except the testicular findings in two male animals of the high dose group that were judged as incidental and not related to the test substance. In a 90-day gavage study, clinical signs related to the treatment with 1000 mg/kg dose level included hypoactivity, ataxia, and salivation with a sign of recovery. Slight decreases in food consumption and body weight gains were noted in the first two weeks of dosing that were restricted to the 1000 mg/kg/day group. In a 13-week inhalation study, changes in haematology parameters were noted in the 2500 ppm female rats, however, their biological significance was viewed as questionable. Furthermore, the inhalation exposure of iso-butanol up to 2500 ppm (*ca.* 7.5 mg/L) did not cause any systemic toxicity in a 2-generation reproduction toxicity study in rats. An indication of systemic toxicity (ataxia and hypoactivity) was reported in a 13-week oral gavage study in rats for the alcohol metabolite of nBA (n-butanol) during the final six weeks of a study. This study was concluded not to be robust enough for hazard assessment (OECD, 2001). Whereas no CNS effects were observed in a metabolic precursor of n-butanol, butyl acetate. In a thirteen-week inhalation study, exposure to butyl acetate produced transient hypoactivity (during exposure only) at and above 7185 mg/m^3 along with decreased body weight and food consumption, but no post exposure neurotoxicity was observed up to the highest dose level of 14370 mg/m^3. A concurrent subchronic neurotoxicity study under the same exposure conditions showed no evidence of cumulative neurotoxicity based upon functional observational battery endpoints, quantitative motor activity, neuropathology and scheduled-controlled operant behavior endpoints with NOAEL of 2395 mg/m^3 for systemic effects in rats, and a NOAEL of 14370 mg/m^3 for post exposure neurotoxicity in rats (OECD, 2001). Overall, a lack of systemic toxicity observed for iso-butanol and n-butanol suggests that the potential to see systemic toxicity with iBA that is different from that predicted by the read-across approach is negligible.

Conclusion

The variable part of the category approach is the length and/or configuration of the side chain of the parent ester and the alcohol metabolite, as well as their impacts on physico-chemical properties and consequent biological properties. Despite these variations, the available data support a lack of systemic toxicity for all the category members across the tested species. The repeated dose toxicity studies are available for AA, MA, EA, nBA and 2EHA (OECD 422 study in progress) for the oral route. For the inhalation route, studies are available for all the category members except for iBA. The available repeated dose toxicity studies on the acrylate category members have dosing periods ranging from 28 days to 2 years. Results demonstrate similar effects in rats and mice via both the oral and inhalation routes. The most predominant effects are based on the irritant (local reactive) properties of this class of chemical, rather than on intrinsic potential to cause systemic toxicity. None of the studies available for the category members produced evidence of reproductive toxicity or carcinogenicity viewed as relevant to humans. These repeated dose toxicity studies have also reported a similar and common profile of target organs (i.e. a lack of systemic toxicity). Thus, the results of the collection of sub-chronic and chronic studies conducted on these substances are consistent and can be regarded as offering a true picture of repeated dose toxicity for the category. In order to fill the data-gap for iBA, a category based read-across is applied to the sub-chronic repeated dose toxicity studies available for oral and inhalation routes for nBA. Overall, the read-across approach is applied with a high level of confidence.

For this endpoint, the common primary metabolic pathway of the category members (i.e. common functional groups and rapid metabolism by ester cleavage leading to the common metabolite AA) is considered as the most relevant aspect of the category approach. Qualitatively, this aspect can be categorised as scenario 3 “(Bio) transformation to common compound(s)”, whereas AA is the toxicologically relevant metabolite for systemic effects.

5.4.2.3 Genetic toxicity

Table 15 - Summary of genetic toxicity data

CAS	79-10-7	96-33-3	140-88-5	141-32-2	106-63-8	1663-39-4	103-11-7
	Acrylic Acid	**Methyl acrylate**	**Ethyl acrylate**	**n-Butyl acrylate**	**Isobutyl acrylate**	**tert-Butyl acrylate**	**2-Ethylhexyl acrylate**
Bacterial reverse mutation assay	Negative (purity ca. 99.3 %) *[BASF, 1977]*	Negative (purity ca. 99.6 %) *[BASF, 1977]*	Negative *[Rohm&Haas, 1981]*	Negative (purity ca.99.6 %) *[BASF, 1977]*	Negative *[NTP. 1982]*	Negative (purity 99.6 %) *[BASF, 2002]*	Negative [NTP, 1985]
In vitro clastogenicity							
Chromosomal aberration test	Positive *[Celanese, 1986]*	Positive at > 60 % cytotoxicity *[Moore, 1989]*	Positive *[Moore, 1989]*	Negative *[Wiegand, 1989]*			Inconclusive [Dearfield, 1989]
Micronucleus test				Negative *[Wiegand, 1989]*			Negative [Dearfield, 1989; BAMM 2018]
In vitro mutagenicity							
HPRT	Negative (purity 99.92 %) *[BAMM, 1988]*	Negative *[Moore, 1989]*	Negative *[Moore, 1989]*			Negative (purity 99.83 %) *[BASF, 2014]*	Negative [UCC, 1980; BAMM, *2018]*
MLA (TK)	Positive *[Moore, 1988]*	Negative [BAMM, 2019] Positive *[Moore, 1988]*	Negative [BAMM, 2019] Positive *[Moore, 1988]*	Negative (purity 99.78 %) *[BASF, 2016]*			Positive *[Rohm&Haas, 1984; Dearfield, 1989]*
UDS	Negative *[BAMM, 1988]*						
In vivo genotoxicity							
Chromosomal aberration test	Negative (purity 99.8 %) *[Celanese, 1986]*		Negative *[Kligerman, 1991; NTP 1990]*	Negative (purity 99.5 %) *[BASF, 1978]*			Inconclusive *[Rohm&Haas, 1984]*
Micronucleus test		Negative *[Sofuni 1984; Hachiya, 1981]*	Negative (purity 98.5 %) [Ashby, 1989; Kligerman, 1991)		Negative (purity > 99.5 %) *[BASF, 2001]*	Negative (purity 99.69 %) *[BASF, 2001]*	
TGR (OECD TG 488)			Negative (purity 99.87 %) *[BAMM, 2015]*				
UDS							Negative *(purity 99.9 %) [BASF, 2002]*
Dominant lethal assay	Negative *[McCarthy, 1992]*						

Discussion

AA and all the acrylate esters within the category members are non-mutagenic in bacterial cells (i.e. Ames test).

AA and the acrylate esters were positive in some of the *in vitro* clastogenicity and mammalian gene mutation tests. However, the positive results were due to excessive cytotoxicity. AA was positive in the mouse lymphoma assay, small colonies were induced preferentially and therefore the *in vitro* mutagenic potential of AA seems to be associated with its clastogenicity.

MA was negative in gene mutation assays in mammalian cells (HGPRT and XPRT assays) but was positive in the mouse lymphoma TK mutation assay in the absence of metabolic activation. However, these positive results were observed at clearly cytotoxic concentrations (≤ 50% cell survival) and the majority of the mutant colonies were small colonies, suggesting that methyl acrylate acts via a clastogenic mechanism *in vitro*. Moreover, in a 2019 study, MA was demonstrated to be negative at the same test concentrations by avoiding the previously observed cytotoxicity with supplemental GSH. *In vivo*, methyl acrylate was negative in several mouse micronucleus assays.

For EA *in vitro* identical results were observed compared to methyl acrylate: negative HPRT assay, positive mouse lymphoma TK mutation assay with small mutant colonies. Again in a 2019 mouse lymphoma TK mutation assay, EA was demonstrated to be negative at the same test concentrations by avoiding the previously observed cytotoxicity with supplemental GSH. Several negative *in vivo* mouse micronucleus assays and chromosome aberration tests. In addition ethyl acrylate was tested in the in vivo gene mutation assay in *gpt* Delta Mice according to OECD TG 488. The mutant frequencies (6-thioguanine and Spi- selection) in the liver and stomach of all groups treated by oral gavage with ethyl acrylate were not increased; therefore ethyl acrylate was negative for genotoxicity in this test system.

nBA was negative in an *in vitro* micronucleus assay, a mammalian cell gene mutation assay (thymidine kinase (TK) locus and structural chromosome aberrations) (OECD TG 490) and in an *in vitro* UDS assay in Syrian hamster embryo fibroblasts. *In vivo*, n-butyl acrylate showed no genotoxic effects after vapor inhalation exposure in rats and hamsters in a chromosome aberration assay.

iBA was not clastogenic *in vivo* in a mouse micronucleus test.

tBA was not clastogenic *in vivo* in a mouse micronucleus test and not mutagenic in a HPRT test in V79 cells.

EHA did not induce gene mutations in a HGPRT test and was negative in a 2018 mouse lymphoma TK mutation assay. The *in vitro* chromosomal aberration test gave inconclusive results but this endpoint was shown to be negative in a 2018 in vitro micronucleus test. Inconclusive results were also obtained from the *in vivo* chromosomal aberration assay. The *in vivo* UDS assay was negative.

iBA

The similarity in physico-chemical properties supports the similarity in toxicokinetic properties and bioavailability between nBA and iBA. Furthermore, the common metabolic pathway was supported by the aforementioned *in vitro* hydrolysis assays, with very similar half-lives in rat liver microsomes and blood for these two substances as well as in in vitro tests showing a comparable conjugation with GSH. This provides evidence which supports the reason why they have similar mutagenicity profiles in the mammalian cell gene mutation assays. The genotoxicity profiles following in vivo exposure are expected to be similar between nBA and iBA, as evidenced by the negative *in vivo* chromosome aberration tests for

nBA and negative *in vivo* micronucleus test result for iBA. Both nBA and iBA are negative in the Ames tests.

The structural alerts for genotoxicity further support the similarity between nBA and iBA. The protein binding alters for chromosome aberration were flagged for all the category substances (Michael addition to alpha, beta - unsaturated carboxylic acids and esters) using QSAR Toolbox. No alerts were identified for *in vitro* mutagenicity (Ames test) or *in vivo* mutagenicity (Micronucleus) for any of the category members (Annex 2).

A lack of genotoxic concern is demonstrated for the alcohol metabolites of nBA and iBA (n-butanol and iso-butanol). An entire battery of negative *in vitro* tests and a negative *in vivo* micronucleus test indicate that n-butanol is not genotoxic (OECD, 2001). Iso-butanol was not genotoxic in the *in vitro* experiments using human, rodent, and bacterial cells nor in *in vivo* experiments in mice (ECHA, 2019c).

The available evidence indicates that the iso structure of the side chain of iBA does not have a significant impact on the read-across approach for genotoxicity. A weight of evidence-based conclusion is that none of the category members are classified as genotoxicants. Therefore, the likelihood that the mutagenicity of iBA cannot be reliably predicted based upon the proposed read-across approach is considered very low. This is also supported by data from an oral gavage transgenic rodent gene mutation assay (TG488) for EA that confirms that the mode of action for EA-induced forestomach tumours is via a secondary, non-DNA-reactive (i.e. non-genotoxic) mechanism.

Conclusion

For this endpoint, a data-gap filling is addressed by a category based read-across approach. In RAAF nomenclature, this approach is described in scenarios 4 (different compounds have qualitatively similar properties) and governed by AE 4.2 and 4.3 (common underlying mechanism, qualitative and quantitative aspects). Here, the common underlying mechanism is a direct electrophilic reaction of the intact ester. The variable part of the category approach is the length or configuration of the side chain of the parent ester and the alcohol metabolite and their impact on physico-chemical properties and consequent biological properties. Despite the variation, the available data support a weight of evidence-based conclusions that there is no genotoxicity concern for all the category members. There is a data gap for *in vitro* mutagenicity in mammalian cells for iBA, which is assessed by a direct read-across from a reliable mammalian cell gene mutation tests with nBA (OECD 490; 2016). nBA is considered to be a close analogue within the category without a tertiary structure. Overall, this read-across approach is applied with a high level of confidence.

5.4.2.4 Carcinogenicity

Table 16 - Summary of available data on carcinogenicity

Substance	AA	MA	EA	nBA	iBA	tBA	2EHA
Oral	Negative (rat) 26-28 months Drinking water NOAEL ≥ 78 mg/kg bw/day (OECD 451) (BASF AG, 1989a, BASF AG, 1993c, Hellwig J et al., 1993)	No data	Negative (rat) 2 years (drinking water) NOAEL ≥ 150 mg/kg bw/day (no guideline) (Borzelleca JF et al., 1964) --- Neoplastic effects (rat, stomach) 103 weeks (gavage) No NOAEL identified (similar to OECD 451) (NTP, 1986a) --- Neoplastic effects (mouse, stomach) 103 weeks (gavage) No NOAEL identified (similar to OECD 451) (NTP 1986a)	No data	No data	No data	No data
Inhalation	No data	Negative (rat) 2 year (whole body, vapour) NOAEC ≥ 0.519 mg/L (similar to OECD 453) (BASF AG, 1985a)	Negative (rat) 27 months (whole body, vapour) NOAEC ≥ 0.92 mg/L (similar to OECD 453) (IATG, 1983c,d) --- Negative (mouse) 27 months (whole body, vapour) NOAEC ≥ 0.92 mg/L (similar to OECD 453) (IATG, 1983c,d)	Negative (rat) 2 year (whole body, vapour) NOAEC ≥ 0.773 mg/L (similar to OECD 453) (Inbifo, 1985a,b)	No data	No data	No data

Table 17 - Summary of available data on carcinogenicity (cont.)

Substance	AA	MA	EA	nBA	iBA	tBA	2EHA
Dermal	Increased incidence of lymphosarcoma (C3H females) (mouse) 21 months (3 times/ week) No NOAEL identified (no guideline) (BAMM, 1990e) ---------- Negative (mouse) 21 months (3 times/ week) NOAEL ≥ 52 mg/kg bw/day (no guideline) (BAMM, 1990e) ---------- Negative (mouse, male) Entire lifetime (3 times/ week) NOAEL ≥ 10 mg/kg bw/day (no guideline) (IATG, 1982a)	No data	Negative (mouse) 20 weeks (3 times/ week) NOAEL ≥ 3000 mg/kg bw/day (no guideline) (Nylander-French L. A. and French JE, 1998)	Negative (mouse) Entire lifetime (3 times/week) ≥ 8 mg/kg bw/day (no guideline) (IATG, 1982a)	No data	No data	Negative (mouse) 24 months (3 times/ week) NOAEL 919 mg/kg bw/day (BASF AG, 1992). ---------- Cell papillomas and squamous cell carcinomas at 750 mg/kg bw/day (C3H/HeJ male mice; study not reliable) Lifespan (3 times/ week), no NOAEL identified (Union Carbide Corp, 1979) ---------- Squamous cell carcinomas, melanocarcinomas and fibrosarcomas without any dose dependency (C3H/HeJ male mice) 2-year (3 times/ week) NOAEL = 24.8 mg/kg bw/day (study and model deficiencies) (BASF AG 1986, Wenzel-Hartung et al 1989).

Discussion

Carcinogenicity studies are available for the category members in rats and/or mice for oral, inhalation or dermal routes except for iBA and tBA. Overall, none of the category members are considered to be carcinogens following systemic exposure. Carcinogenicity studies for some of category members confirmed that tumour formation may be associated with localised irritation at the site of contact for dosing at dose levels in excess of the maximum tolerated dose (MTD). There is no evidence of tumour formation at a site distant from the route of entry for any of the acrylate esters. The forestomach carcinogenicity observed in the gavage studies with EA were concluded to be secondary to a site-specific and concentration-dependent irritating effect of the substance. This was supported by negative data from a transgenic rodent gene mutation assay (TG488). No EA-induced gene mutations were observed in the stomach, bone marrow or liver of gpt-delta mice exposed via oral gavage. This confirms that the mode of action for EA-induced forestomach tumours is via a secondary, non-DNA-reactive (i.e. non-genotoxic) mechanism. In a 2-year inhalation study in rats with MA, a dose-related degeneration of the olfactory epithelium (primarily the anterior portion) and a subsequent regeneration and replacement with respiratory epithelium was also observed at the highest dose level of 0.519 mg/L. However, no differences in the incidence of preneoplastic or neoplastic lesions were observed in this study. In a dermal mouse carcinogenicity study with 2EHA, skin tumours were induced that are considered to be associated with the highly irritative properties of 2EHA at dose levels in excess of the maximum tolerated dose (MTD). At a low concentration of 2.5% of 2EHA, a transient irritation was observed but this did not develop into any tumour formation. Other long-term studies with different mouse strains did not confirm tumour induction in mouse skin. Since none of the category members are considered to be genotoxic, and since EA has been confirmed not to induce gene mutation *in vivo*, it is concluded that the observed tumours at the site of contact are due to irritation and not a DNA-reactive mode of action. In conclusion, the available data support a lack of carcinogenicity associated with systemic exposure of the category across the tested species.

5.4.2.5 Toxicity for reproduction

5.4.2.5.1 Fertility

Table 18 - Summary of reproductive toxicity data - fertility

Substance	Study design	Results	Reference
AA	2-gen (OECD 416) rat Oral (drinking water)	NOAEL (general): P: 240 mg/kg bw/d F1: 53 mg/kg bw/d F2: 53 mg/kg bw/d NOAEL (fertility) P/F1: 460 mg/kg bw/d	BASF AG, 1994b
	1-gen (similar to OECD 415) rat Oral (drinking water)	NOAEL (general): P: 83 mg/kg bw/d F1: 250 mg/kg bw/d NOAEL (fertility) P: 250 mg/kg bw/d)	Inter-Company Acrylate Testing Group, 1980b
MA	2-gen (OECD 416) rat Inhalation (vapour, whole body)	NOAEC (general): P/F1: 0.019 mg/L NOAEC (fertility): P/F1: 0.268 mg/L NOAEC (development): F1/F2: 0.092 mg/L	Basic Acrylic Monomer Manufacturers, 2009
	13-wk, rat (oral) drinking water *12-wk, rat (inhalation)* *2-yr, rat (inhalation)*	*NOAEL 20 mg/kg bw/d* *NOAEC 2.24 mg/L* *NOAEC 0.52 mg/L*	*Dow Chemical, 1981a* *BASF AG 1978b and 1980c* *BASF AG, 1985a* *Reininghaus W, Koestner A and Klimisch H-J, 1991*

Table 19 - Summary of reproductive toxicity data – fertility (cont.)

Substance	Study design	Results	Reference
EA	*13-wk, rat* *Oral (gavage)* *27-month, rat (inhalation)*	*NOAEL 20 mg/kg bw/d* *NOAEC 0.92 mg/L*	*National Toxicology Programme (NTP), 1986a* *Dow Chemical USA, 1983a*
nBA	EOGRTS (OECD 443) rat Oral (gavage)	NOAEL (general): P/F1: 150 mg/kg bw/d NOAEL (fertility): P: 150 mg/kg bw/d	Acrylate Reach TF, 2017a
	13-wk, rat (inhalation)	*NOAEC 2.86 mg/L*	*BASF AG, 1979b*
iBA			
tBA	Screening (OECD 413/422) rat Inhalation (vapour, whole body)	NOAEC (general): P/F1: 0.319 mg/L NOAEC (fertility) P: 0.319 mg/L	BASF AG, 2004a
2EHA	OECD 422 Study rat (gavage)	In Progress	ARTF, TBD
	90-day, rat (inhalation)	*NOAEC ca. 0.75 mg/L*	BASF AG, 1989c

EOGRTS: Extended One Generation Reproductive Toxicity Study
Text in italics indicates results taken from non-reproductive toxicity studies

Discussion

Five members of the category have been tested in reproductive toxicity studies (AA, MA, nBA, tBA and 2-EHA (on-going)) conducted according to established study designs. Overall, the available systemic toxicity studies for the category indicate no concerns for reproductive and developmental toxicity. A lack of intrinsic reproductive and developmental toxicity is commonly observed for all category members in the rat, mouse and rabbit.

AA has been tested in a two-generation reproduction study via drinking water. The NOAEL for reproductive effects was 460 mg/kg bw/day (the highest dose tested), while the NOAEL with respect to general toxicity of AA was 240 mg/kg bw/day for the F0 generation parental animals and 53 mg/kg bw/day for the F1 and offspring. Clear signs of toxicity in the highest dose group in F0 and F1 parents were observed including reduced food and/or water consumption, impairment of body weight/body weight gain and gross and histopathological findings in the fore- and the glandular stomach. The observed effects are considered to be a consequence of the administration of an acid solution (indicative of the irritating properties of AA). The observation of impaired pup development is considered to be a secondary non-specific consequence of maternal toxicity.

A two-generation reproduction study has been conducted for MA in rats via inhalation route. In this study, the NOEC for parental systemic toxicity was determined to be 0.02 mg/L (5 ppm) and was based on histologic changes in the nasal tissues (severe degeneration and atrophy of the olfactory epithelium) seen at higher concentrations. The NOEC for developmental toxicity was 0.09 mg/L (25 ppm), based on decreases in pup body weight at 75 ppm which were concluded to be secondary to parental toxicity. The NOEC for reproductive toxicity was 0.27 mg/L (75 ppm), the highest concentration tested.

For nBA, an extended one generation study according to OECD 443 is available. Crl:CD(SD) rats were exposed to nBA at dose levels of 20, 50 and 150 mg/kg bw/day by oral (gavage). There was no evidence of reproductive toxicity at any dose level based on the evaluation of reproductive performance in the F0 generation, as well as the results of the sperm measurements and oestrous cyclicity in the F0 and F1 generations. Therefore, the NOAEL for F0 and F1 reproductive toxicity was concluded to be the highest dose level of 150 mg/kg/day. A 13-week inhalation toxicity study in rats did not give any evidence for any impairment of the investigated reproductive organs of both sexes up to the highest dose level of 546 ppm (2.86 mg/L). Evidence of systemic absorption included effects on organ weight, haematology and clinical chemistry parameters.

tBA was tested in a combined sub-chronic toxicity study and reproductive and developmental toxicity screening study in rats by inhalation (design based on OECD TG 413 and 422). In the high dose group of 956 mg/m^3 (180 ppm), tBA induced maternal toxicity including death of two females during gestation, which indicates that the maximum tolerated dose was exceeded. The effects were slight irritation of the eyes and upper respiratory tract, significantly retarded body weight development, mild impairment of renal function in the males and a reduced general state of health. Whereas the cohabitation and fertility of both male and female rats were not affected, substantially impaired pre- and post-natal development of the offspring was observed at this overtly maternally toxic concentration. No treatment-related effects were found in the male and female rats exposed to 106 mg/m^3 (20 ppm) and 319 mg/m^3 (60 ppm) of the test compound. Thus, the No Observed Adverse Effect Level (NOAEL) was concluded to be 319 mg/m^3 (60 ppm).

There is no reproductive toxicity study available for 2EHA (Study on-going). However, a lack of reproductive concern was demonstrated in a 90-day repeated dose inhalation toxicity study in rats. No treatment-related changes were observed for the testes weights as well as the gross and microscopic pathology for testes, seminal vesicles, ovaries, and uteri up to the highest dose level tested (0.750 mg/L).

There is a data gap for reproductive toxicity for EA, iBA, tBA and 2EHA for oral and/or inhalation routes and the read-across approach is applied within the category to predict their reproductive toxicity properties.

EA

For inhalation route, the 2-generation reproduction toxicity study in rats is available for MA. This study is used to predict the reproductive toxicity of EA and nBA for inhalation route. The only structural difference between MA and EA is the length of the carbon chain. Based on the physico-chemical properties, MA and EA are hydrophilic (the water solubility ranging from 1.7 – 60 g/L with log Pow of 0.74 – 2.38), which shows a clear correlation with the carbon chain length. The variation in the physico-chemical properties are not considered to have any consequence with respect to differences in toxicokinetics or bioavailability, as supported by their similar systemic toxicity profiles. As evidenced in the recent *in vitro* hydrolysis assays, EA has the shorter half-lives than MA in rat liver microsomes, with the lower GSH depletion levels in the rat forestomach (ARTF, 2017e; ARTF, 2018). A dose-dependent GSH depletion potency of EA was also confirmed in the male mice forestomach (ARTF, 2017d). The half-lives in rat blood were shown to be within a similar range, with a slower hydrolysis rate than seen in the rat liver microsomes. The findings from the hydrolysis assays suggest that the difference in the side chain length in the acrylate esters would have no significant consequence on the hydrolysis rate.

All the acrylate esters in this category are considered to be highly similar regarding *in silico* toxicodynamics. The structural alerts using the QSAR Toolbox showed a similarity in protein binding reactivity (Michael addition) with a low level of toxicity indicated by the assignment of Cramer Class I. None of the category substances, including AA, are flagged as potential receptor binders (Annex).

The repeated dose toxicity studies indicate that the systemic toxicity of the category members is manifested mainly as reduction in bodyweight gain. Overall, the systemic toxicity for EA and MA is considered to be minimal. The irritancy is confirmed in the repeated dose toxicity studies with localised irritation at the site of contact following oral and inhalation administration of EA. In the oral gavage 2-year carcinogenicity studies, EA induced marked local irritation and cellular proliferation which led to forestomach tumours at high concentrations in rats and mice. In 90-day and two-year inhalation studies in rats with nBA, the observed effects were primarily irritation of eyes and nasal mucosa and mortality associated with irritation of the respiratory tract, reduced bodyweight gain and changes in clinical chemistry parameters. In addition to the reduction in bodyweight gain, organ weight changes were also noted, and the severity of nasal mucosa effects

increased in a concentration-dependent manner in the two-year study. Localised irritation at the site of contact and minimal systemic toxicity were also observed in the repeated dose toxicity studies with MA and the common metabolite AA.

There are no reproductive and developmental toxicity concerns for the alcohol metabolites of EA and MA that are relevant for human (ethanol and methanol, respectively). No fertility or developmental effects of ethanol were seen at inhalation exposures up to 16000 ppm (30,400 mg/m^3) with the lowest reported NOAEL for fertility by the oral route of 2000 mg/kg bw in rats, equivalent to a blood alcohol concentration of 1320 mg/L. Most of the available studies use very high doses and few are individually robust enough to allow a NOAEL to be established. However, the collective weight of evidence is that the NOAEL for the developmental effects in animals is high, typically ≥ 6400mg/kg bw, which is much higher dose level compared to the maternally toxic dose level of 3600 mg/kg bw (OECD, 2004b). Methanol exhibits some reproductive and developmental effects depending on the tested species. Teratogenic effects are observed in the rodent studies. However, based on major species differences between humans and rodents (i.e. metabolic pathway/enzymes, mode of action, toxicokinetics), the overall weight of evidence along with the evaluation of reproductive toxicity provided by the Committee for Risk Assessment published in 2014 concluded that methanol does not appear to be toxic to reproduction (ECHA, 2019). Overall, a lack of toxicity for reproduction for ethanol and methanol suggests that any unknown reproductive and developmental toxicity of EA from the read-across approach for this endpoint is considered negligible.

Due to the similarity in the toxicity profiles across the acrylate category and for the corresponding alcohols, together with the rapid metabolism rates observed in the comparative hydrolysis assay, the uncertainties associated with the read-across based prediction of reproductive toxicity of EA are considered to be minimal.

iBA

There are no data available to directly assess reproductive toxicity of iBA. Therefore, a read-across approach is taken to predict the reproductive toxicity of this substance based on the extended one generation reproduction toxicity study with nBA and the 2-generation reproduction toxicity study in rats via inhalation route for MA. In the MA study, the only notable effect on the reproductive and developmental parameters is a decreased pup body weight at the highest dose level of 75 ppm (0.269 mg/L) that was concluded to be secondary non-specific consequences of maternal toxicity. A lack of potential for reproductive toxicity for nBA was demonstrated in the oral gavage extended one-generation study. Furthermore, in the 13-week inhalation toxicity study in rats for nBA (the analytical concentration levels of 0.11, 0.57, 1.11, 2.86 mg/L/day), there is no indication to suggest any impairment of the reproductive organs under the condition of this study. Although the study does not directly assess potential adverse effects of nBA on sexual function, fertility and teratogenicity, the results of the study supports no concerns for the reproductive organs for iBA via inhalation route. This study could be used as a bridging data to support the read-across between iBA and MA. Following acute exposure, both iBA and nBA exhibits low toxicity.

Due to a very similar physico-chemical properties between iBA and nBA, their toxicokinetic profiles are expected to be similar. The only structural difference between iBA and nBA is the presence of tertiary structure of the side chain of iBA.

All the acrylate esters in this category including nBA and iBA are considered to be highly similar regarding *in silico* toxicodynamics. The structural alerts using the QSAR Toolbox showed that none of the category substances, including AA, are flagged as potential receptor binders (Annex).

There are no reproductive and developmental toxicity concerns for the alcohol metabolites of iBA and nBA (iso-butanol and n-butanol, respectively). In the guideline compliant 2-generation reproductive toxicity study in rats via the inhalation route, iso-butanol up to the highest dose level of 7.5 mg/L did not cause any parental systemic, reproductive, or neonatal toxicity in both the F1 and F2 generations following exposure via whole-body exposure. In two GLP compliant developmental toxicity studies via inhalation route in rats and rabbits, no evidence of teratogenicity or foetotoxicity was reported up to the highest dose level of iso-butanol (10 mg/L). Based on the available rat studies, n-butanol is concluded not to be a reproductive toxicant (OECD, 2004a); n-butanol produced only mild foetotoxicity and developmental alterations at or near the maternally toxic (even lethal) dose of 8000 ppm (24000 mg/m³) throughout gestation. The alcohol metabolite of MA (methanol) exhibits some reproductive and developmental effects depending on the tested species. Teratogenic effects are observed in rodent studies. However, based on major species differences between humans and rodents (i.e. metabolic pathway/enzymes, mode of action, toxicokinetics), the overall weight of evidence along with the evaluation of reproductive toxicity provided by the Committee for Risk Assessment published in 2014, leads to a conclusion that methanol is not toxic to reproduction (ECHA, 2019a).

Due to the similarity in the toxicity profiles across the acrylate category and for the corresponding alcohols, together with the rapid metabolism rates observed in the comparative hydrolysis assay, the uncertainties associated with the read-across based prediction of reproductive toxicity of nBA are considered to be minimal. Since the similarity in the metabolism was demonstrated between nBA and iBA, it is logical to also support the read-across between nBA and iBA.

tBA

The available combined repeated dose inhalation toxicity study and reproductive and developmental toxicity screening test in rats suggests no reproductive toxicity concern for tBA. Lower foetal weights decreased number of liveborn, and an increased number of stillborn pups were observed at a dose level where significantly lower maternal body weight was also observed. Therefore, they are concluded to be secondary non-specific consequences of maternal toxicity.

The read-across approach is taken to predict the reproductive toxicity of the substance using the multi-generation studies available for the acrylate esters in the category. A lack of intrinsic reproductive and developmental toxicity is considered to be a common profile across the category members including AA, with irritancy-associated site of contact effects a common finding. The read-across strategy is applied for the oral gavage extended one generation study for nBA, supplemented by the reproductive organ data from the 90-day repeated dose inhalation toxicity study for nBA and 2EHA. In these studies there were no indications to suggest any impairment of reproductive function up to the highest dose levels tested (2.86 mg/L).

There is a difference in the structure between the target and source substances (side chain length and presence of the tertiary structure). However, they all have a common protein binding reactivity (i.e. Michael addition on conjugated systems with electron withdrawing group due to the carbon double bond), which further supports the prediction of similarity in systemic toxicity. tBA was found to be relatively stable in the *in vitro* hydrolysis assays, whereas a rapid hydrolysis was observed for the other acrylate esters in the category. nBA showed the most rapid metabolism in the rat liver microsomes.. Metabolism in rat whole blood was slower compared to that in rat liver microsomes, which was a common observation for the tested acrylate esters. The data indicate that ester cleavage is a common hydrolysis pathway within the category.

A lack of potential for reproductive toxicity for nBA was demonstrated in the oral gavage extended one-generation study and also in the combined repeated dose inhalation toxicity study for tBA. Although there appears to be a difference in metabolism rate between nBA and tBA, this did not have any consequence with respect to differences in reproductive toxicity.

There are no toxicity concerns for reproduction for the alcohol metabolite of tBA and nBA (tert-butanol and n-butanol, respectively). In the oral gavage reproduction / developmental toxicity screening test in rats (OECD 421), there were no adverse effects of tert-butanol on any reproductive parameters including mating index, fertility index, pregnancy index, or gestation index up to the highest dose level of 1000 mg/kg bw/day. A significant reduction in mean litter size, a decrease in the number of live born per pregnancy, an increase in the number of stillborn pups, increased pup mortality up to PND 4, and a decrease in mean pup body weight at birth which continued to weaning were observed at 1000 mg/kg bw/day at which the significant maternal toxicity was observed. In this study, the NOAEL for developmental/reproductive effects was 400 mg/kg bw/day. An inhalation developmental toxicity study was conducted for tert-butanol at exposure concentrations equivalent to 3098 mg/kg bw/day. An increased number of skeletal variations and visceral variations were reported at the higher exposure concentrations along with lower foetal bodyweight gain and clinical signs in dams at all dose levels. The NOAEC from this study was 1239 mg/kg bw/day, higher than the guideline recommended limit dose level of 1000 mg/kg bw/day. In two oral developmental toxicity studies in mice, a sign of delayed foetal development was noted at maternally toxic dose levels. No classification for reproductive toxicity was warranted for tert-butanol according to the REACH registration dossier (ECHA, 2019b). Based on the available rat studies, n-butanol is concluded not to be a reproductive toxicant (OECD, 2001); n-butanol produced only mild foetotoxicity and developmental alterations at or near the maternally toxic (even lethal) dose of 8000 ppm (24000 mg/m³) throughout gestation.

Overall, a lack of reproductive and developmental toxicity observed for tert-butanol and the alcohol metabolites derived from the source substances indicates that the uncertainties associated with the read-across based prediction for the reproductive toxicity of tBA are minimal.

2EHA

In the available 90-day sub-chronic inhalation toxicity study in rats for 2EHA, no adverse effects were observed in the testes weights, gross and microscopic pathology for testes, seminal vesicles, ovaries, and uteri up to the highest dose level of 0.753 mg/L. Although this study did not directly assess the effects on sexual function or fertility, the results showed no indication of a reproductive toxicity concern for 2EHA. Furthermore, no foetotoxicity or teratogenicity concerns were identified in the inhalation prenatal developmental toxicity in rats with 2EHA. An OECD 422 study is on-going. In order to fulfill the data requirement under REACH, the reproductive toxicity of 2EHA is predicted based on the reproductive toxicity studies available within the category, which is the oral gavage extended one generation study for nBA giving no indication of reproductive toxicity A lack of intrinsic reproductive toxicity is considered to be a common feature across the category members including AA. The similarity in the developmental toxicity profiles between 2EHA and nBA is demonstrated by the available OECD 414 studies.

The structural difference of 2EHA from the source substance is the presence of the tertiary structure of the side chain. However, they all have a common protein binding reactivity (i.e. Michael addition on conjugated systems with electron withdrawing group due to the carbon double bond), which further supporting the similarity in systemic toxicity. In the *in vitro* hydrolysis assays, a rapid hydrolysis was observed for nBA and 2EHA. nBA showed the most rapid metabolism in the rat liver microsomes and the formation of AA was commonly observed both in rat liver microsomes and blood test systems (ARTF, 2018; BASF SE, 2017b; Roos, 2015). Despite the presence of steric hindrance, 2EHA showed a rapid metabolism under the condition of the assays.

In a recent *in vivo* comparative study in rats, similar toxicokinetic profiles between 2EHA and 2-ethylhexanol were reported (ARTF, 2017f). There are no toxicity concerns for reproduction for the alcohol metabolite of 2EHA, 2-ethylhexanol. In a dietary developmental toxicity study in mice, 2-ethylhexanol showed no effects on any gestational parameters examined up to 191 mg/kg bw/day. In an oral gavage developmental toxicity study in rats, a sign of teratogenicity was noted

in foetuses from the highest dose dams (1300 mg/kg bw/day) where severe toxicity including mortality was observed. In a dermal developmental toxicity study in rats, 2-ethylhexanol had no adverse effect on the maternal gestational parameters, or maternal organ weights, foetal weight, sex ratio, viability, or the incidence of malformations and variations up to the highest dose level of 2520 mg/kg bw/day. In a rat inhalation study, no indication of toxicity for reproduction was observed at 850 mg/m^3. It is concluded that 2-ethylhexanol exhibited no adverse developmental effect in the absence of maternal toxicity (ECHA, 2019c).

Overall, the lack of reproductive and developmental toxicity observed for 2-ethylhexanol and the corresponding alcohol metabolites derive from the source substances indicates that the uncertainties associated with the read-across based prediction for the reproductive toxicity of 2EHA are minimal.

5.4.2.5.2 Developmental toxicity

Table 20 - Summary of reproductive toxicity data – developmental toxicity

Substance	Study Design	Results	Reference
AA	OECD 414 (rat) Inhalation (vapour, whole body)	NOAEC: maternal toxicity: 0.12 mg/L teratogenicity: ≥ 1.08 mg/L foetotoxicity: ≥ 1.08 mg/L	Inter-Company Acrylate Study Group, 1983a
	OECD 414 (rabbit) Inhalation (vapour, whole body)	NOAEC: maternal toxicity: 0.075 mg/L teratogenicity: ≥ 0.673 mg/L foetotoxicity: ≥ 0.673 mg/L	Basic Acrylic Monomer Manufacturers, 1993
MA	Similar to OECD 414 (rat) Inhalation (vapour, whole body)	NOAEC: maternal toxicity: *ca.* 0.089 mg/L teratogenicity: ≥ 0.357 mg/L foetotoxicity: 0.179 mg/L	Saillenfait AM *et al.*, 1999
	OECD 414 (rabbit) Inhalation (vapour, whole body)	NOAEC: maternal toxicity: 0.0553 mg/L teratogenicity: ≥ 0.1556 mg/L foetotoxicity ≥ 0.1556 mg/L	Acrylate Reach TF, 2009
EA	Similar to OECD 414 (rat) Inhalation (vapour, whole body)	NOAEC: maternal toxicity: 0.41 mg/L teratogenicity: ≥ 0.82 mg/L foetotoxicity: 0.41 mg/L	Saillenfait AM *et al.*, 1999
	Similar to OECD 414 (rat) Inhalation (vapour, whole body)	NOAEC: maternal toxicity: 0.21 mg/L teratogenicity: ≥ 0.62 mg/L foetotoxicity: ≥ 0.62 mg/L	IATG, 1980

Table 21 - Summary of reproductive toxicity data – developmental toxicity (cont.)

nBA	Similar to OECD 414 (rat) Inhalation, (vapour, whole body)	NOAEC: maternal toxicity: 0.13 mg/L developmental: 0.13 mg/L teratogenicity: 1.13 mg/L	BASF AG, 1979b
	Similar to OECD 414 (rat) Inhalation, (vapour, whole body)	NOAEC: maternal toxicity: not derived developmental: 0.52 mg/L teratogenicity: 1.57 mg/L	Saillenfait AM *et al.*, 1999
	OECD 443 (rat) Oral (gavage)	NOAEL: maternal toxicity: 150 mg/kg bw/d neonatal toxicity: 150 mg/kg bw/d	ARTF, 2017a
	OECD 414 (rabbit) Oral (gavage)	NOAEL: maternal toxicity: 400 mg/kg bw/d developmental: 400 mg/kg bw/d	ARTF, 2017b
	Similar to OECD 414 (mouse) Oral (gavage)	NOAEL: maternal toxicity: 100 mg/kg bw/d developmental: 1000 mg/kg bw/d teratogenicity: 2000 mg/kg bw/d	Rohm & Haas Co. 1979
iBA	No data		
tBA	Screening (OECD 422) rat Inhalation (vapour, whole body)	NOAEC: Maternal toxicity: 0.319 mg/L Developmental: 0.319 mg/L	BASF AG, 2004a
	OECD 414 (rat) Oral (gavage)	NOAEL: Maternal toxicity: ≥ 120 mg/kw bw/d Developmental: ≥ 120 mg/kg bw/d	Acrylate REACH TF, 2017c
2EHA	Similar to OECD 414 (rat) Inhalation (vapour, whole body)	NOAEC: maternal toxicity: 0.56 mg/L teratogenicity: ≥ 0.75 mg/L	Saillenfait AM *et al.*, 1999

Discussion

Developmental toxicity studies are available for at least two species for the category substances AA, MA and nBA and in rats for EA, tBA and 2EHA. No developmental toxicity studies are available with iBA. Therefore, a data gap was identified for the developmental toxicity in the rat for iBA as well as in the rabbits for EA, iBA, tBA and 2EHA. In addition to this, a read-across approach was applied for tBA to demonstrate similarity in developmental toxicity (i.e. a lack of concern) in the rat based on the oral and inhalation studies for EA, nBA and 2EHA. This supports the read-across strategy for a rabbit study.

No prenatal developmental toxicity was observed in rats and rabbits following inhalation exposure of AA. The overall weight of evidence based on the data on AA and several acrylate esters tested in the rat, mouse and rabbit gives no indications that the members of this class of chemicals are developmental toxicants. Developmental effects were only seen at concentrations/doses causing overt maternal toxicity indicating that they are highly likely to be secondary non-specific consequences of maternal stress.

MA, EA and nBA

There are no studies available for the prenatal developmental toxicity in rabbits for EA. A data-gap was filled by read across to two guideline prenatal developmental toxicity studies in rabbits that are available for the acrylate esters within the category (nBA and MA). An oral gavage developmental toxicity study with nBA showed no evidence of developmental toxicity up to the highest dose level of 400 mg/kg/day. At this dose level, a lower mean food consumption was noted during the entire treatment period (gestation days 7 - 29); however, the differences were not statistically significant and not of sufficient magnitude to affect mean body weights at this dosage. In a prenatal developmental toxicity study in rabbits, vapours of MA revealed no influence on sex distribution of the foetuses and foetal body weights up to the highest dose level of 0.1556 mg/L, at which a severe degeneration and atrophy of the olfactory epithelium were observed in the dams. The rabbit study for MA was also used for a read-across to nBA.

EA, MA and nBA showed no signs of foetotoxicity or teratogenicity in the rat via the inhalation route. A lower foetal body weights was observed following exposure to EA vapour at 0.82 mg/L at which a significant decrease in maternal bodyweight was observed throughout the exposure period. In another inhalation prenatal developmental toxicity study in rats, exposure to EA vapour resulted in a low incidence of malformed fetuses at 0.72 mg/L at which a significant maternal toxicity, including decreased body weight gain and good consumption, was also observed. Lower foetal body weights were observed following the dosing of MA at the highest concentration of 0.82 mg/L at which a significant decrease in maternal bodyweight was observed throughout the exposure period. Inhalation of 0.71 - 1.31 mg/L of nBA caused a significant reduction in maternal body weight gain and irritation to the nose and eyes. In this study, a dose-dependent increase in post-implantation loss was observed at 0.13 mg/L but this was not observed in another developmental toxicity study in rats up to the highest dose level of 1.57 mg/L. Similar to the observation in the rat study for MA, foetal body weight was significantly reduced at 1.05 – 1.57 mg/L of nBA at which a dose-dependent lower maternal weights were also observed. Furthermore, an oral gavage prenatal developmental toxicity study in mice with nBA showed no signs of developmental toxicity under the maternally toxic dose level up to 1000 mg/kg bw/day. Overall, a lack of developmental toxicity is observed in studies with EA, MA and nBA, which appears to be a common finding across the category substances.

All the acrylate esters in this category including EA, MA and nBA are considered to be very similar regarding *in silico* toxicodynamics. The structural alerts using the QSAR Toolbox showed that none of the category substances, including AA, are flagged as potential receptor binders (Annex).

The available reproductive and developmental toxicity information for the common metabolite AA and the alcohol metabolites of acrylate esters within the category show no concern of developmental toxicity as discussed in the section 5.4.2.5.1.

Due to the similarity in the systemic toxicity profiles and comparable rapid metabolism rates observed in the hydrolysis assay across the acrylate category and the lack of concern for the systemic toxicity of the corresponding alcohol metabolites, the uncertainties associated with the read-across based prediction for the reproductive toxicity of EA and nBA are minimal given the rapid metabolism rates observed in the comparative hydrolysis assay.

iBA

There are no data available to directly assess developmental toxicity of iBA. Therefore, a read-across approach is applied to predict the developmental toxicity of iBA based on the available developmental toxicity studies for nBA (the oral prenatal developmental toxicity study in mice, two inhalation prenatal developmental toxicity studies in rats and the oral prenatal developmental toxicity study in rabbits). No foetotoxicity or teratogenicity concern was identified in these

studies. Although excessively high dose levels were employed in the oral gavage developmental toxicity study in mice with nBA (up to 4000 mg/kg bw/day), the results of this study showed no developmental effects including morphological alterations at maternally toxic dose levels.

All the acrylate esters in this category are considered to be very similar regarding *in silico* toxicodynamics. The structural alerts using the QSAR Toolbox showed that none of the category substances, including AA, are flagged as potential receptor binders (Annex).

The available reproductive and developmental toxicity information for the common metabolite AA and the corresponding alcohol metabolites of acrylate esters within the category show no concern of developmental toxicity as discussed in the section 5.4.2.5.1.

Due to the similarity in the systemic toxicity profiles within the category and their corresponding alcohol metabolites, uncertainties associated with the read-across between MA and nBA are negligible. Moreover, since similarity in metabolism was demonstrated between nBA and iBA, it is logical to also support the read-across between MA and iBA.

tBA

A data gap was identified for the developmental toxicity in the rabbit. Therefore, a read-across approach is applied to predict the developmental toxicity of this substance based on the oral prenatal developmental toxicity study in mice and rabbits for nBA.

The available combined repeated dose inhalation toxicity and reproductive and developmental toxicity study (OECD 413/422) in rats indicates no reproductive and developmental toxicity concern for tBA. Lower foetal weights, decreased number of liveborn, and an increased number of stillborn pups were observed at a dose level where significantly lower maternal weight gain was also observed. Therefore, these developmental effects are concluded to be secondary non-specific consequences of maternal toxicity. Furthermore, the oral gavage prenatal developmental toxicity study in rats, a recent OECD 414 guideline compliant study, showed no indication of foetotoxicity or teratogenicity up to the highest dose level of 120 mg/kg bw/day. The only notable findings in this study was test substance-related microscopic findings in the non-glandular forestomach in all treated groups. Inhaled MA was not toxic to the embryo or foetuses, except at concentrations that produced overt maternal toxicity in a developmental toxicity study in rats. nBA showed no indication of developmental toxicity in the rat or rabbit. In the available inhalation prenatal developmental toxicity in rats. Overall, a lack of intrinsic reproductive and developmental toxicity is considered to be a common finding across the category members including AA, with irritancy-related site of contract effects seen as a common observation.

The available studies for the common metabolite AA and the corresponding alcohol metabolites of the acrylate esters within the category show no concerns for developmental and reproductive toxicity as discussed in the section 5.4.2.5.1.

Overall, a lack of reproductive and developmental toxicity observed for AA and the alcohol metabolites of source substances indicate that the likelihood of tBA causing reproductive toxicity not predicted by the proposed read-across approach is very low.

2EHA

A data gap was identified for the developmental toxicity in the rabbit. A read-across approach is applied to predict the developmental toxicity of this substance based on the oral prenatal developmental toxicity study in mice and rabbits with nBA as well as the inhalation study in rabbits with MA.

In the available inhalation prenatal developmental toxicity in rats, 2EHA showed no embryotoxic, teratogenic or foetotoxic effects up to the highest dose tested (0.75 mg/L). A

slightly lower non-statistically significant foetal bodyweight gain was judged to be secondary to maternal toxicity.

In the two inhalation prenatal developmental toxicity studies for rats, nBA showed no teratogenicity effects up to the dose levels of 0.13 mg/L. In the oral prenatal developmental toxicity study in mice, nBA showed no teratogenicity at the dose levels where no maternal toxicity was observed up to the 2000 mg/kg bw/day. Furthermore, in an oral gavage prenatal developmental toxicity in rabbits, nBA showed non-adverse lower mean body weight gains and corresponding lower mean food consumption at 400 mg/kg/day group. No evidence of developmental toxicity was noted up to the highest dose level of 400 mg/kg/day (ARTF 2017b). A similarity in metabolism pathway between rats and mice could be assumed for 2EHA based on the *in vivo* studies for EA (ARTF, 2017d; ARTF, 2017e), where a similar level of GSH depletion was demonstrated using the same study design.

The available studies for the common metabolite AA and the corresponding alcohol metabolites of acrylate esters within the category show no concern for reproductive and developmental toxicity as discussed in the section 5.4.2.5.1.

The likelihood of EHA causing reproductive toxicity not predicted by the proposed read-across approach is very low

Toxicity for reproduction conclusion

For this endpoint, the common primary metabolic pathway of the category members (i.e. common functional groups and rapid metabolism by ester cleavage leading to the common metabolite AA) is considered as the most relevant aspect of the category approach. Qualitatively, this aspect can be categorised as scenario 3 "(Bio) transformation to common compound(s)", whereas AA is the toxicologically relevant metabolite for local and systemic effects.

The variable part of the category approach is the length or configuration of the side chain of the parent ester and the alcohol metabolite and their impacts on physico-chemical properties and subsequent properties. Despite the variation, the available data support a lack of toxicity for reproduction for all the category members across the tested species. Overall, the read-across is applied with a high level of confidence.

5.4.2 Category justification for environmental fate

A summary of the relevant environmental fate properties is given in the table below. Read-across is not applied to any of the environmental fate endpoints. There are data available on all members of the category, for all of the required environmental endpoints. The data that are available are derived from studies of appropriate quality to warrant a high degree of reliability and accordingly, all have Klimisch ratings of 1 or 2. QSAR estimations have been utilised to address the hydrolysis endpoint (tBA, iBA), phototransformation in air, and to support the discussion on bioaccumulation.

Table 22 - Summary of environmental fate and the relevant physico-chemical properties

Substance (MW)	AA (72.1)	MA (86.1)	EA (100.1)	tBA (128.2)	iBA (128.2)	nBA (128.2)	2EHA (184.3)
Physico-chemical							
Vapour pressure [hPa]	5.29 (25 °C)	90 (20 °C)	40 (21 °C)	20 (23 °C)	10 (25 °C)	5 (22 °C)	0.24 (25 °C)
Henry's Law constant [Pam³/mol]	0.029	9.3	12.5	21.9	21.9	21.9	461
Water solubility [g/L]	1 000 (25 °C)	60 (25 °C)	20 (20 °C)	2 (20 °C)	1.8 (25 °C)	1.7 (20 °C)	0.01 (25 °C)
Partition coefficient (Log Pow)	0.46	0.74	1.18	2.32	2.38	2.38	4.00
Degradation							
Hydrolysis (DT_{50}) pH3 pH7 pH11	 > 1 yr > 1 yr > 1 yr	 > 28 d > 28 d 1.8 h	 > 1 yr > 1 yr 182 h	 - > 1 yr (QSAR) -	 - > 1 yr (QSAR) -	 > 1 yr > 1 yr 4.05 h	 22.2 d 8.75 d 18.5 h
Phototransformation in air (DT_{50}) (24-h day, 0.5^6 OH/cm³) (QSAR)	39.6 h	40.9 h	35.4 h	39.7 h	27.98 h	27.98 h	19.15 h
Biodegradation in water (screening) % degradation (day)	Readily biodegradable 95 (9 d) 81 (28 d)	Readily biodegradable 90-100 (28 d)	Readily biodegradable 80-90 (28 d)	Moderately biodegradable 59 (28 d)	Readily biodegradable 87 (28 d)	Readily biodegradable 80-90 (28 d)	Readily biodegradable 70-80 (15 d)
Environmental distribution							
Adsorption/Desorption Measured Koc Calculated Koc	 42.8 1.2	 NDA 6.4	 42.2 11.9	 NDA 26.1	 NDA 33.8	 88.4 35.4	 NDA 360
Mackay I calculation (%) Air Water Soil Sediment	 1.3 98.7 0.02 0.02	 81.9 18 0.01 0.01	 87.5 12.4 0.01 0.02	 97.8 2.1 0.1 0.1	 95.78 4.06 0.08 0.08	 94.55 5.24 0.1 0.1	 91 1.12 3.38 3.92
Bioaccumulation							
BCF (QSAR)	3.16	3.16	2.0	15.8	17.3	17.3	70

Discussion

Abiotic degradation

- Hydrolysis: Acrylic acid and the majority of acrylate esters are stable at neutral and acidic pH. Hydrolysis is therefore negligible and does not significantly contribute to the degradation of the substances under these pH conditions. The exception is 2-ethylhexyl acrylate (CAS No. 103-11-7) which has been shown to hydrolyse slowly in contact with water.

  Under alkaline conditions at pH 11, the acrylate esters hydrolyse. This is partly due to decreasing alkoxide stability during ester cleavage. The most common base hydrolysis mechanism is that of BAC2 which involves cleavage of the carbonyl - alkoxide oxygen bond to form the free alkoxide. The alkoxide formed is more basic the longer the chain length and, as stronger bases make poorer leaving groups, the rate of cleavage and hence hydrolysis is slower.

  In conclusion, hydrolysis is expected to play a role in the degradation of acrylate esters under alkaline conditions. For 2EHA, a slower hydrolysis also occurs at neutral and acidic pH.

- Photodegradation: The acrylate esters do not possess UV-absorbing structures. Therefore, direct photolysis is not expected to occur to any significant degree.

  There are no measured data available for any of the substances in the category. Half-lives for these reactions have been estimated using SRC AOP v1.92 and range from 19.15 h for 2EHA to 40.9 h for MA. The half-lives generally decrease with increasing chain length and molecular weight.

Biodegradation

Results from biodegradation studies on substances within the category consistently indicate a high potential for ready biodegradability, except for tert-butyl acrylate which, due to the steric inhibition caused by the highly branched tert-butyl moiety, is moderately biodegradable with a longer lag-phase. The required biodegradation degree of 60 % TIC/TOC was reached within 28 days in two out of three tests on tert-butyl acrylate indicating complete, albeit slower, mineralisation and as such can be expected to be ultimately biodegradable in the environment. The formation of persistent breakdown products is not expected.

Therefore, it may be assumed that the persistence in water, in soil and in sediment is not an element of concern for acrylate esters. A well-documented and reliable test on biodegradation in soil was performed on acrylic acid. During the study, the metabolism of [^{14}C]-acrylic acid was investigated in a Milton sandy loam soil, under aerobic conditions, for up to 28 days after treatment. Acrylic acid rapidly metabolised. After 3 days no acrylic acid was detected in soil extracts. The half-life for acrylic acid was estimated to be less than 1 day. Most of the AA was mineralised to carbon dioxide. The remainder probably became incorporated into soluble or insoluble organic material (Huntingdon Research Centre Ltd., 1992).

Environmental distribution

As detailed above, the category have no persistence concern based on its rapidly biodegradation. Their low affinity with organic matter (measured and calculated Koc values from 6.4 for MA to 360 for 2EHA) also limit their presence in the soil and sediment compartments. Furthermore, acrylic ester would rather be mobile in soil and sediment rely upon their high solubility in water (> 1,7 g/L except for 2EHA which has a solubility 2 orders of magnitude below),

The water and the atmosphere are the targeted compartments: the solubility is high and the Henry's law constant increases within the category. AA is expected to remain in water unlike 2EHA which is very volatile (H of 461 $Pa.m^3/mol$)

Hydrolysis of the acrylic esters can be expected under alkaline conditions and also under acidic and neutral conditions for 2EHA.

Bioaccumulation

The experimental Log K_{OW} and the correlated calculated BCF increases with alkyl chain length and molecular weight. Log K_{OW} values range between 0.46 for acrylic acid and 2.38 for n-butyl acrylate indicate a relatively low bioaccumulation potential for the category members up to and including n-butyl acrylate. The Log K_{OW} of 4.00 for 2-ethyhexyl acrylate would indicate an increased potential for bioaccumulation compared to the other members of the category. However, the models do not take into account hydrolysis of the ester in organism by esterases (see metabolism) that can be expected. Thus, the potential for bioaccumulation of 2-ethyhexyl acrylate under environmental conditions is expected to be lower than estimated by the model calculations. In conclusion, the calculated BCF values range from 3.16 for acrylic acid and methyl acrylate, to 70 for 2-ethyhexyl acrylate, demonstrating that none of the category members have a BCF triggering a bioaccumulation potential according to both CLP and REACh regulations (BCFs < 500).

Conclusions

Available data shows that, with the exception of 2EHA which hydrolyses slowly in contact with water, abiotic degradation by hydrolysis is only expected to play an important role in the degradation of acrylate esters in alkaline environment.

All substances within the category have nevertheless a high potential for ready biodegradability in water, except for tert-butyl acrylate which can be considered to be moderately biodegradable.

Additionally, none of the substances are expected to bioaccumulate according to PBT criteria.

Adsorption to soil, sediments and suspended solids of acrylic acid and the acrylate esters is not to be expected.

The acrylate esters are expected to evaporate slowly but to an increasing extent with increasing chain length and molecular weight. Based on the physical chemical properties of the acrylate esters, the atmosphere is the main target compartment for distribution and only small amounts will remain in the hydrosphere and geosphere.

Overall, these data serve to demonstrate that there are clear similarities and trends in the environmental fate properties of the members of the category, related to molecular weight, molecular size and hydrolysis and strongly supports the hypothesis that read-across between category members is justified for ecotoxicity endpoints.

5.4.3 Read-across justification for ecotoxicological information

In RAAF nomenclature, the read-across approach for ecotoxicity endpoints is described in scenario 6 (different compounds have quantitatively similar properties) and governed by AE 6.3 (common underlying mechanism, quantitative aspects). Although acrylic acid itself is noted to be more harmful to the algae with lower ErC50 and NOEC values compared to its esters (and therefore is not considered within the justification), the available data indicates toxicity at the same order of magnitude across all three trophic levels for all acrylate esters within the category. The only outliers to this trend display a lower level of toxicity. It can therefore be concluded that read across is applied with a high level of certainty and is suitably precautionary.

Table 23 - Summary of relevant ecotoxicity endpoints

Parameter	AA	MA	EA	nBA	iBA	tBA	2EHA
Short-Term Toxicity Testing on Fish (LC_{50}) (Fresh Water)	27 mg/L	3.4 mg/L	4.6 mg/L	5.2 mg/L	2.1 mg/L	2.37 mg/L	1.81 mg/L
Short-Term Toxicity Testing on Fish (LC_{50}) (Marine Water)	236 mg/L	1.1 mg/L	2.0 mg/L	2.1 mg/L	**Read-across from nBA**	**Read-across from nBA**	**Read-across from MA, EA and nBA**
Long-Term Toxicity Testing on Fish	No data available	No data available	No data available	No data available	No data available	No data available	No data available
Short-Term Toxicity Testing on Invertebrates (EC_{50})	47 mg/L (fresh water) 97 mg/L (LC50) (marine water)	2.6 mg/L (fresh water) 1.6 mg/L (marine water)	7.9 mg/L (fresh water)	8.2 mg/L (fresh water)	**Read-across from nBA**	8.74 mg/L (fresh water)	1.3 mg/L
Long-Term Toxicity Testing on Invertebrates	12 mg/L (NOEC)	**Read-across from nBA and EA**	0.19 mg/L (NOEC)	0.136 mg/L (NOEC)	**Read-across from nBA and EA**	**Read-across from nBA and EA**	**Study on-going**
Growth Inhibition Study Aquatic Plants (E_rC_{50})	0.13 mg/L	3.55 mg/L	4.5 mg/L (Cell number)	2.65 mg/L (Cell number)	5.28 mg/L	14.6 mg/L	1.71 mg/L
Algae (NOEC)	0.03 mg/L (E_rC_{10})	No data available	No data available	No data available	0.82 mg/L	3.85 mg/L	0.45 mg/L
Activated Sludge Respiration Inhibition	EC_{20} (30 min) 900 mg/L	EC_{10} (3d) > 100 mg/L	EC_{10} (72h) > 100 mg/L	EC_0 (3d) > 150 mg/L	EC_{20} (30 min) > 1000 mg/L	EC_{20} = *ca.* 950 mg/L	EC_{20} (30 min) > 1000 mg/L

5.4.3.1 Fish

5.4.3.1.1 Short-Term Toxicity to Fish

There are data available for all substances in the category. The data available on these substances are derived from studies of the appropriate duration and quality to warrant a high degree of reliability and accordingly have Klimisch reliability ratings of 1 or 2. All studies were conducted for an exposure duration of 96 hours.

Table 24 - Summary on short-term toxicity data on fish - freshwater species

Substance	Fish Species	LC_{50} (mg/L)*	Study Type	Reference
AA	Rainbow trout (*Oncorhynchus mykiss*)	27	EPA OTS 797.1400 (Flow-through)	Analytical Bio-Chemistry Laboratories, Inc., 1990a
MA	Rainbow trout (*Oncorhynchus mykiss*)	3.4	OECD 203 (Flow-through)	Basic Acrylic Monomer Manufacturers, 1995a
EA	Rainbow trout (*Oncorhynchus mykiss*)	4.6	OECD 203 (flow-through)	Analytical Bio-Chemistry Laboratories, Inc., 1990b
tBA	Golden orfe (*Leuciscus idus*)	2.37†	DIN 38412, part 15 (Static)	BASF AG, 1978a
iBA	Fathead minnow (*Pimephales promelas*)	2.1	ASTM, 1980 (Flow-through)	Russom CL *et al.*, 1988
nBA	Rainbow trout (*Oncorhynchus mykiss*)	5.2	EPA OTS 797.1400 (Flow-through)	Analytical Bio-Chemistry Laboratories, Inc., 1990c
2EHA	Rainbow trout (*Oncorhynchus mykiss*)	1.81	OECD 203 (Semi-static)	BASF AG, 1999

* mean measured concentration

† estimated (recalculated (geometric mean))

Table 25 - Summary on short-term toxicity data on fish - marine Species

Substance	Fish Species	LC_{50} (mg/L)*	Study Type	Reference
AA	Sheepshead minnow (*Cyprinodon variegatus*) Saltwater	236	OECD 203 (Flow-through)	Wildlife International Ltd., 1995a
MA	Sheepshead minnow (*Cyprinodon variegatus*) Saltwater	1.1	OECD 203 (Flow-through)	Wildlife International Ltd., 1995b
EA	Sheepshead minnow (*Cyprinodon variegatus*) Saltwater	2.0	EPA OTS 797.1400 (Flow-through)	Wildlife International, Ltd., 1995c
tBA	-	**RA to nBA**	-	-
iBA	-	**RA to nBA**	-	-
nBA	Sheepshead minnow (*Cyprinodon variegatus*) Saltwater	2.1	OECD 203 (Flow-through)	WildLife International Ltd., 1996a
2EHA	-	**RA to MA, EA and nBA**	-	-

* mean measured concentration

Discussion

As stated above, data are available for this endpoint on all members of the category with all substances having studies on freshwater species available and a number of studies on marine species also having been conducted. Although acrylic acid itself yields different result with regards to its ecotoxic effects on both marine and freshwater species of fish, with LC_{50} levels two and one orders of magnitude larger respectively indicating lower levels of toxicity, the acrylate substances all give remarkably similar results, all with LC_{50} values of the same order of magnitude, across all the studies conducted. This close similarity between the results of the acrylate esters supports the use of read across between the substances. The use of read-across to address the toxicity of the substances to marine fish is technically not required to address any REACH endpoints; however, the use of the endpoint data on nBA to both iBA and tBA is considered appropriate. Although there are differences in the branching structure of the molecules they are the most structurally similar within the category. Similarly read across from MA, EA and nBA to 2EHA is considered appropriate given that all LC50 results for freshwater and marine water are of the same order of magnitude, indicating a consistent level of toxicity across all members of the category.

Conclusion

The results of all studies conducted on the esters yield extremely similar results across all substances within the category across both freshwater and marine species, supporting the broader use of the category approach. Read across to data on the toxicity of the substances to marine species is not technically required but the use of data on MA, EA and nBA, to address the endpoints for other substances, is considered valid.

5.4.3.1.2 Long-Term Toxicity to Fish

Discussion

There are no data available, on long-term toxicity to fish, for any of the substances in the category. The substances are handled primarily, if not exclusively, in closed systems and therefore environmental exposure would be limited. The volatility of the substances provides for volatilization of any releases to the air. The substances are slowly photodegradable and are generally biodegradable with the majority of the category being considered readily biodegradable - accidental releases to the environment would not result in accumulation or persistence. The relatively high water solubility and corresponding low Log K_{OW} indicate that no bioaccumulation potential exists.

Additionally, it can be seen that all short-term ecotoxicity L(E)C_{50} values, which are available from studies conducted with the acrylate esters of the category, are in the range 1.1-8.74 mg/L across all three trophic levels (fish, daphnia and algae) with the exception of the ErC50 value for tBA (14.6 mg/L) which is an order of magnitude higher. It can therefore be concluded that all of the acrylate ester category members exert a similar level of toxicity as each other across all trophic levels. In line with this, the NOEC values from long term toxicity studies conducted with aquatic invertebrates with EA (0.19 mg/L) and nBA (0.136 mg/L) are used to address the same endpoint data requirement for MA, iBA, tBA. The long-term toxicity study on aquatic invertebrates for 2EHA is currently ongoing. Additionally, it is postulated that long-term studies with fish would generate results of the same order of magnitude as the long-term studies conducted with invertebrates and, as such, no vertebrate testing is considered to be scientifically justified since the environmental hazard can suitably be determined using the available long-term invertebrate data. This is further substantiated since two of the three available algal NOECs are of the same order of magnitude (iBA: 0.82 mg/L and 2EHA: 0.45 mg/L, respectively). The third available algal NOEC is an order of magnitude higher (3.85 mg/L, tBA) indicating lower concern from this study.

Conclusion

Acute effect levels were determined to be in the same range of concentrations for all three trophic levels and it is considered that a similar trend would be observed for long-term effects. As such, information on long-term toxicity to aquatic invertebrates was used to assess long-term toxicity towards aquatic organisms. Further testing in vertebrates is, therefore, not necessary or justified and similarly the need to demonstrate the suitability of read across within the category for this endpoint is not considered necessary at this time.

5.4.3.2 Aquatic Invertebrates

5.4.3.2.1 Short-Term Toxicity to Aquatic Invertebrates

There are data available for all substances in the category. The data available on these substances are derived from studies of the appropriate duration and quality to warrant a high degree of reliability and accordingly have Klimisch reliability ratings of 1 or 2. The exposure time was 48 h in freshwater studies and 96h in studies conducted on marine species.

Table 26 - Summary of short-term toxicity data on aquatic invertebrates - freshwater species

Substance	Invertebrate Species	48h EC_{50} (mg/L)*	Study Type	Reference
AA	*Daphnia magna*	47†	EU Method C.2 (Static, closed vessels)	Huels AG, 1995b
MA	*Daphnia magna*	2.6	OECD 202 (Flow-through)	Basic Acrylic Monomer Manufacturers, 1995b
EA	*Daphnia magna*	7.9	EPA OTS 797.1300 (Flow-through)	Analytical Bio-Chemistry Laboratories, Inc., 1990g
tBA	*Daphnia magna*	8.74	OECD 202 (Static, closed vessels)	BASF AG, 2001b
iBA	*Daphnia magna*	9.7†	OECD 202 (Static, open)	BASF AG, 1988b
nBA	*Daphnia magna*	8.2	EPA OTS 797.1300 (Flow-through)	Analytical Bio-Chemistry Laboratories, Inc., 1990d
2EHA	*Daphnia magna*	1.3	OECD 202 (Static, closed vessels)	BASF AG, 2001c

* mean measured concentration
† nominal concentration

Table 27 - Summary of short-term toxicity data on aquatic invertebrates - marine species

Substance	Invertebrate Species	96h EC_{50} (mg/L)*	Study Type	Reference
AA	*Mysidopsis bahia* Saltwater	97	EPA OTS 797.1930 (Flow-through)	Wildlife International Ltd., 1996b
MA	*Mysidopsis bahia* Saltwater	1.6	EPA OTS 797.1930 (Flow-through)	Basic Acrylic Monomer Manufacturers, 1996a
EA	-	**RA to MA**	-	-
tBA	-	**RA to MA**	-	-
iBA	-	**RA to MA**	-	-
nBA	-	**RA to MA**	-	-
2EHA	-	**RA to MA**	-	-

* mean measured concentration

Discussion

As stated above, data are available for this endpoint on all members of the category with all substances having studies on freshwater species available and two studies on marine species also having been conducted on different substances. Although acrylic acid itself yields different result with regards to its ecotoxic effects on both marine and freshwater species of invertebrates, with EC_{50} levels at least an order of magnitude larger (indicating similar lower levels of toxicity), the acrylate substances all give remarkably similar results across all the studies conducted even taking into account the slight differences in methodology (flow through vs static test etc.). This close similarity between the results of the acrylate esters supports the use of read across between the substances. The use of read-across to address the toxicity of the substances to marine invertebrates is technically not required to address any REACH endpoint. If required the use of the endpoint data on MA to the other substances could be considered appropriate. Although there are differences structure of the molecules in the category the broad similarity in the results in freshwater species suggests that a similar trend could be observed in marine species.

Conclusion

The results of all studies conducted on the esters yield extremely similar results across all substances within the category across both freshwater and marine species, supporting the broader use of the category approach. Read across to data on the toxicity of the substances to marine species is not technically required but the use of data on MA to address the endpoints for other substances is considered valid.

5.4.3.2.2 Long-Term Toxicity to Aquatic Invertebrates

There are data available for the substances AA, EA and nBA in the category, as outlined in the table below. The data available on these substances are derived from studies of the appropriate duration and quality to warrant a high degree of reliability and accordingly have Klimisch reliability ratings of 1. The studies were conducted with an exposure time of 21 days.

Table 28 - Summary of long-term toxicity data on aquatic invertebrates

Substance	Invertebrate Species	NOEC (mg/L)*	Study Type	Reference
AA	*Daphnia magna* Freshwater	19	EPA OTS 797.1330 (Flow-through)	ABC Laboratories California, 1996
MA	-	**RA to EA and nBA**	-	-
EA	*Daphnia magna* Freshwater	0.19	EPA OTS 797.1330 (Flow-through)	ABC Laboratories, Inc., 1997
tBA	-	**RA to EA and nBA**	-	-
iBA	-	**RA to EA and nBA**	-	-
nBA	*Daphnia magna* Freshwater	0.136	OECD 211 (Flow-through)	BASF SE, 2009d
2EHA	-	**Study ongoing**	-	-

* mean measured concentration
RA: endpoint addressed using read-across data

Discussion

As stated above, data are available for this endpoint on three members of the category

The result of the study on acrylic acid is two orders of magnitude larger than the results of the studies conducted on the two acrylate ester substances indicating a comparatively lower level of toxicity when compared to the acrylate esters that were tested. The results of the studies on the two acrylate esters give remarkably similar results. This close similarity between the results of the acrylate esters supports the use of read across between the substances although no results from studies on the other substances in the category are available to further support this conclusion.

The results of the study conducted on acrylic acid indicate that the substance poses less concern than the larger parent acrylate ester and, as such, read across to EA and nBA is considered to present an appropriately precautionary approach in this instance.

As stated above, it can be seen that all-short term ecotoxicity L(E)C_{50} values, that are available from studies conducted with the acrylate esters of the category, are generally of the same order of magnitude across all three trophic levels (fish, daphnia and algae). It can therefore be concluded that all of the acrylate ester category members exert a similar level of toxicity as each other across all trophic levels. In line with this, the NOEC values from long term toxicity studies conducted with aquatic invertebrates with EA (0.19 mg/L) and nBA (0.136 mg/L) are used to address the same endpoint data requirement for MA, iBA, tBA. The long term toxicity study on

aquatic invertebrates for 2EHA is currently ongoing. This is further substantiated since two of the three available algal NOECs are of the same order of magnitude (iBA: 0.82 mg/L and 2EHA: 0.45 mg/L, respectively). The third available algal NOEC is an order of magnitude higher (3.85 mg/L, tBA) which indicates a lower toxicity.

Conclusion

Given that the results of all studies across all three trophic levels are broadly the same, and that there is no marked difference between any of the substances within the category, it is concluded that this trend would extend to long-term testing on aquatic invertebrates. Similarly, the results of both long-term daphnia studies conducted on the acrylate esters yield very similar results, supporting the broader use of the category approach. As such, it is considered justified to use read across to the data on EA and nBA to address this endpoint for the other category substances. Read across is applied with a high level of confidence.

5.4.3.3 Algae and Aquatic Plants

There are data available for all substances in the category. The data available on these substances are derived from studies of the appropriate duration and quality to warrant a high degree of reliability and accordingly have Klimisch reliability ratings of 1 or 2.

Table 29 - Summary of toxicity data on algae and aquatic plants

Substance	Results (72 hr)*			Remarks	Reference
	ECr_{50} (mg/L)	**ECr_{10} (mg/L)**	**NOEC (mg/L)**		
AA	0.13†	0.03†	0.008†	EU Method C.3 *Scenedesmus subspicatus* (Freshwater) Static	BASF AG, 1994a
	0.205†	0.031†		EU Method C.3 *Scenedesmus subspicatus* (Freshwater) Static	Huels AG, 1995c
MA	3.55			OECD 201 *Pseudokirchneriella subcapitata* (Freshwater) Static	Basic Acrylic Monomer Manufacturers, 1995c
EA	4.5 (cell number)			OECD 201 *Selenastrum capricornutum* (Freshwater) Static	Analytical Bio-Chemistry Laboratories, Inc., 1990e
tBA	14.6	5.61	3.85 (fluorescence)	OECD 201 *Desmodesmus subspicatus* (Freshwater) Static	BASF AG, 2002a
iBA	5.28	2.09	0.82	OECD 201 *Desmodesmus subspicatus* (Freshwater) Static	BASF AG, 2002b
nBA	2.65			OECD 201 Pseudokirchneriella subcapitata (Freshwater) Static	Analytical Bio-Chemistry Laboratories, Inc., 1990f
2EHA	1.71	0.8	0.45	OECD 201 *Scenedesmus subspicatus* (Freshwater) Static	BASF AG, 2002c

* mean measured concentration
† nominal concentration

Discussion

Data of the effects of the substances on the growth of freshwater algae are available for each substance under consideration and as such read-across does not technically require justification. Acrylic acid itself was found to exert greater toxicity to the species tested, with the results being an order of magnitude lower than the acrylate esters. Amongst the acrylate esters, the results were broadly similar and with the exception of the larger EC50 and NOEC for tBA were of the same order of magnitude. No obvious structural or physico-chemical reason is apparent for this slight difference.

Studies on some of the substances (AA, tBA, iBA and 2EHA) also calculated ErC_{10} and NOEC values Those results vary somewhat and are, in some cases, an order of magnitude different however overall they are of sufficiently similar levels so as to suggest that read across to a worst case scenario in the absence of other data would be appropriate.

Conclusion

There are appropriate and applicable acute data on all members of the category such that read-across is not required. The results across the different substances are all broadly similar. As such there is no reason to suggest that read-across for chronic data within the category should not be considered appropriate.

5.4.3.4 Sediment Organisms

There are no data available, on sediment organism toxicity, for any of the substances in the category.

There is no concern for relevant exposure of sediment organisms to the substances of the category. None of the category substances are PBT or vPvB. Furthermore, due to the physico-chemical properties, if the substances are released into water, they are predicted to partition only to a small degree into sediment. Additionally, all of the substances are generally biodegradable, have a short half-life in the environment, and do not bioaccumulate. Hence, this margin of safety is sufficient and further testing in sediment organisms is unnecessary.

Furthermore, significant exposure of the sediment compartment is unlikely.

6. Uncertainties

6.1 Read-across for toxicological information

A qualitative uncertainty analysis was performed for the read-across based predictions of (eco)toxicological properties for acrylate esters, as summarised in Table 30 - 30. Uncertainties associated with the read-across based toxicity prediction is scored according to the RAAF guidance (ECHA, 2017); 1: not acceptable, 2: not acceptable in its current form; 3: acceptable with just sufficient confidence, 4: acceptable with medium confidence, 5: acceptable with high confidence.

The read-across within the acrylate esters between MA, EA and nBA is considered justified with a high level of confidence, since their only structural difference is the length of the carbon chain, with a clear correlation with the carbon chain length and similarity in the metabolism in the in vitro assays. This is reflected in the similarity in the systemic toxicity.

In general, there are low uncertainties associated with the read-across approach for the toxicological data from nBA to iBA. nBA is considered to be the closest analogue of iBA due to the similarity in the toxicokinetic and toxicodynamic properties. Therefore, the read-across between the two substances should be supported by a high level of confidence hence to address

the REACH data requirement and subsequent hazard classifications. Indeed, nBA and iBA were categorised for hazard assessment due to their structural similarity in the OECD SIDS (OECD, 2002) and weight of evidence approach was applied to assess their toxicological properties.

The currently available data set is considered sufficient to address the REACH data requirement for genotoxicity for nBA and tBA, on the basis that the sufficient exposure is demonstrated in their *in vivo* genotoxicity studies. This is considered to be a reasonable and pragmatic approach given the apparent variation in the genotoxicity results within the category. The negative gene mutation assay in transgenic mouse for EA could potentially be used to read-across to MA to strengthen the conclusion drawn for mammalian cell mutagenicity (i.e. positive results linked to the excess cytotoxicity).

Some inconsistency was noted for the read-across approach within the original category report, which are related to the route of exposure for the source substances. Currently an update including the read-across for iBA to the oral OECD TG 443 in rats for nBA is pending whereas this study was used for read-across to tBA and 2EHA.

For tBA, multiple source substances were used for the read-across strategy for developmental toxicity in rats where the guideline compliance study conducted with a preferred route of exposure is already available. This read-across approach was interpreted as to bridge the read-across strategy for the rabbit studies.

Upon completion of an oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in rats for 2EHA, a similarity in the toxicity for reproduction between 2EHA and the other acrylate esters within the category could be assessed to use this study to bridge the read-across strategy for the relevant systemic toxicity endpoints.

Table 30 - Proposed assessment options for the read-across strategy within the category

	Endpoint	Exposure route	Substances with read-across	Assessment Option	Comments
Toxicological information	Sensitisation	Dermal	iBA	5	Strong evidence for hypothesised skin sensitisation potency. Due to the similarity in the physchem data with the source substance nBA and the expected protein binding reactivity and dermal absorption commonly seen for the acrylate esters within the category, the read across is justified with a high level of confidence. Therefore, the prediction for skin sensitisation potency and the associated hazard classification based on this study is also considered reliable. There is no indication that the tertiary structure of the side chain of iBA would have a significant impact on the read-across approach for skin sensitisation.
			tBA	4	Strong evidence for hypothesised skin sensitisation potency. Due to the similarity in the physchem data with the source substance nBA with the expected protein binding reactivity and dermal absorption, the read across to the source substance nBA is justified with a high level of confidence. The variation in the metabolism rate is not directly relevant for the prediction for skin sensitisation given the nBA and iBA themselves are considered to be haptens. Therefore, the prediction for skin sensitisation potency and the associated hazard classification based on this study is also considered reliable. There is no indication that the tertiary structure of the side chain of iBA would have a significant impact on the read-across approach for skin sensitisation.
	Repeated dose toxicity	Oral, inhalation	iBA	5	Strong evidence to support similarity in systemic toxicity profiles with the source substance BA based on the structural similarity, available toxicology data and similarity in metabolism. The systemic toxicity between nBA and iBA are considered to be very similar from the toxicokinetic and toxicodynamic perspectives. Based on their very similar and rapid metabolism rates observed in the comparative hydrolysis assays, the likelihood of seeing toxicity different from that predicted by the read-across is very low. No adverse systemic toxicity is identified for the alcohol metabolites within the category that would give a significant impact on the read-across approach.
	In vitro mutagenicity in mammalian cells	N/A	iBA	5	Strong evidence to support the similarity in genotoxicity profiles with the source substance nBA both in vitro and in vivo, based on the structural similarity and associated alerts, available genotoxicity data and similarity in metabolism. There is no indication that the tertiary structure of the side chain of iBA would have a significant impact on the read-across approach for genotoxicity.
	Toxicity for reproduction	Oral, inhalation	EA	5	Strong evidence to support similarity in systemic toxicity profiles and lack of toxicity for reproduction with the source substance MA based on the structural similarity, available toxicology data and similarity in metabolism. Uncertainties associated with the read-across based prediction of reproductive toxicity of EA is considered to be minimal due to its rapid metabolism rate observed in the comparative hydrolysis assay. No adverse systemic toxicity is identified for the alcohol metabolites within the category that would give a significant impact on the read-across approach.

Table 30 Proposed assessment options for the read-across strategy within the category (cont.)

	Endpoint	Exposure route	Substances with read-across	Assessment Option	Comments
	Toxicity for reproduction	Inhalation	nBA	5	Strong evidence to support similarity in systemic toxicity profiles and lack of reproductive toxicity concerns with the source substance MA based on the structural similarity, available toxicology data and similarity in metabolism. Uncertainties associated with the read-across based prediction of reproductive toxicity of nBA is considered to be minimal due to its rapid metabolism rate observed in the comparative hydrolysis assay. No adverse systemic toxicity is identified for the alcohol metabolites within the category that would give a significant impact on the read-across approach.
		Oral, inhalation	iBA	5	Strong evidence to support similarity in systemic toxicity profiles and lack of reproductive toxicity concerns with the source substance MA and nBA based on the structural similarity, available toxicology data and similarity in metabolism. The systemic toxicity between nBA and iBA are considered to be very similar from the perspectives of toxicokinetic and toxicodynamic. Uncertainties associated with the read-across based prediction of reproductive toxicity of iBA is considered to be minimal due to its very similar and rapid metabolism rates observed in the comparative hydrolysis assays. No adverse systemic toxicity is identified for the alcohol metabolites within the category that would give a significant impact on the read-across approach.
		Oral, inhalation	tBA	4	The read-across justification may be weakened by a difference in the hydrolysis rate between tBA and the acrylate esters within the category. Date are not available to extend the read across for tBA to dose levels beyond those used in the existing inhalation studies for tBA. However, a read-across to the oral study for nBA is supported based on a common systemic toxicity profile within the category (a lack of systemic toxicity). No adverse systemic toxicity is identified for the alcohol metabolites within the category that would give a significant impact on the read-across approach.

		Oral, inhalation	2EHA	4	Strong evidence to support similarity in lack of systemic toxicity for the source substances MA and nBA based on the structural similarity, available toxicology data and similarity in metabolism. The presence of the tertiary structure of the side chain of 2EHA is not considered to have any significant impact on the toxicology profile given the lack of systemic toxicity observed with the target and source substances. There is currently a data gap for reproductive toxicity. Upon a completion of an oral OECD 422 study in rats, similarity to the toxicity of other category substances could be demonstrated to strengthen the read-across justification. No adverse systemic toxicity is identified for the alcohol metabolites within the category that would give a significant impact on the read-across approach.
Ecotoxicological information	Long-term toxicity testing on invertebrates	N/A	MA, iBA, tBA, 2EHA	5	Due to the similarity in physchem and environmental fate properties observed between the members of the category the substances are expected to behave in the same way when exposed to environmentally relevant media. There is strong evidence showing that the category substances exhibit the same degree of environmental toxicity across all three trophic levels with little intersubstance variation. This trend was mirrored in the available long-term aquatic (eco)toxicity tests (a long-term toxicity test on *Daphnia magna* is ongoing for 2EHA). The read across strategy is applied with a high level of confidence

7. Conclusions for C&L

The classification and labelling of the substances of the acrylate category are presented below.

Evaluation of the chemicals in this category leads to the conclusions that [1] data currently exist to adequately represent the toxicological and ecological profile of major portions of this category, [2] there is a concurrence and similarity among the existing data for the various endpoints and [3] toxicokinetic data shows that all acrylate esters are rapidly absorbed and metabolised to acrylic acid and their associated alcohol.

Table 31 - Summary of classification and labelling of the category members

Substance	Harmonised Classification in Annex VI of CLP/ EU- GHS (1272/2008/EC)	Self-Classification Derived According to Annex I of CLP/EU-GHS (1272/2008/EC) Based on Available Data
AA	**Index Number : 607-061-00-8** Flam. Liq. 3 (H226) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Corr. 1A (H314) STOT SE 3 (H335) Aquatic Acute 1 (H400)	Flam. Liq. 3 (H226) Acute Tox. 4 (H302) Acute Tox. 4 (H332) Skin Corr. 1A (H314) STOT SE 3 (H335) Aquatic Acute 1 (H400) Aquatic Chronic 2 (H411)
MA	**Index Number : 607-034-00-0** Flam. Liq. 2 (H225) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) STOT SE 3 (H335)	Flam. Liq. 2 (H225) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 3 (H331) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) STOT SE 3 (H335) Aquatic Chronic 3 (H412)
EA	**Index Number : 607-032-00-X** Flam. Liq. 2 (H225) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) STOT SE 3 (H335)	Flam. Liq. 2 (H225) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 3 (H331) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) STOT SE 3 (H335) Aquatic Chronic 3 (H412)
tBA	**Index Number : 607-245-00-8** Flam. Liq. 2 (H225) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Skin Sens. 1 (H317) STOT SE 3 (H335) Aquatic Chronic 2 (H411)	Flam. Liq. 2 (H225) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 3 (H331) Skin Irrit. 2 (H315) Skin Sens. 1 (H317) STOT SE 3 (H335) Aquatic Chronic 2 (H411)

iBA	**Index Number : 607-115-00-0** Flam. Liq. 3 (H226) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Skin Sens. 1 (H317)	Flam. Liq. 3 (H226) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Skin Sens. 1 (H317) STOT SE 3 (H335) Aquatic Chronic 3 (H412)
nBA	**Index number : 607-062-00-3** Flam. Liq. 3 (H226) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) STOT SE 3 (H335)	Flam. Liq. 3 (H226) Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) STOT SE 3 (H335) Aquatic Chronic 3 (H412)
2EHA	**Index Number : 607-107-00-7** Skin Irrit. 2 (H315) Skin Sens. 1 (H317) STOT SE 3 (H335)	Skin Irrit. 2 (H315) Skin Sens. 1 (H317) STOT SE 3 (H335) Aquatic Chronic 3 (H412)

Table 32 - Summary of classification and labelling of the metabolites of the category members

	Harmonised classification in Annex VI of CLP/ EU- GHS (1272/2008/EC)	Self-classification derived according to Annex I of CLP/EU-GHS (1272/2008/EC) based on available data
AA	**Index Number : 607-061-00-8** Flam. Liq. 3 (H226) Acute Tox. 4 (H302) Acute Tox. 4 (H312) Acute Tox. 4 (H332) Skin Corr. 1A (H314) STOT SE 3 (H335) Aquatic Acute 1 (H400)	Flam. Liq. 3 (H226) Acute Tox. 4 (H302) Acute Tox. 4 (H332) Skin Corr. 1A (H314) STOT SE 3 (H335) Aquatic Acute 1 (H400) Aquatic Chronic 2 (H411)
Methanol	**Index number : 603-001-00-X** Flam. Liq. 2 (H225) Acute Tox. 3 (H301) Acute Tox. 3 (H311) Acute Tox. 3 (H331) STOT SE 1	**n/a**
Ethanol	**Index Number : 603-002-00-5** Flam. Liq. 2	**n/a**
t-Butanol	**Index Number : 603-005-00-1** Flam. Liq. 2 (H225) Acute Tox. 4 (H332) Eye Irrit. 2 (H319) STOT SE 3 (H335)	**n/a**
i-Butanol	**Index Number : 603-108-00-1** Flam. Liq. 3 (H226) Skin Irrit. 2 (H315) Eye Dam. 1 (H318) STOT SE 3 (H335) STOT SE 3 (H336)	**n/a**
n-Butanol	**Index Number : 603-004-00-6** Flam. Liq. 3 (H226) Acute Tox. 4 (H302) Skin Irrit. 2 (H315) Eye Dam. 1 (H318) STOT SE 3 (H335) STOT SE 3 (H336)	**n/a**
2-Ethyl-Hexanol	no substance-specific entry in Annex VI	Acute Tox. 4 (H332) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) STOT SE 3 (H335)

Discussion

All of the acrylate esters have harmonised classifications, as detailed in Annex VI of EC Regulation 1272/2008. The harmonised classifications are applied as a minimum. Where available data supports a more stringent classification this is adopted, as for acute inhalation toxicity. A STOT SE 3 classification is adopted across the category in consideration of available data on the effects of each of the substances with regards to respiratory tract irritation. Additionally, an environmental classification is assigned to each member of the category on the basis of the application of the results of the long-term ecotoxicity tests across the category and consideration of the substance specific ready biodegradability study results.

8. PBT and VPVB assessment

8.1 Assessment of PBT/vPvB Properties – Comparison with the Criteria of Annex XIII

8.1.1 Persistence Assessment

In valid studies a high grade of biodegradation was obtained. Thus, all members of the category are rapidly biodegradable and, hence, not persistent.

8.1.2 Bioaccumulation Assessment

The logP for all members of the category is below 4.5. Based on that, a bioaccumulation potential is not expected. Thus, the substances in the category are not expected to bioaccumulate.

8.1.3 Toxicity Assessment

All available NOECs and EC_{10} values of the substances of the category are above 0.01 mg/L. According to the presently available data, none of the category members are CMR substances nor are any members classified for target organ toxicity (STOT RE category 1 or 2, acute or chronic).

8.1.4 Summary and Overall Conclusions on PBT or vPvB Properties

The substances in the lower alkyl acrylate category are not PBTs or vPvBs.

9. Dose Descriptors

Use of dose descriptors from the read-across substances is considered appropriate given the rationale stated throughout this document. Where necessary assessment factors are applied in the Chemical Safety Assessment in order to ensure that the resultant Risk Characterisation Ratios are sufficiently protective.

Table 33 - Self-classification (dose descriptor)

	79-10-7	**96-33-3**	**140-88-5**	**1663-39-4**	**106-63-8**	**141-32-2**	**103-11-7**
	AA	**MA**	**EA**	**tBA**	**iBA**	**nBA**	**2EHA**
Flammability	Flam. Liquid 3	Flam. Liquid 2	Flam. Liquid 2	Flam. Liquid 2	Flam. Liquid 3	Flam. Liquid 3	-
Acute oral toxicity	Acute Tox. 4* (1000 - < 2000 mg/kg bw)	Acute Tox. 4* (ca. 768 mg/kg bw)	Acute Tox. 4 (1120 mg/kg bw)	Acute Tox. 4* (ca. 1047 mg/kg bw)	- * (4895 mg/kg bw)	- * 3150 mg/kg bw)	- (4435 mg/kg bw)
Acute dermal toxicity	- (> 2000 mg/kg bw)	Acute Tox 4* (1250 mg/kg bw)	Acute Tox 4* (3049 mg/kg bw)	Acute Tox 4* (ca. 2000 mg/kg bw)	Acute Tox 4* (793 - 4000 mg/kg bw)	Acute Tox 4 (2000 mg/kg bw)	- (ca. 7522 mg/kg bw)
Acute inhalation toxicity	Acute Tox. 4* (> 5.1 mg/L air)	Acute Tox. 3 (< 10.8 mg/L air)	Acute Tox. 3 (< 9.1 mg/L air)	Acute Tox. 3 (7.0 mg/L air)	Acute Tox. 4* (ca. 10.5 mg/L air)	Acute Tox. 4 (10.3 mg/L air)	-
Skin corrosion/irritation	Skin Corr. 1A*	Skin Irrit. 2*	Skin Irrit. 2*	Skin Irrit. 2*	Skin Irrit. 2*	Skin Irrit. 2*	Skin Irrit. 2*
Serious eye damage/irritation	-	Eye Irrit. 2*	Eye Irrit. 2*	- *	- *	Eye Irrit. 2*	-
Sensitising	- *	Skin Sens. 1B* (EC_3 19.6%)	Skin Sens. 1B* (EC_3 36.8%)	Skin Sens. 1B*#	Skin Sens. 1B*†	Skin Sens. 1B* (EC_3 11.2%)	Skin Sens. 1B* (EC_3 9.7%)
Specific target organ toxicity - single	STOT Single Exp. 3*	STOT Single Exp. 3*	STOT Single Exp. 3*	STOT Single Exp. 3*	STOT Single Exp. 3	STOT SE 3*	STOT SE 3*
Short-term aquatic	Aquatic Acute 1*	-	-			-	-
Long-term aquatic	Aquatic Chronic 2	Aquatic Chronic 3	Aquatic Chronic 3	Aquatic Chronic 2*	Aquatic Chronic 3	Aquatic Chronic 3	Aquatic Chronic 3

* Annex VI classification

supporting data available from Magnusson & Kligman Maximisation test and Freund's complete adjuvant test

† read across from other category members

10. Conclusion

The acrylic acid and lower alkyl acrylate esters category is defined as a structurally related group of seven substances including AA, MA, EA, tBA, iBA, nBA and 2EHA. The short-chain acrylate esters in this category are classed as alpha, beta-unsaturated esters with having potential Michael acceptors capable of electrophilic attack of protein and other cellular macromolecules. AA is a common major metabolite in the category that is considered to be the most relevant compound for systemic toxicity for the category.

The read-across within the category that is presented in this report is supported by the common toxicokinetics and toxicodynamics behaviour exhibited by the members of the category. The shared chemical reactivity and primary metabolic pathway (to acrylic acid and the relevant alcohol) result in the similarity of their toxicological properties. The potential toxicity from the remaining parental acrylate esters is considered to be minimal due to a fast metabolism with short half-lives. Alcohols are not expected to make a significant contribution to the systemic toxicity profiles of acrylate esters.

All data on environmental fate are available and there are only few data gaps in the ecotoxicology dataset of the acrylate esters. Similar patterns have been demonstrated for the ecotoxicity: all the members of the category are toxic for the aquatic organisms. In the environment, the acrylate esters will have a similar behaviour: they are all rapidly biodegradable, have a low potential of bioaccumulation and adsorption to soil is not expected.

The read across strategy that has been applied within the category has been demonstrated to provide an acceptable level of confidence. Read-across from the studies on the source substances are considered as an appropriate adaptation to the standard information requirements of Annex VII, VIII, IX and X of the REACH Regulation for the target substance, in accordance with the provisions of Annex XI, 1.5 of the REACH Regulation.

11. References

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Annex 1. Data matrix for acrylic acid and lower alkyl acrylates

Physico-chemical properties

Property	AA	MA	EA	tBA	iBA	nBA	2EHA
Physical state (20 °C, 101.3 kPa)	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Freezing point [°C]	13	-76.5	-71.2	-69	-61	-64.6	-90
Boiling point [°C]	141	80.1	99.8	119.2	132	147	215
Relative density	1.05	0.95	0.92	0.87	0.89	0.90	0.88
Vapour pressure [hPa]	5.29 (25 °C)	90 (20 °C)	40 (21 °C)	20 (23 °C)	10 (25 °C)	5 (22 °C)	0.24 (25 °C)
Water solubility [g/L]	1 000 (25 °C)	60 (25 °C)	20 (20 °C)	2 (20 °C)	1.8 (25 °C)	1.7 (20 °C)	0.01 (25 °C)
Partition coefficient n-octanol/water (Log value)	0.46	0.74	1.18	2.32	2.38	2.38	4.00
Surface tension	Not surface active	Not surface active	Not surface active	Not surface active	Not surface active	Not surface active	Not surface active
Flammability	Flammable	Highly flammable	Highly flammable	Highly flammable	Flammable	Flammable	Not-flammable (Combustible liquid – GHS)
Self-ignition temp. [° C]	438	468	372	400	350	275	252
Flashpoint [°C]	48.5	-2.8	9	14	30	37	86
Explosiveness	Non explosive	Non explosive	Non explosive	Non explosive	Non explosive	Non explosive	Non explosive
Oxidising properties	Not oxidising	Not oxidising	Not oxidising	Not oxidising	Not oxidising	Not oxidising	Not oxidising
Dissociation constant (pKa)	4.26 (25 °)	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Viscosity [mPa.s]	1.149 (25 °C)	0.472 (25 °C)	0.5351 (25 °C)	0.9 (20 °C)	0.82 (20 °C)	0.88 (20 °C)	1.75 (20 °C)

Environmental fate properties

Substance (MW)	AA (72.1)	MA (86.1)	EA (100.1)	tBA (128.2)	iBA (128.2)	nBA (128.2)	2EHA (184.3)
Physico-chemical							
Vapour pressure [hPa]	5.29 (25 °C)	90 (20 °C)	40 (21 °C)	20 (23 °C)	10 (25 °C)	5 (22 °C)	0.24 (25 °C)
Henry's Law constant [Pam³/mol]	0.029	9.3	12.5	21.9	21.9	21.9	461
Water solubility [g/L]	1 000 (25 °C)	60 (25 °C)	20 (20 °C)	2 (20 °C)	1.8 (25 °C)	1.7 (20 °C)	0.01 (25 °C)
Partition coefficient (Log Pow)	0.46	0.74	1.18	2.32	2.38	2.38	4.00
Degradation							
Hydrolysis (DT_{50}) pH3 pH7 pH11	 > 1 yr > 1 yr > 1 yr	 > 28 d > 28 d 1.8 h	 > 1 yr > 1 yr 182 h	 - > 1 yr (QSAR) -	 - > 1 yr (QSAR) -	 > 1 yr > 1 yr 4.05 h	 22.2 d 8.75 d 18.5 h
Phototransformation in air (DT_{50}) (24-h day, 0.5^6 OH/cm³) (QSAR)	39.6 h	40.9 h	35.4 h	39.7 h	27.98 h	27.98 h	19.15 h
Biodegradation in water (screening) % degradation (day)	Readily biodegradable 95 (9 d) 81 (28 d)	Readily biodegradable 90-100 (28 d)	Readily biodegradable 80-90 (28 d)	Moderately biodegradable 59 (28 d)	Readily biodegradable 87 (28 d)	Readily biodegradable 80-90 (28 d)	Readily biodegradable 70-80 (15 d)
Environmental distribution							
Adsorption/Desorption Measured Koc Calculated Koc	 42.8 1.2	 NDA 6.4	 42.2 11.9	 NDA 26.1	 NDA 33.8	 88.4 35.4	 NDA 360
Mackay I calculation (%) Air Water Soil Sediment	 1.3 98.7 0.02 0.02	 81.9 18 0.01 0.01	 87.5 12.4 0.01 0.02	 97.8 2.1 0.1 0.1	 95.78 4.06 0.08 0.08	 94.55 5.24 0.1 0.1	 91 1.12 3.38 3.92
Bioaccumulation							
BCF (QSAR)	3.16	3.16	2.0	15.8	17.3	17.3	70

Ecotoxicological properties

Substance (MW)	**AA (72.1)**	**MA (86.1)**	**EA (100.1)**	**tBA (128.2)**	**iBA (128.2)**	**nBA (128.2)**	**2EHA (184.3)**
Short-Term Toxicity Testing on Fish (LC_{50}) (Fresh Water)	27 mg/L	3.4 mg/L	4.6 mg/L	5.2 mg/L	2.1 mg/L	2.37 mg/L	1.81 mg/L
Short-Term Toxicity Testing on Fish (LC_{50}) (Marine Water)	236 mg/L	1.1 mg/L	2.0 mg/L	2.1 mg/L	**Read-across from nBA**	**Read-across from nBA**	**Read-across from MA, EA and nBA**
Long-Term Toxicity Testing on Fish	No data available	No data available	No data available	No data available	No data available	No data available	No data available
Short-Term Toxicity Testing on Invertebrates (EC_{50})	47 mg/L (fresh water) 97 mg/L (LC50) (marine water)	2.6 mg/L (fresh water) 1.6 mg/L (marine water)	7.9 mg/L (fresh water)	8.2 mg/L (fresh water)	**Read-across from nBA**	8.74 mg/L (fresh water)	1.3 mg/L
Long-Term Toxicity Testing on Invertebrates	12 mg/L (NOEC)	**Read-across from nBA and EA**	0.19 mg/L (NOEC)	0.136 mg/L (NOEC)	**Read-across from nBA and EA**	**Read-across from nBA and EA**	**Study ongoing**
Growth Inhibition Study Aquatic Plants (E_rC_{50})	0.13 mg/L	3.55 mg/L	4.5 mg/L (Cell number)	2.65 mg/L (Cell number)	5.28 mg/L	14.6 mg/L	1.71 mg/L
Algae (NOEC)	0.03 mg/L (E_rC_{10})	No data available	No data available	No data available	0.82 mg/L	3.85 mg/L	0.45 mg/L
Activated Sludge Respiration Inhibition	EC_{20} (30 min) 900 mg/L	EC_{10} (3d) > 100 mg/L	EC_{10} (72h) > 100 mg/L	EC_0 (3d) > 150 mg/L	EC_{20} (30 min) > 1000 mg/L	EC_{20} = *ca.* 950 mg/L	EC_{20} (30 min) > 1000 mg/L

Human health properties

	AA	MA	EA	nBA	iBA	tBA	2EHA
Acute oral (LD50: mg/kg bw)	>1000<2000 (rat)	768 (rat)	1120 (rat)	3150 (rat)	4895 (rat)	1047 (rat)	4435 (rat)
Acute inhalation (LD50: mg/L)	> 5.1 (rat)	10.4 (rat)	9.1 (rat)	10.3 (rat)	10.5 (rat)	7.01 (rat)	> 1.19 mg/L air (rat)
Acute dermal (LD50: mg/kg bw)	>2000 (rabbit)	1250 (rabbit)	3049 (rat)	2000 (rabbit)	793 (rabbit)	2000 (rabbit)	7522 (rabbit)
Skin irritation	Corrosive	Irritating	Irritating	Irritating	Irritating	Irritating	Irritating
Eye irritation	Corrosive	Serious eye damage	Irritating	Irritating	Not irritating	Not irritating	Not irritating
Skin sensitisation	Not sensitising	Sensitising EC3 = 19.6%	Sensitsing EC3 = 36.8%	Sensitising EC3 = 11.2%	**RA (n-Butyl acrylate)**	**RA (n-Butyl acrylate)**	Sensitising EC3 = 9.7%
Repeated dose toxicity (oral NOAEL)	83 mg/kg bw (similar to OECD 408, rat) 40 mg/kg bw (similar to OECD 452, rat)	5 mg/kg bw (similar to OECD 408, rat)	<20 mg/kg bw (similar to OECD 408, rat) 55 mg/kg bw (similar to OECD 408, rat)	84 mg/kg bw (similar to OECD 408, rat)	**RA (n-Butyl acrylate)**	-	-
Repeated dose toxicity (inhalation NOAEC)	Systemic: >0.22 mg/L (>75 ppm) Local: 0.07 mg/L (25 ppm) (similar to OECD 413, rat) Systemic: 0.015 mg/L (5 ppm) Local: <0.015 mg/L (<5 ppm) (similar to OECD 413, mice)	Systemic and local: 0.08 mg/L (23 ppm) (similar to OECD 413, rat)	Systemic: 0.10 mg/L (25 ppm) Local: 0.02 mg/L (5 ppm) (similar to OECD 413 and 453, rat and mice)	Systemic: 0.57 mg/L (108 ppm) Local: 0.11 mg/L (21 ppm) (similar to OECD 413, rat)	**RA (n-Butyl acrylate)**	Systemic and local: 0.32 mg/L (60 ppm) (OECD 413/422 study, rat)	Systemic: 0.23 mg/L (30 ppm) Local: 0.075 mg/L (10 ppm) (OECD 413, rat)
Genetic toxicity							
- Ames test	Negative	Negative	Negative	Negative	Negative	Negative	Negative

	AA	**MA**	**EA**	**nBA**	**iBA**	**tBA**	**2EHA**
- In vitro clastogenicity	CA: Positive	CA: Positive at >60% cytotoxicity	CA: Positive	MN: Negative CA: Negative SCE: Positive Mammalian cell gene mutation assay (thymidine kinase (TK) locus and structural chromosome aberrations): Negative (OECD TG 490)	Waived – *in vivo* study available	Waived – *in vivo* study available	MN: Negative CA: Inconclusive
- *In vitro* mutagenicity in mammalian cells	TK: Positive HPRT: Negative	TK: Negative and Positive at cytotoxicity HPRT: Negative	TK: Negative and Positive at cytotoxicity HPRT: Negative	UDS: Negative Mammalian cell gene mutation assay (thymidine kinase (TK) locus and structural chromosome aberrations): Negative (OECD TG 490)	**RA (Methyl acrylate and Ethyl acrylate)**	HPRT: Negative	TK: Positive HPRT: Negative
- In vivo genotoxicity	CA: Negative DLA: Negative	MN: Negative	CA: Negative MN: Negative OECD TG 488 (gpt Delta mouse): Negative	CA: Negative	MN: Negative	MN: Negative	CA: Inconclusive UDS: Negative

	AA	MA	EA	nBA	iBA	tBA	2EHA
Carcinogenicity	Negative (Rat, oral) Negative (Mice, dermal)	Negative (Rat, inhalation)	Negative (Rat, oral), forestomach tumors at cytotoxic concentrations Negative (Rat, inhalation) Negative (Mice, dermal)	Negative (Rat, inhalation) Negative (Mice, dermal)			Negative (Mice, dermal), skin tumors at doses exceeding the MTD and in an immunocompromised mouse model only

	AA	MA	EA	nBA	iBA	tBA	2EHA
Fertility	NOAELs: P (general): 240 mg/kg bw F1 (general): 53 mg/kg bw F2 (general): 53 mg/kg bw P/F1 (fertility): 460 mg/kg bw (rat. oral, OECD 416) NOAELs: P (general): 83 mg/kg bw P (fertility): 250 mg/kg bw F1 (general): 250 mg/kg bw (oral, OECD 415)	NOAELs: Parental: 0.02 mg/L (5 ppm) Fertility: >0.27 mg/L (75 ppm) Developmental: 0.09 mg/L (25 ppm) (rat, inhalation, OECD 416)	No effects reproduction organs in repeated dose toxicity studies **RA (Methyl acrylate)**	No effects reproduction organs (Inhalation, similar to OECD 413) P0 NOAEL systemic ≥150 mg/kg P0 reproductive effects: ≥150 mg/kg P0 LOAEL local effects (non-glandular stomach) 150 mg/kg F1 NOAEL systemic ≥150 mg/kg F1 LOAEL local effects (non-glandular stomach) 150 mg/kg (EOGRTS, OECD 443, oral, rats)	**RA (Methyl acrylate)**	NOAELs: Parental: 0.32 mg/L (60 ppm) Fertility: 0.32 mg/L (60 ppm) (inhalation, OECD 413/422 study) **RA (Methyl acrylate, n-Butyl acrylate, and 2-Ethylhexyl acrylate)**	No effects reproduction organs (Inhalation, OECD 413) **RA (Methyl acrylate and n-Butyl acrylate**

	AA	MA	EA	nBA	iBA	tBA	2EHA
Developmental (rat) NOAEC/NOAEL	NOAELs: Maternal: 0.12 mg/L Developmental: 1.1 mg/L (inhalation, rat, OECD 414)	NOAELs: Maternal: 0.09 mg/L (25 ppm) Developmental: 0.18 mg/L (50 ppm) (inhalation, rat, similar to OECD 414)	NOAELs: Maternal: 0.41 mg/L (100 ppm) Developmental: >0.82 mg/L (200 ppm) (inhalation, rat, similar to OECD 414) NOAELs: Maternal: 0.21 mg/L (50 ppm) Developmental: >0.62 mg/L (150 ppm) (inhalation, rat, similar to OECD 414)	NOAELs: Maternal: 100 mg/kg bw Developmental: 1000 mg/kg bw (oral, mice, similar to OECD 414) NOAELs: Maternal: 0.13 mg/L (25 ppm) Developmental: 0.13 mg/L (25 ppm) (inhalation, rat, similar to OECD 414) Maternal: <0.52 mg/L (<100 ppm) Developmental: 0.52 mg/L (100 ppm) (inhalation, rat, similar to OECD 414)	**RA (n-Butyl acrylate)**	Maternal: NOAEL systemic: ≥120 mg/kg NOEL local effects (non-glandular stomach) 30 mg/kg NOAEL developmental: ≥ 120 mg/kg (OECD 414, rat, oral) NOAELs: Maternal: 0.32 mg/L (60 ppm) Developmental: 0.32 mg/L (60 ppm) (inhalation, OECD 413/422 study) **RA (Methyl acrylate and n-Butyl acrylate)**	NOAELs: Maternal: 0.56 mg/L (75 ppm) Developmental: 0.75 mg/L (100 ppm) (inhalation, similar to OECD 414)

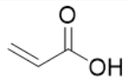
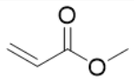
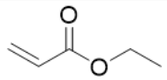
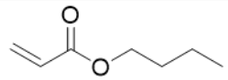
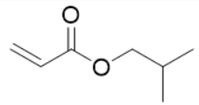
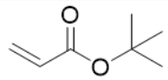
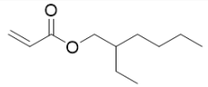
	AA	MA	EA	nBA	iBA	tBA	2EHA
Developmental (rabbit) NOAEC/NOAEL	NOAEC Maternal: 0.075 mg/L NOAEC Developmental: 0.673 mg/L (inhalation, OECD 414)	NOAEC Maternal: 0.06 mg/L (15 ppm) NOAEC Developmental: >0.16 mg/L (45 ppm) (inhalation, OECD 414)	**RA (Methyl – and n-Butyl acrylate)**	NOAEL maternal systemic: ≥ 400 mg/kg NOAEL developmental toxicity: ≥ 400 mg/kg (oral, OECD 414)	**RA (Methyl – and n-Butyl acrylate)**	**RA (Methyl – and n-Butyl acrylate)**	**RA (Methyl – and n-Butyl acrylate)**

CA = Chromosome aberration; HPRT = In Vitro Mammalian Cell Gene Mutation Tests using the Hprt gene; DLA = Dominant Lethal Assay, MN = Micronucleus; RA = Read-across, TK = In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene, UDS = Unscheduled DNA Synthesis

EU CLP Classification and labelling (self-classification)

	AA	MA	EA	nBA	iBA	tBA	2EHA
Flammability	Flam. Liquid 3	Flam. Liquid 2	Flam. Liquid 2	Flam. Liquid 3	Flam. Liquid 3	Flam. Liquid 2	-
Acute oral toxicity	Acute Tox. 4	Acute Tox. 4	Acute Tox. 4	-	-	Acute Tox. 4	-
Acute dermal toxicity	-	Acute Tox. 4	Acute Tox. 4	-	Acute Tox. 4	Acute Tox. 4	-
Acute inhalation toxicity	Acute Tox. 4	Acute Tox. 3	Acute Tox. 3	Acute Tox. 4	Acute Tox. 4	Acute Tox. 3	-
Skin corrosion/irritation	Skin Corr. 1A	Skin Irrit. 2	Skin Irrit. 2	Skin Irrit. 2	Skin Irrit. 2	Skin Irrit. 2	Skin Irrit. 2
Serious eye damage/irritation	-	Eye Irrit. 2	Eye Irrit. 2	Eye Irrit. 2	-	-	-
Sensitising	-	Skin Sens. 1B	Skin Sens. 1B	Skin Sens. 1B	Skin Sens. 1B	Skin Sens. 1B	Skin Sens. 1B
Specific target organ toxicity - single	STOT Single Exp. 3	STOT Single Exp. 3	STOT Single Exp. 3	STOT Single Exp. 3	STOT Single Exp. 3	STOT Single Exp. 3	STOT Single Exp. 3
Short-term aquatic	Aquatic Acute 1	-	-	-	-	-	-
Long-term aquatic	Aquatic Chronic 2	Aquatic Chronic 3	Aquatic Chronic 3	Aquatic Chronic 3	Aquatic Chronic 3	Aquatic Chronic 2	Aquatic Chronic 3

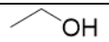
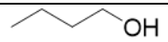
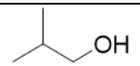
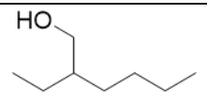
Annex 2. QSAR Toolbox output for acrylate esters and acrylic acids

Substance name	Acrylic acid	Methyl acrylate	Ethyl acrylate	n-Butyl acrylate	Isobutyl acrylate	tert-Butyl acrylate	2-Ethylhexyl acrylate
Structure							
CAS number	**79-10-7**	**96-33-3**	**140-88-5**	**141-32-2**	**106-63-8**	**1663-39-4**	**103-11-7**
Organic functional groups	Alkene; Carboxylic acid; Acrylic acids	Alkene; Carboxylic acid ester; Acrylate	Alkene; Carboxylic acid ester; Acrylate	Alkene; Carboxylic acid ester; Acrylate	Alkane, branched with tertiary carbon; Alkene; Carboxylic acid ester; Acrylate; Isobutyl	Alkane, branched with tertiary carbon; Alkene; Carboxylic acid ester; Acrylate; tert-Butyl	Alkane, branched with tertiary carbon; Alkene; Carboxylic acid ester; Acrylate
Acute aquatic toxicity classification by Verhaar (Modified) v. 3.2	Class 3 (unspecific reactivity)	Class 3 (unspecific reactivity)	Class 3 (unspecific reactivity)	Class 3 (unspecific reactivity)	Class 3 (unspecific reactivity)	Class 3 (unspecific reactivity)	Class 3 (unspecific reactivity)
Aquatic toxicity classification by ECOSAR	Not Related to an Existing ECOSAR Class	Acrylates	Acrylates	Acrylates	Acrylates	Acrylates	Acrylates
DART scheme v. 1.3	Not known precedent reproductive and developmental toxic potential	Known precedent reproductive and developmental toxic potential; Vinyl amide, aldehyde and ester derivatives (21a)	Known precedent reproductive and developmental toxic potential; Vinyl amide, aldehyde and ester derivatives (21a)	Known precedent reproductive and developmental toxic potential; Vinyl amide, aldehyde and ester derivatives (21a)	Known precedent reproductive and developmental toxic potential; Vinyl amide, aldehyde and ester derivatives (21a)	Known precedent reproductive and developmental toxic potential; Vinyl amide, aldehyde and ester derivatives (21a)	Known precedent reproductive and developmental toxic potential; Vinyl amide, aldehyde and ester derivatives (21a)
Estrogen Receptor Binding v. 2.2	Non binder, non cyclic structure	Non binder, non cyclic structure	Non binder, non cyclic structure	Non binder, non cyclic structure	Non binder, non cyclic structure	Non binder, non cyclic structure	Non binder, non cyclic structure
Repeated dose (HESS) v. 3.10	Not categorized	Not categorized	Urethane (Renal toxicity) Alert	Not categorized	Not categorized	Not categorized	Not categorized
Toxic hazard classification by Cramer v. 2.4	Intermediate (Class II)	Low (Class I)	Low (Class I)	Low (Class I)	Low (Class I)	Low (Class I)	Low (Class I)
DNA binding OASIS v. 1.6	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
DNA binding OECD v. 2.3	No alert found	Michael addition >> Polarised Alkenes-Michael addition >> Alpha, beta-unsaturated esters	Michael addition >> Polarised Alkenes-Michael addition >> Alpha, beta- unsaturated esters	Michael addition >> Polarised Alkenes-Michael addition >> Alpha, beta- unsaturated esters	Michael addition >> Polarised Alkenes-Michael addition >> Alpha, beta- unsaturated esters	Michael addition >> Polarised Alkenes-Michael addition >> Alpha, beta- unsaturated esters	Michael addition >> Polarised Alkenes-Michael addition >> Alpha, beta- unsaturated esters
DNA Alerts for Ames by OASIS v.1.4	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
DNA Alerts for CA and MNT by OASIS v.1.1	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found

Protein binding OASIS v. 1.6	No alert found	Michael addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds	Michael addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds	Michael addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds	Michael addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds	Michael addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds	Michael addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds
Protein binding OECD v. 2.3	No alert found	Michael addition >> Polarised Alkenes >> Polarised alkene - esters	Michael addition >> Polarised Alkenes >> Polarised alkene - esters	Michael addition >> Polarised Alkenes >> Polarised alkene - esters	Michael addition >> Polarised Alkenes >> Polarised alkene - esters	Michael addition >> Polarised Alkenes >> Polarised alkene - esters	Michael addition >> Polarised Alkenes >> Polarised alkene - esters
Protein binding potency GSH v. 3.4	Not possible to classify according to these rules (GSH)	Highly reactive (GSH) >> Acrylates (MA)	Highly reactive (GSH) >> Acrylates (MA)	Highly reactive (GSH) >> Acrylates (MA)	Highly reactive (GSH) >> Acrylates (MA)	Moderately reactive (GSH) >> Alkyl 2-alkenoates (MA)	Highly reactive (GSH) >> Acrylates (MA)
Protein binding potency Cys (DPRA 13%) v. 1.0	Grey zone 9-21% (DPRA 13%) >> alpha, beta-unsaturated acids	DPRA above 21% (DPRA 13%) >> Conjugated alpha, beta-unsaturated esters (reactive)	DPRA above 21% (DPRA 13%) >> Conjugated alpha, beta-unsaturated esters (reactive)	DPRA above 21% (DPRA 13%) >> Conjugated alpha, beta-unsaturated esters (reactive)	DPRA above 21% (DPRA 13%) >> Conjugated alpha, beta-unsaturated esters (reactive)	Out of mechanistic domain	DPRA above 21% (DPRA 13%) >> Conjugated alpha, beta-unsaturated esters (reactive)
Protein binding potency Lys (DPRA 13%) v. 1.0	DPRA less than 9% (DPRA 13%) >> No protein binding alert	DPRA above 21% (DPRA 13%) >> Conjugated alpha,beta-unsaturated esters (reactive)	DPRA above 21% (DPRA 13%) >> Conjugated alpha,beta-unsaturated esters (reactive)	DPRA above 21% (DPRA 13%) >> Conjugated alpha,beta-unsaturated esters (reactive)	DPRA above 21% (DPRA 13%) >> Conjugated alpha,beta-unsaturated esters (reactive)	Out of mechanistic domain	DPRA above 21% (DPRA 13%) >> Conjugated alpha,beta-unsaturated esters (reactive)
Protein binding alerts for Chromosome aberration by OASIS v. 1.5	AN2 >> Michael addition to alpha, beta-unsaturated acids and esters >> alpha, beta - Unsaturated Carboxylic Acids and Esters	AN2 >> Michael addition to alpha, beta-unsaturated acids and esters >> alpha, beta - Unsaturated Carboxylic Acids and Esters	AN2 >> Michael addition to alpha, beta-unsaturated acids and esters >> alpha, beta - Unsaturated Carboxylic Acids and Esters	AN2 >> Michael addition to alpha, beta-unsaturated acids and esters >> alpha, beta - Unsaturated Carboxylic Acids and Esters	AN2 >> Michael addition to alpha, beta-unsaturated acids and esters >> alpha, beta - Unsaturated Carboxylic Acids and Esters	AN2 >> Michael addition to alpha, beta-unsaturated acids and esters >> alpha, beta - Unsaturated Carboxylic Acids and Esters	AN2 >> Michael addition to alpha, beta-unsaturated acids and esters >> alpha, beta - Unsaturated Carboxylic Acids and Esters
Protein binding alerts for skin sensitisation by OASIS v. 1.7	No alert found	Michael Addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds	Michael Addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds	Michael Addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds	Michael Addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds	Michael Addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds	Michael Addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds
In vitro mutagenicity (Ames test) alerts by ISS v. 2.4	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
In vivo mutagenicity (Micronucleus) alerts by ISS v. 2.4	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found

Oncologic Primary Classification v. 4.1	No alert found	Acrylate Reactive Functional Groups	Acrylate Reactive Functional Groups	Acrylate Reactive Functional Groups	Acrylate Reactive Functional Groups	Acrylate Reactive Functional Groups	Acrylate Reactive Functional Groups
Carcinogenicity (genotox and nongenotox) alerts by ISS v 2.4	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	Structural alert for nongenotoxic carcinogenicity; Substituted n-alkylcarboxylic acids (Nongenotox)

Annex 3. QSAR Toolbox output for the hydrolised alcohols for acrylate esters (protein binding reactivity)

Substance name	**Methanol**	**Ethanol**	**n-butanol**	**iso-butanol**	**tert-butanol**	**2-ethylhexanol**
Structure						
CAS number	1455-13-6	64-17-5	4712-38-3	78-83-1	75-65-0	104-76-7
Chemical name	Methyl acrylate	Ethyl acrylate	n-Butyl acrylate	Isobutyl acrylate	tert-Butyl acrylate	2-Ethylhexyl acrylate
Parental acrylates						
Other identifier						
SMILES	CO	CCO	CCCCO	CC(C)CO	CC(C)(C)O	CCCCC(CC)CO
Protein binding by OASIS	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
Protein binding potency Cys (DPRA 13%)	DPRA less than 9% (DPRA 13%) >> Alcohols	DPRA less than 9% (DPRA 13%) >> Alcohols	DPRA less than 9% (DPRA 13%) >> Alcohols	DPRA less than 9% (DPRA 13%) >> Alcohols	DPRA less than 9% (DPRA 13%) >> Alcohols	DPRA less than 9% (DPRA 13%) >> Alcohols
Protein binding potency Lys (DPRA 13%)	DPRA less than 9% (DPRA 13%) >> Alcohols	DPRA less than 9% (DPRA 13%) >> Alcohols; DPRA less than 9% (DPRA 13%) >> Nonionic surfactants	DPRA less than 9% (DPRA 13%) >> Alcohols	DPRA less than 9% (DPRA 13%) >> Alcohols	DPRA less than 9% (DPRA 13%) >> Alcohols	DPRA less than 9% (DPRA 13%) >> Alcohols
Protein binding potency GSH	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)
Protein binding by OECD	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
Protein binding alerts for skin sensitization according to GHS	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
Protein Binding Potency h-CLAT	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
Protein binding alerts for skin sensitization by OASIS	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
Protein binding alerts for Chromosomal aberration by OASIS	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found