Read-across illustrative example

Part 2
Example 1 – Analogue approach: similarity based on breakdown products

Read-across illustrative example

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EDITORIAL NOTE

This illustrative example is intended to highlight crucial aspects of a hypothetical read-across situation in respect to documentation and scientific justification. The example is intended to highlight various considerations that are crucial for the assessment of a hypothetical read-across situation. The argumentation is in some aspects generic and intended to give the reader an idea of what aspects need to be addressed; rather than going into detail on how to address them. Each read-across situation may contain various case-specific considerations that may put different emphasis on various aspects covered by this example.

This illustrative example puts forward a read-across hypothesis developed between two structurally similar substances based on the similarity of their breakdown products.

This read-across approach intends to exemplify how to develop a case in order to use the results of an existing study performed with the source substance to predict the properties of the target substance.

Furthermore, this case also provides an illustration of an approach demonstrating how results from a study proposed to be performed with the source substance can be considered as adequate for fulfilling information requirements that apply to the target substance.

1. Read-across hypothesis and justification

This read-across is based on the hypothesis that source and target substances have similar toxicological properties because they hydrolyse to a common product and non-common products predicted to have no toxicological effects. This prediction is supported by toxicological data on the substances themselves and on the hydrolysis products of the substances, known rapid and extensive hydrolysis and subsequent metabolism of the substances.

The target substance is a multi-constituent substance (Table 1) composed of four main constituents. All four constituents of the target substance are esters of the acid X differing only in the number of the repetitive moiety Y (3, 4, 5 and 6) in the side chains and, consequently, in the chain length. The major constituent of the target substance (constituent 1) is identical to the source substance (mono-constituent, Table 2). Therefore, the source and the target substances share structural similarities with common functional groups, esters, and side chains varying in their length. Moreover, the side chains are chemically simple structures which have no structural alerts for toxicity and which are closely related to substances of known low toxicity.

Therefore, read-across from the existing sub-chronic toxicity study on the source substance, and testing for pre-natal developmental toxicity with the source substance is considered as an appropriate adaptation to the standard information requirements of Annex IX, 8.6.2 and 8.7.2 of the REACH Regulation for the target substance, 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.

2. The justification of the proposed read-across approach is elaborated in the next chapters.Target and source substances

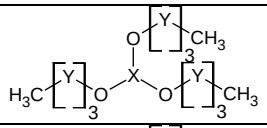
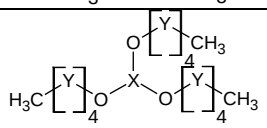
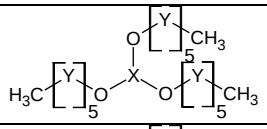
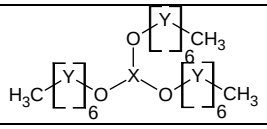
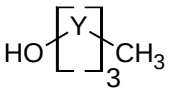
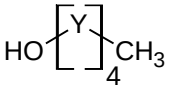
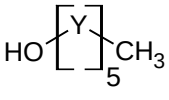
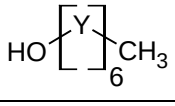
2.1 Substance identity

a. Target substance

The target substance (Table 1) is a multi-constituent substance (EC No/CAS No/IUPAC name). The constituents of the target are esters of the acid X with three identical side chains for each constituent. The side chains differ only in the number of the repetitive moiety Y (3, 4, 5 and 6) for each of the four constituents of the target substance and, consequently, in the chain length.

The typical concentration and concentration range for each of the four constituents and all identified impurities of the target substance are shown in Table 1.

Table 1. Composition of the target substance

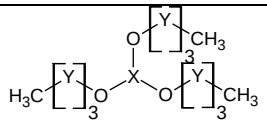
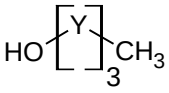
Target substance (EC No, CAS No, IUPAC name)				
Main constituents				
Constituent No	**Molecular structure**	**Min conc. (% w / w)**	**Max conc. (% w / w)**	**Typical conc. (% w / w)**
1 (EC No, CAS No, IUPAC name)		50	60	55
2 (EC No, CAS No, IUPAC name)		15	20	16
3 (EC No, CAS No, IUPAC name)		10	15	12
4 (EC No, CAS No, IUPAC name)		10	15	12
Identified impurites				
Impurity No	**Molecular structure**	**Min conc. (% w / w)**	**Max conc. (% w / w)**	**Typical conc. (% w / w)**
1 (EC No, CAS No, IUPAC name)		2	4	3
2 (EC No, CAS No, IUPAC name)		0.5	0.8	0.7
3 (EC No, CAS No, IUPAC name)		0.3	0.7	0.6
4 (EC No, CAS No, IUPAC name)		0.3	0.7	0.6

b. Source substance

The source substance (Table 2) is a mono-constituent substance, identical to the major constituent of the target substance (constituent 1, Table 1) (EC No/CAS No/IUPAC name). It contains three ester functional groups with three identical side chains, containing three Y moieties.

The typical concentration and concentration range of the single constituent and identified impurity of the source substance are shown in Table 2.

Table 2. Composition of the source substance

Source substance (EC No, CAS No, IUPAC name)				
Main constituents				
Constituent No	**Molecular structure**	**Min conc. (% w / w)**	**Max conc. (% w / w)**	**Typical conc. (% w / w)**
1 (EC No, CAS No, IUPAC name)		96	98	97
Identified impurities				
Impurity No	**Molecular structure**	**Min conc. (% w / w)**	**Max conc. (% w / w)**	**Typical conc. (% w / w)**
1 (EC No, CAS No, IUPAC name)		2	4	3

Note and comments:

Information on substance identity for each substance included in the category or analogue approach should be provided. Identifiers such as the CAS No and EC No and the chemical structures should be used to identify the substances.

c. Purity and impurities

The source and target substances are characterised by similar impurities. The impurities present in both target (Table 1) and source (Table 2) substances are the corresponding alcohol side chains containing the repetitive moiety Y. These impurities are not classified for any hazard and are not considered as hazardous for the chemical safety assessment of the target and source substances.

Note and comments:

The identification of the impurities (by chemical name, CAS number, EC number and/or molecular formula) and their hazardous profile should be considered in the proposed read-across approach. Impurities that are relevant for the classification and/or PBT assessment shall always be specified (by the same identifiers), independently from their concentration.

2.2 Structural similarity

a. Structural similarity and functional groups

The basic structures of the target and source substances are the same: the acid X of the substance forms three ester bonds with three alcohol side chains. The side chains of source and target differ in the number of a repetitive moiety (Y), being 3 for the source and 3 to 6 for the corresponding constituents of the target. The source substance is identical to the major constituent (55%) of the target substance.

b. Common breakdown products

Hydrolysis occurs rapidly when the substances come into contact with water; enzymatic reactions are not involved. The target and the source substances hydrolyse rapidly ($t_{90\%}$< 5 min) to the corresponding acid X and the alcohol side chains. The resulting acid X is a common hydrolysis product for both substances and will therefore exhibit similar toxicokinetic behaviour. This hydrolysis also results in the formation of the similar alcohol side chains which only differ in the number of repetitive moieties (3 for the source and 3 to 6 for the corresponding constituents of the target substance).

3. Physicochemical properties

Physicochemical data shows that the physicochemical profiles of the target and source substances are similar as outlined in the data matrix. The structural differences in side chains do not significantly influence the physicochemical properties of both substances. Thus, the calculated partition coefficient n-octanol/water (Log K_{ow}) is negative for both (although increasing with the molecular weight) and none of the substances are volatile (*Ref. 1-8*).

Furthermore, all the constituents of the target substance, including its main constituent, which

corresponds to the source substance, undergo rapid hydrolysis in contact with water producing the same acid X and very similar alcohol side chains. These side chains differ in length by the number of a repetitive moiety (Y) and some trends in the physicochemical parameters for these side chain hydrolysed products can be observed (see data matrix on side chains in Section 7, *Ref. 9*). For example, the partition coefficient n-octanol/water (Log K_{ow}) is always negative but increases with *increase* of the molecular weight. The same is observed for the vapour pressure, although none of the alcohol side chains are volatile. Additionally, the water solubility is high for all of them (*Ref. 29-41*).

Note and comments:

A study summary must be included in the physical and chemical properties section of the technical dossier – IUCLID section 4 – to report the experimental data from the studies.

4. Toxicokinetics

No experimental data on absorption, distribution and excretion is available for the source and target substances and their hydrolysis products. The toxicokinetics assessment is based on physicochemical properties of the substances. Although the hydrolysis study indicates very rapid breakdown of both substances, exposure to the parent forms of the source and target substances cannot be completely neglected. Therefore, the toxicokinetics of both the parent, *i.e.* unhydrolysed forms of the substances, and the hydrolysis products is discussed below.

4.1 Target and source substances

Based on the physico-chemical characteristics (large molecular weight, and low Log K_{ow}), and the evidence of rapid hydrolysis, the absorption after oral administration of the target substance is expected to be low. The absorption after oral administration of the source substance is expected to be higher than that of the constituents of the target substance due to lower molecular weight. The low vapour pressure indicates that both substances are non-volatile at room temperature and thus the exposure of the substances via inhalation route is unlikely. Therefore, absorption via inhalation is not further discussed.

Dermal absorption is expected to be very low for both substances based on large molecular weight and low Log K_{ow}. Based on the low Log K_{ow} and high water solubility, both substances are expected to remain in the body fluids, and low or no bioaccumulation is expected.
The hydrolysis study indicates rapid and complete breakdown of both the target and source substance. Toxicokinetic information of the hydrolysis products are provided in section 4.2.

4.2 Hydrolysis products

An *in vitro* hydrolysis study with the target substance shows rapid hydrolysis for each constituent ($t_{90\%}$ = < 5 min) in simulated gastro-intestinal fluids (at pH 2, 4, 7 and 9), resulting in the corresponding acid X and the alcohol side chains (*Ref. 9*). This hydrolysis study suggests minimal exposure to the parent compound after oral administration. Since the source substance corresponds to the main constituent of the target substance (constituent 1, Fig. 1), the hydrolysis rate for the source substance is similar to that observed for the target substance. The results of the *in vitro* hydrolysis study conducted with the target substance demonstrate that the rate of hydrolysis of the ester bond is not dependent on the length of the side chain (for side chains up to 6 Y moieties, $t_{90\%}$ = < 5 min.) (*Ref. 9*).

The target and the source substances hydrolyse rapidly to the corresponding acid X and the alcohol side chains. The resulting acid X is a common hydrolysis product for both substances and will therefore exhibit similar toxicokinetic behaviour.

The other hydrolysis products are the alcohol side chains, which differ only in the length (structures shown in data matrix). The oral absorption of these side chains is expected to increase as the hydrophobicity increases. Supporting information indicates that side chains with 5, 6 and 7 moieties rapidly break into smaller side chains which are well-characterised non-toxic compounds (*Ref. 42*). Since the side-chains with length 3 and 4 repeat units are intermediates in the breakdown pathway of the longer side chains, it can be concluded that they are metabolised in the same way, and that differences in the number of repeat units will not lead to any significant difference in toxicokinetic properties.

Note and comments:

A robust study summary must be included in the toxicokinetic section of the technical dossier – IUCLID section 7.1 – to report the experimental data from the *in vitro* hydrolysis study referred to in this paragraph.

Uncertainty:
No data on toxicokinetics of the target and source substances is available. The metabolism of the side chains is supported only by literature data. However, no other metabolic pathways are expected for these kinds of substance.
If no data on toxicokinetics is available, an assessment based on physicochemical and existing toxicological data should be provided.
Details of the physicochemical properties of the source substance should be provided in the read-across justification document, attached to the IUCLID dossier.
A robust study summary must be included in the toxicokinetics, metabolism and distribution section of the technical dossier – IUCLID section 7.1 – to report the experimental data from the studies, or the assessment of the toxicological properties.

5. Comparison of data from human health endpoints

5.1 Toxicity data of the target and source substances

Experimental data obtained with the source and target substances indicate that both substances have low oral and dermal acute toxicity (LD_{50} > 2 000 mg/kg bw). The substances are not irritating to skin and eye, are not sensitising, and are negative in the Ames test, *in vitro* chromosome aberration and *in vitro* mammalian gene mutation tests (*Ref. 10-25*).

For the source substance a reliable sub-chronic toxicity study (OECD 408) is available. NOAEL of 450 mg/kg bw/day was obtained based on slightly decreased mean body weight and food consumption, and liver hypertrophy. No adverse effects were observed in the reproductive organs examined (*Ref. 28*).

Results from a combined repeated dose toxicity with the reproduction/developmental toxicity screening test (OECD 422) are available for both the target and source substances. Both substances showed moderate signs of maternal toxicity at the highest dose tested (1 000 mg/kg bw/day) with slight weight loss and decreased food consumption. No reproductive/developmental adverse effects were observed. Maternal NOAEL for both substances is 650 mg/kg bw/day and for reproduction/development > 1 000 mg/kg bw/day (*Ref. 26-27*).

The results suggest similar local and systemic toxicity profiles for both substances and thus

support the proposed read-across.

5.2 Toxicity of the hydrolysis products

No toxicological data is available for the acid X. However, for the purpose of this read-across, the toxicological properties of the acid X are or will be indirectly tested as a consequence of the hydrolysis of the target and source substances.

Toxicity data is available for the side chains containing 3, 5 and 6 Y moieties. The acute oral toxicity studies (LD_{50} 10 500 mg/kg bw for 3 Y moiety, and > 3 000 mg/kg for 6 Y moiety), oral repeated dose toxicity studies (NOAEL > 1 500 mg/kg bw/day for 3 Y moiety, and > 2 000 mg/kg bw/day for 5 Y moiety) and pre-natal developmental toxicity study (NOAEL > 1 000 mg/kg bw/day for 3 and 6 Y moieties) demonstrate the absence of toxicity in these studies for these side chains. No adverse effects were observed in any of these studies. While the reliability of some of these studies are assigned a Klimisch score of 2 due to non-guideline studies and limited reporting of some experimental conditions and results, the data is considered as adequate and reliable enough to serve as supporting data for this read-across hypothesis (*Ref. 43-48*). Based on the available information on the toxicity of the side chains containing 3, 5 and 6 Y moieties, no toxicity is also expected for the other side chains because they are expected to be rapidly metabolised by the same pathway (*Ref. 42*).

Note and comments:

Robust study summaries must be included in the technical dossier – IUCLID section 7.
It is not sufficient to quote references in the literature. A study summary for each reference should be reported in the IUCLID dossier under the relevant endpoint.

5.3 Classification and labelling

The substances and their breakdown products are not classified for any human health hazard.

Note and comments:

The classification applying to a substance used as source substance in a read-across approach also applies to the substance used as target substance when this classification is relevant to the endpoint(s) that is/are read across.

6. Conclusion

The structural similarities between the source and the target substances and the similarities in their breakdown products presented above support the read-across hypothesis. Adequate, reliable and available scientific information indicates that the source and target substances and their subsequent degradation products have similar toxicity profiles.

The source substance is identical to the major constituent (55%) of the target substance. Hydrolysis data shows that both source and target substances are subject to rapid hydrolysis to acid X and the alcohol side chains containing 3, 4, 5 and 6 Y moieties. Thus, systemic exposure will be predominantly to these breakdown products.

In the combined repeated dose and reproduction/developmental toxicity studies conducted with target or source substance, only mild systemic toxicity was observed for both substances (NOAELs 650 mg/kg bw/day). Signs of moderate systemic toxicity were observed in the oral sub-chronic repeated dose toxicity study (90-days) conducted with the source substance (NOAEL 450 mg/kg bw/day). No toxicity was observed in sub-chronic repeated dose toxicity studies conducted with the breakdown products containing 3 and 5 Y moieties (NOAELs 1 500 and 2 000 mg/kg bw/day, respectively). Furthermore, based on the metabolism study, the side chains are expected to follow the same metabolic pathways leading to rapid metabolic clearance.

The hypothesis of rapid, and common, metabolic clearance of the side chains of different length is consistent with the toxicity data available on the side chains. The results of the studies performed with the parent substance are consistent with the results obtained from studies on the breakdown products.

Therefore, based on the considerations above, it can be concluded that the results of the oral sub-chronic repeated dose toxicity study conducted in the rat with the source substance is likely to predict the properties of the target substance and are considered as adequate to fulfil the information requirement of Annex IX, 8.7.2. The dose descriptor obtained from the existing sub-chronic repeated dose toxicity study performed on the source substance is considered as an appropriate starting point for deriving a DNEL. The remaining uncertainty associated with this read-across approach is accounted for by using the appropriate assessment factors, as recommended in ECHA Guidance R.8.

Note and comments:

The establishment of Derived No-Effect Levels (DNELs) is considered to be outside of the scope of this document and is therefore not further discussed.

A prenatal developmental toxicity study is proposed to be performed with the source substance. Prenatal developmental toxicity for this substance would be mediated by systemically available chemicals (*i.e.* primarily the breakdown products of the substances), and thus much of the justification for read-across in respect of the sub-chronic repeated dose toxicity endpoint is relevant for prenatal developmental toxicity. In the light of the information on the basis for read-across for repeated dose toxicity between the source and the target substance presented above, and the available information showing an absence of reproductive or developmental toxicity of these substances from reproductive toxicity screening studies, the results from the proposed study are considered as adequate to fulfil the information requirement of Annex IX, 8.7.2 applying to the target substance.

The future results obtained with the source substance will be accepted as valid for the target substance in both possible scenarios: (i) developmental effects not observed and (ii) developmental effect observed.

No classification and labelling criteria are fulfilled by the source or target substances.

7. Data matrix

Matrix 1 - Toxicity data on the source and target substance:

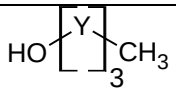
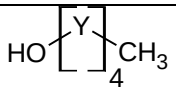
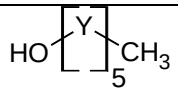
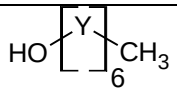
CORRESPONDING STANDARD INFORMATION REQUIRED	**SOURCE**	**TARGET**
Information on the physicochemical properties		
Water solubility	Water solubility (g/L) at 20°C; Waived based on rapid hydrolysis. 1 000 g/L (calculated, EPIWIN 40, WSKOW v1.41) *Ref. 1*	Water solubility (g/L) at 20°C; Waived based on rapid hydrolysis. >800 g/L (calculated, EPIWIN 40, WSKOW v1.41) *Ref. 2*
Partition coefficient n-octanol/water	Log K_{ow} -3.2 (calculated, EPIWIN 40, KOWWIN v1.67) *Ref. 3*	Log K_{ow} <-2.5 (calculated, EPIWIN 40, KOWWIN v1.67) *Ref. 4*
Vapour pressure	EU A.4 1 Pa at 20°C 4 Pa at 50°C	EU A.4 1 Pa at 20°C 3 Pa at 50°C
Molecular weight	500 g/mole *Ref. 7*	500 – 950 g/mole *Ref. 8*
Toxicological information		
Toxicokinetics	Hydrolysis Read-across from the target substance.	Hydrolysis An *in vitro* study in GI-fluids show rapid hydrolysis. $T_{1/2}$ <5 min. *Ref. 9*
Toxicokinetics	Assessment based on phys-chem properties: Low oral, dermal and inhalation absorption. Small volume of distribution, low/no bioaccumulation potential. Excretion via bile.	Assessment based on phys-chem properties: Low oral, dermal and inhalation absorption. Small volume of distribution, low/no bioaccumulation potential. Excretion via bile.
Acute toxicity, oral	Rat (Wistar), male/female Oral: gavage OECD guideline 401 (Acute Oral Toxicity) LD_{50} > 2 000 mg/kg 1 (reliable without restriction) Key study *Ref. 10*	Rat (Wistar), male/female Oral: gavage OECD guideline 401 (Acute Oral Toxicity) LD_{50} > 2 000 mg/kg 1 (reliable without restriction) Key study *Ref. 11*

Acute toxicity, dermal	Rat (Fischer 344), male/female Coverage: semiocclusive OECD guideline 402 (Acute Dermal Toxicity) LD_{50} > 2 000 mg/kg Key study 1 (reliable without restriction) *Ref. 12*	Rat (Fischer 344), male/female Coverage: semiocclusive OECD guideline 402 (Acute Dermal Toxicity) LD_{50} > 2 000 mg/kg Key study 1 (reliable without restriction) *Ref. 13*
Skin irritation or skin corrosion	Rabbit (New Zealand White) Coverage: semiocclusive OECD guideline 404 (Acute Dermal Irritation/Corrosion) Not irritating Key study 1 (reliable without restriction) *Ref. 14*	Rabbit (New Zealand White) Coverage: semiocclusive OECD guideline 404 (Acute Dermal Irritation/Corrosion) Not irritating Key study 1 (reliable without restriction) *Ref. 15*
Skin sensitisation	Guinea pig (Dunkin-Hartley) male/female Guinea pig maximisation test OECD guideline 406 (Skin Sensitisation) Not sensitising 1st reading (0 h after challenge): 0/20 (test group) 1st reading (0 h after challenge): 0/10 (negative control) 2nd reading (24 h after challenge): 0/20 (test group) 2nd reading (24 h after challenge): 0/10 (negative control) 3rd reading (72 h after challenge): 0/20 (test group) 3rd reading (72 h after challenge): 0/10 (negative control) Key study 1 (reliable without restriction) *Ref. 16*	Guinea pig (Dunkin-Hartley) male/female Guinea pig maximisation test OECD guideline 406 (Skin Sensitisation) Not sensitising 1st reading (0 h after challenge): 0/20 (test group) 1st reading (0 h after challenge): 0/10 (negative control) 2nd reading (24 h after challenge): 0/20 (test group) 2nd reading (24 h after challenge): 0/10 (negative control) 3rd reading (72 h after challenge): 0/20 (test group) 3rd reading (72 h after challenge): 0/10 (negative control) Key study 1 (reliable without restriction) *Ref. 17*
Eye irritation/corrosion	Rabbit (New Zealand White) OECD guideline 405 (Acute Eye Irritation/Corrosion)	Rabbit (New Zealand White) OECD guideline 405 (Acute Eye Irritation/Corrosion)

	Not irritating Key study 1 (reliable without restriction) *Ref. 18*	Not irritating Key study 1 (reliable without restriction) *Ref. 19*
Mutagenicity; *In vitro* gene mutation study in bacteria	OECD guideline 471 (Bacterial Reverse Mutation Test) *S. typhimurium* TA 98, 100, 1535, 1537, and *E. coli* WP2 uvrA (with and without metabolic activation) Doses: 1st study: 0, 10, 20, 100, 500, 2 500 and 5 000 µg/plate 2nd study: 0, 10, 20, 100, 500, 2 500 and 5 000 µg/plate Test results: negative for TA 98, TA 100, TA 1535, TA 1537, and *E. coli* WP2 uvrA, with and without S9. Key study 1 (reliable without restriction) *Ref. 20*	OECD guideline 471 (Bacterial Reverse Mutation Test) *S. typhimurium* TA 98, 100, 1535, 1537, and *E. coli* WP2 uvrA (with and without metabolic activation) Doses: 1st study: 0, 10, 20, 100, 500, 2 500 and 5 000 µg/plate 2nd study: 0, 10, 20, 100, 500, 2 500 and 5 000 µg/plate Test results: negative for TA 98, TA 100, TA 1535, TA 1537, and *E. coli* WP2 uvrA, with and without S9. Key study 1 (reliable without restriction) *Ref. 21*
Mutagenicity; *In vitro* gene mutation study in mammalian cells	OECD guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test) Negative (non-mutagenic) Test results: negative for Chinese hamster ovary (CHO) cells with and without metabolic activation. Cytotoxicity: no Key study 1 (reliable without restriction) *Ref. 22*	OECD guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test) Negative (non-mutagenic) Test results: negative for Chinese hamster ovary (CHO) cells with and without metabolic activation. Cytotoxicity: no Key study 1 (reliable without restriction) *Ref. 23*
Mutagenicity; *In vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study	OECD guideline 473 (*In vitro* Mammalian Chromosome Aberration Test) Negative Test results: negative for human lymphocytes with and without metabolic activation. Cytotoxicity: no Key study	OECD guideline 473 (*In vitro* Mammalian Chromosome Aberration Test) Negative Test results: negative for human lymphocytes with and without metabolic activation. Cytotoxicity: no Key study

	1 (reliable without restriction) *Ref. 24*	1 (reliable without restriction) *Ref. 25*
Short-term repeated dose toxicity study (28-day) / Screening study for reproductive/developmental toxicity	Rat, (Wistar) male/female Sub-acute (oral: gavage) Doses: 0, 200, 650, 1 000 mg/kg bw/day OECD guideline 422 (Combined Repeated Dose Toxicity with the Reproduction/Developmental Toxicity Screening Test) $NOAEL_{systemic}$ 650 mg/kg bw/day based on slight weight loss and decreased food consumption. $NOAEL_{reproductive/developmental}$ > 1 000 mg/kg bw/day. Key study 1 (reliable without restriction) *Ref. 26*	Rat, (Wistar) male/female Sub-acute (oral: gavage) Doses: 0, 200, 650, 1 000 mg/kg bw/day OECD guideline 422 (Combined Repeated Dose Toxicity with the Reproduction/Developmental Toxicity Screening Test) $NOAEL_{systemic}$ 650 mg/kg bw/day based on slight weight loss and decreased food consumption. $NOAEL_{reproductive/developmental}$ > 1 000 mg/kg bw/day. Key study 1 (reliable without restriction) *Ref. 27*
Sub-chronic repeated dose toxicity study (90-day)	Rat (Sprague-Dawley) male/female Sub-chronic (oral: gavage) Doses: 0, 100, 400, 1 000 mg/kg bw/day OECD guideline 408 (Repeated Dose 90-day Oral Toxicity in Rodents) NOAEL 450 mg/kg bw/day based on liver hypertrophy, and decreased body weight and food consumption. Key study 1 (reliable without restriction) *Ref. 28*	Read-across from the source substance.
Pre-natal developmental toxicity study	Testing proposal Rat (Sprague-Dawley), oral: gavage route. OECD guideline 414 (Prenatal Developmental Toxicity study) Test material: Registered substance	Testing proposal (read-across) Rat (Sprague-Dawley), oral: gavage route. OECD guideline 414 (Prenatal Developmental Toxicity study) Test material: Source substance (read-across)

Matrix 2 - Toxicity data on the side chains:

CORRESPONDING STANDARD INFORMATION REQUIRED				
Information on the physicochemical properties				
Water solubility	EU A.6 Water solubility at 20°C: 1 100 g/L *Ref. 29*	EU A.6 Water solubility at 20°C: 1 000 g/L *Ref. 30*	EU A.6 Water solubility at 20°C: 900 g/L *Ref. 31*	EU A.6 Water solubility at 20°C: 800 g/L *Ref. 32*
Partition coefficient n-octanol/water	Log K_{ow} -1.12 *Ref. 33*	Log K_{ow} -0.6 *Ref. 34*	Log K_{ow} -0.5 *Ref. 35*	Log K_{ow} -0.3 *Ref. 36*
Vapour pressure	EU A.4 1 Pa at 20°C *Ref. 37*	EU A.4 0.1 Pa at 20°C *Ref. 38*	EU A.4 0.03 Pa at 20°C *Ref. 39*	EU A.4 0.01 Pa at 20°C *Ref. 40*
Molecular weight	150 g/mole *Ref. 41*	200 g/mole *Ref. 41*	250 g/mole *Ref. 41*	300 g/mole *Ref. 41*
Toxicological endpoints				
Toxicokinetics	No data	No data	Cleavage to smaller side chains. *Ref. 42*	Cleavage to smaller side chains. *Ref. 42*
Acute toxicity, oral	Rat (Sprague-Dawley) male/female Oral: gavage OECD 401 (Acute Oral Toxicity) LD_{50} 10500 mg/kg 1 (reliable without restrictions) Key study *Ref. 43*	No data	No data	Rat (unknown strain), male Oral: gavage Similar to OECD 401 (Acute Oral Toxicity) with limitations one dose (3 000 mg/kg), N=5, no adverse effects observed LD_{50} > 3 000 mg/kg Supporting study 2 (reliable with restrictions due to non-guideline study

				and limited reporting of study details) *Ref. 44*
Short-term repeated dose toxicity study (28-day)	No data	No data	Rat, (Wistar) male Sub-acute (oral: gavage) Doses: 0, 1 000, 2 000 mg/kg bw/day Similar to OECD guideline 407 (Repeated Dose 28-day Oral Toxicity Study in Rodents), deviation only two doses tested NOAEL > 2 000 mg/kg bw/day, no adverse effects observed Supporting study 2 (reliable with restrictions due to non-guideline study and limited reporting of study details) *Ref. 46*	No data
Sub-chronic repeated dose toxicity study (90-day)	Rat (Wistar) male/female Sub-chronic (oral: gavage Doses: 0, 500, 1 000, 1 500 mg/kg bw/day OECD guideline 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents) NOAEL > 1 500 mg/kg bw/day,	No data	No data	No data

	no adverse effects observed Supporting study 2 (reliable with restrictions due to limited reporting of study details) *Ref. 45*			
Pre-natal developmental toxicity study	Rat (Wistar) male/female Oral: gavage Doses: 0, 100, 500, 1 000 mg/kg bw/day OECD guideline 414 (Prenatal Developmental Toxicity study) NOAEL >1000 mg/kg bw/day No maternal or developmental effects observed Supporting study 2 (reliable with restrictions due to limited reporting of study details) *Ref. 47*	No data	No data	Rat (Wistar) male/female Oral: gavage Doses: 0, 100, 500, 1 000 mg/kg bw/day OECD guideline 414 (Prenatal Developmental Toxicity study) NOAEL >1000 mg/kg bw/day No maternal or developmental effects observed Supporting study 2 (reliable with restrictions due to limited reporting of study details) *Ref. 48*

8. References

1 Water solubility, EPIWIN 40 (see IUCLID Section 4.8, Study a).
2 Water solubility, EPIWIN 40 (see IUCLID Section 4.8, Study b).
3 Log K_{ow}, EPIWIN 40 (see IUCLID Section 4.7, Study a).
4 Log K_{ow}, EPIWIN 40 (see IUCLID Section 4.7, Study b).
5 Vapour pressure (see IUCLID Section 4.6, Study a).
6 Vapour pressure (see IUCLID Section 4.6, Study b).
7 Molecular weight.
8 Molecular weight.
9 Hydrolysis (see IUCLID Section 7.1.1, Study a).
10 Acute oral toxicity (see IUCLID Section 7.2.1, Study a).
11 Acute oral toxicity (see IUCLID Section 7.2.1, Study b).
12 Acute dermal toxicity (see IUCLID Section 7.2.3, Study a).
13 Acute dermal toxicity (see IUCLID Section 7.2.3, Study b).
14 Skin irritation (see IUCLID Section 7.3.1, Study a).
15 Skin irritation (see IUCLID Section 7.3.1, Study b).
16 Skin sensitisation (see IUCLID Section 7.4.1, Study a).
17 Skin sensitisation (see IUCLID Section 7.4.1, Study b).
18 Eye irritation (see IUCLID Section 7.3.2, Study a).
19 Eye irritation (see IUCLID Section 7.3.2, Study b).
20 Bacterial reverse mutation test (see IUCLID Section 7.6.1, Study a).
21 Bacterial reverse mutation test (see IUCLID Section 7.6.1, Study b).
22 *In vitro* Mammalian Cell Gene Mutation Test (see IUCLID Section 7.6.1, Study c).
23 *In vitro* Mammalian Cell Gene Mutation Test (see IUCLID Section 7.6.1, Study d).
24 *In vitro* Mammalian Chromosome Aberration Test (see IUCLID Section 7.6.1, Study e).
25 *In vitro* Mammalian Chromosome Aberration Test (see IUCLID Section 7.6.1, Study f).
26 Combined repeated dose toxicity with the reproduction/developmental toxicity screening test (see IUCLID Sections 7.5.1 and 7.8.1, Study a).
27 Combined repeated dose toxicity with the reproduction/developmental toxicity screening test (see IUCLID Sections 7.5.1 and 7.8.1, Study b).
28 Repeated Dose 90-day Oral Toxicity Study in Rodents (see IUCLID Section 7.5.1, Study c).
29 Water solubility (see IUCLID Section 4.8, Study c).
30 Water solubility (see IUCLID Section 4.8, Study d).
31 Water solubility (see IUCLID Section 4.8, Study e).
32 Water solubility (see IUCLID Section 4.8, Study f).
33 Log K_{ow} (see IUCLID Section 4.7, Study c).
34 Log K_{ow} (see IUCLID Section 4.7, Study d).
35 Log K_{ow} (see IUCLID Section 4.7, Study e).
36 Log K_{ow} (see IUCLID Section 4.7, Study f).
37 Vapour pressure (see IUCLID Section 4.6, Study c).
38 Vapour pressure (see IUCLID Section 4.6, Study d).
39 Vapour pressure (see IUCLID Section 4.6, Study e).
40 Vapour pressure (see IUCLID Section 4.6, Study f).
41 Molecular weight
42 Metabolism (see IUCLID Section 7.1.1, Study b).
43 Acute oral toxicity (see IUCLID Section 7.2.1, Study c).
44 Acute oral toxicity (see IUCLID Section 7.2.1, Study d).
45 Repeated Dose 90-day Oral Toxicity Study in Rodents (see IUCLID Section 7.5.1, Study d).
46 Repeated Dose 28-day Oral Toxicity Study in Rodents (see IUCLID Section 7.5.1, Study e).
47 Pre-natal developmental toxicity study (see IUCLID Section 7.8.2, Study a).
48 Pre-natal developmental toxicity study (see IUCLID Section 7.8.2, Study b).