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Toxicity screening of potential bio-based Polar Aprotic Solvents (PAS)

Memo

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1 Introduction

One of the priority areas identified by the Dutch Safe Chemicals Innovation Agenda (SCIA) is solvents [1]. Several polar aprotic solvents (PAS) are of particular concern, being N-methyl pyrrolidone (NMP), dimethylacetamide (DMAc) and dimethylformamide (DMF) because of their hazardous toxicological properties and wide dispersive use. All three substances are categorised as substances of very high concern (SVHC) under the European chemicals legislation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), because of their toxicity for reproduction (NMP [2], DMAc [3] and DMF [4]). As NMP, DMAc and DMF are all SVHC, the aim is to ensure that these solvents are gradually replaced by less hazardous substances [5]. NMP is also under restriction from 2024 in placing on the market for use as a solvent or reactant in the process of coating wires [6].

Bio-based chemicals, made from renewable resources, have a widespread use in a diversity of sectors [7, 8] and are becoming increasingly important as alternative to fossil based compounds in view of the Sustainable Development Goals and the targets of the Paris Climate Agreement [9]. If designed in a proper way, a win-win situation is created where bio-based compounds may be safe *and* sustainable alternatives for substances of concern [10, 11]. They are therefore of special interest for substituting SVHCs under the REACH chemical legislation [12].

Several bio-based chemicals are (highly) polar, which increases the availability of 'new' bio-based substances with unique chemical structures and properties. This could be a solution to finding renewable alternatives to the disputed conventional PAS.

To this end, the Dutch Ministry of Infrastructure and Environment commissioned two studies. First an inventory, carried out by Wageningen Food and Bio-based Research (WFBR), of potential new bio-based alternatives for NMP, DMAc and DMF, published recently [13]. The term 'new' refers to the fact that these substances, albeit originating from emerging or existing bio-based sources, are not yet available on a commercial scale in large quantities. Furthermore, bio-based substances that are produced either via existing or emerging bio-based processes, that have known existing petrochemical counterparts, such as ethanol and succinic acid, are also not considered as 'new'.

The WFBR study resulted into a list of substances or classes of substances that are likely to reach a significant production volume in the coming years. The list has been prioritised by the WFBR according to qualitative criteria like feedstock availability, level of (industrial) development, whether or not a substance is already commercially produced, and its potential to serve as a solvent based on physico-chemical properties (polarity, melting and boiling point). Based on these selection criteria, WFBR identified nineteen substances and substance classes as potential new bio-based alternatives for the currently disputed PAS [13].

To avoid regrettable substitution of the disputed PAS, i.e. the replacement of PAS by a functional substitute but not a safer alternative (think e.g. of chemical structure look-a-likes in the case of a drop-in

substitution), the Dutch Ministry of Infrastructure and Environment commissioned a second study to the Dutch National Institute of Public Health and the Environment (RIVM). RIVM screened the most promising new bio-based alternatives identified by the WFBR study on their environmental health and safety aspects, as described in this report,

2 Methodology

In this chapter, the methodology of our toxicity screening of a selection of potential polar aprotic solvents is explained. First, the substance selection is described and second, the toxicity screening set-up.

2.1.1 *Substance selection*

From the abovementioned WFBR list of nineteen substances and classes of substances (see Annex E – Overview of emerging bio-based substances (and derivatives)), we made a further selection for the toxicity screening. We only took into account these substances and classes of substances that scored high both on polarity and (potential) availability¹.

As many of the substances are still in the R&D phase, we have chosen to include all substances with lowest to highest score for potential PAS replacement, as we are not sure whether low potential may change to higher potential for applicability². Additionally, a chemical had to have a Chemical Abstracts Service (CAS) registry number, to be able to identify the substance and have information on its chemical structure. The combination of these selection criteria then resulted in a list of thirteen potential new bio-based PAS replacements (see Table 1 below). The table below also indicates whether a substance has been registered under REACH in the third column (production volume in tons per annum). REACH registration requires information on intrinsic properties of a substance. The standard information to meet the registration obligations of REACH depends on the quantity of the substance that is manufactured or imported into the EU/EEA. The requirements are described in Annexes VI to X to REACH for the different production/import volumes (see section 2.1 for more information).

¹ "Polarity" was defined in the WFBR study as follows: "based on presence of polar chemical functionalities, such as oxygen containing ether, ester or ketone groups and absence of reactive substituents (e.g. hydroxyl or amine groups"; "Availability" was defined in the WFBR study as follows: "i.e. assessed based on their feedstock availability and process feasibility. Please also see Annex E.

² The PAS alternatives assessment was done by the WFBR based on qualitative assessment factors such as (expected) low melting point, -(expected) boiling point and polarity (see note 1 above).

Table 1: Selected bio-based substances for screening, in their respective chemical structure classes.

Substance	CAS No	Total tonnage/annum [†]
Cyrene	53716-82-8	0 - 10
Isosorbide dimethyl ether	5306-85-4	0 - 10
<i>Tetrahydrofurfuryl alcohol ethers</i>		
Methyl tetrahydrofurfuryl ether*	19354-27-9	n.a.
<i>Lactic acid derivatives</i>		
Butyl lactate	138-22-7	n.a.
Ethyl 2-ethoxypropionate	7737-40-8	n.a.
Ethyl lactate	97-64-3	n.a.
Methyl 2-methoxypropionate	17639-76-8	n.a.
Methyl DL-lactate	547-64-8	n.a.
<i>Levulinic acid derivatives</i>		
Methyl levulinate ethylene ketal	35351-33-8	n.a.
Levulinic ketal	42136-73-2	n.a.
Ethyl levulinate	539-88-8	10-100
Gamma-valerolactone)	108-29-2	n.a.
Methyl levulinate	624-45-3	n.a.

[†]Total tonnage is the total volume manufactured and/or imported in the European Economic Area (EEA) as indicated in the REACH dossier (information is taken from the European Chemicals Agency (ECHA) data base, accessed September 2018, <https://echa.europa.eu/home>).

n.a. means no REACH registration and hence no data available.

*Methyl tetrahydrofurfuryl ether was not specified in the WFBR study [13] as a potential bio-based alternatives for PAS. However, it was confirmed by the author to belong to the category of TetraHydrofurfuryl alcohol esters in this study (personal communication).

2.1.2 Toxicity screening

For the screening on adverse effects for human health and the environment, the focus in this study was on specific endpoints according to REACH Article 57, i.e. carcinogenicity, mutagenicity and/or reprotoxicity (CMR), persistent, bioaccumulative and toxic (PBT), very persistent and very bioaccumulative (vPvB) or equivalent concern (such as endocrine disruption) [14]. These hazard criteria coincide with the hazard criteria for the SVHC substances placed on the REACH candidate list. Note that these hazard criteria also apply to the Dutch substances of very high concern, the so-called ZZS substances (see section 2.2 for more information).

At first, data was screened on the above mentioned endpoints for the selected chemicals (section 2.2.1.3.), other hazard information, when available, is also given. This search was done using a limited number of databases.

The database screening was supplemented with expert judgement to address data gaps (section 2.3), based on the use of Quantitative Structure Activity Relationships (QSARs).

2.2 Database screening

The databases used for screening are listed below. These databases are solid starting points for finding hazard information on human health and

the environmental including toxicity, ecotoxicity, bioaccumulation and biodegradation properties, which are:

- ECHA database
- Dutch RVS database
- OECD eChemPortal

The databases searched are briefly addressed hereafter. It should be realised that there may be some overlap between the databases. No additional literature searches, e.g. open literature sources, were carried out.

2.2.1 *ECHA database*

The European Chemical Agency (ECHA) database consists of information on substances, manufactured in or imported into the European Economic Area (EEA). It consists of:

- C&L Inventory (Classification & Labelling)³
- Registered substances⁴
- SVHC Candidate list⁵
- Authorisation list⁶
- Restrictions list⁷

Below more information can be found per database or list mentioned.

2.2.1.1 C&L Inventory

The Classification, Labelling and Packaging (CLP) Regulation (EC No 1272/2008) is based on the United Nations' Globally Harmonised System (GHS) and its purpose is to ensure a high level of protection of health and the environment, as well as the free movement of substances, mixtures and articles [15]. To this purpose, manufacturers, importers or downstream users ('the notifier') have to classify and label hazardous substances and mixtures. Such notifications are called "notified classifications". For hazards of highest concern (carcinogenicity, mutagenicity, reproductive toxicity (CMR) and respiratory sensitisers) and for other substances on a case-by-case basis, classification and labelling is harmonised throughout the EU to ensure adequate risk management. Such classification is done through harmonised classification and labelling (CLH) and is thus called "harmonised classification". All harmonised classifications are listed in Annex VI to the CLP Regulation and should be applied by all manufacturers, importers or downstream users of such substances and of mixtures containing such substances [15]. For this study we included data from the C&L inventory, which contains both notified and harmonised classification. The hazard classes in CLP cover physical, health, environmental and additional hazards. Only health and environmental hazards are considered for this study; physical hazards (such as corrosivity or flammability) are out of scope.

Abbreviations used in classification and labelling can be found in "Annex A - C&L Abbreviations".

³ <https://echa.europa.eu/information-on-chemicals/cl-inventory-database>

⁴ <https://echa.europa.eu/information-on-chemicals>

⁵ <https://echa.europa.eu/candidate-list-table>

⁶ <https://echa.europa.eu/nl/authorisation-list>

⁷ <https://echa.europa.eu/substances-restricted-under-reach>

2.2.1.2 Registered substances (REACH)

REACH Regulation ((EC) No 1907/2006) requires registration of every chemical produced or imported into the EU above an annual volume of 1 ton [14]. This obligation applies to manufacturers of substances and importers of substances and mixtures. Below 1 ton per annum (tpa) per manufacturer or importer there are no registration obligations but other obligations such as the need to communicate hazards in the supply chain still apply (e.g. as for the CLP regulation). Bio-based chemicals, if produced or imported into the EU in quantities of more than 1 tpa, fall under REACH regulation and should be registered. There may be, however, specific exemptions or adapted data requirements for the registration of bio-based chemicals, such as substances listed under "REACH Annex V.8", which occur in nature and are not chemically modified. More information on REACH and bio-based chemicals can be found elsewhere [10].

ECHA compiled an inventory of substances likely to meet criteria indicating a toxicological concern. The inventory was produced using publicly available databases with experimental data and by using (Q)SAR model results. Indications for hazardous toxicological or ecotoxicological properties together with information on uses and other available relevant information can be cause for a substance to be on the Annex III [14]. The fact that a substance is not in this list does not necessarily mean that the criteria for a specific toxicological concern are necessarily met, it is only an indication that there might be cause for concern. All sources used for compiling Annex III under REACH are described in the REACH document "Preparation of the Annex III inventory – Technical Documentation, 18 May 2016"⁸. The inventory itself can be found (and filtered) at the ECHA website⁹.

2.2.1.3 SVHC Candidate list, Authorisations and Restrictions under REACH

Authorization and restriction are REACH instruments to protect human health and the environment from unacceptable risks posed by chemicals.

SVHC Candidate list and Authorisations

Member States or the European Chemicals Agency (ECHA) may propose a substance to be identified as an SVHC by preparing a dossier in accordance with the requirements set out in Annex XV to REACH.

SVHCs meet the hazard criteria as set out in Article 57 a-f of REACH:

- a) Carcinogenic category 1A or 1B according to Regulation (EC) 1272/2008;
- b) Mutagenic category 1A or 1B according to Regulation (EC) 1272/2008;
- c) Toxic for reproduction category 1A or 1B according to Regulation (EC) 1272/2008;
- d) Persistent, Bioaccumulative and Toxic in accordance with the criteria set out in REACH Annex XIII

⁸https://echa.europa.eu/documents/10162/22332820/annex_iii_preparation_inventory_en.pdf/e42ea5b1-28f0-4390-8d34-6d09e2875ebd

⁹ <https://echa.europa.eu/information-on-chemicals/annex-iii-inventory>

- e) Very Persistent and Very Bioaccumulative in accordance with the criteria set out in REACH Annex XIII;
- f) Substances for which there is scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern to those of other substances listed above (for example respiratory sensitising properties or endocrine disrupting properties).

If a substance is identified as an SVHC, it will be added to the Candidate List for eventual inclusion in the Authorisation List (Annex XIV). Once on this Authorisation list, substances cannot be placed on the market or used after a given date ("sunset date"), unless an authorization is granted for their specific use, or the use is exempted from authorization.

Restrictions (Annex XVII)

Restrictions are normally used to limit or ban the manufacture, placing on the market (including imports) or use of a substance, but can impose any relevant condition, such as requiring technical measures or specific labels. A restriction may apply to any substance on its own, in a mixture or in an article, including those that do not require registration, for example, substances manufactured or imported below one tonne per year or certain polymers.

A Member State, or ECHA, at the request of the European Commission, can start the restriction procedure when they are concerned that a certain substance poses an unacceptable risk to human health or the environment. ECHA can also propose a restriction on articles containing substances that are on the Authorisation List (Annex XIV). Once the restriction has been adopted and put on this list, industry must comply (i.e. manufacturers, importers, distributors, downstream users and retailers).

2.2.2 *Dutch RVS database*

The Dutch RVS ("Risico's van stoffen") database, managed by RIVM, contains current and authorised information on hazards/risks of substances for human health and the environment [16]. For this report we focus on the so called ZZS list and potential ZZS list, that are both part of the RVS database.

The Netherlands has legislation to limit industrial emissions of hazardous substances focusing on 'priority substances', the so called substances of very high concern ('Zeer Zorgwekkende Stoffen'). The ZZS substances are identified based on the same hazard criteria as the SVHC (Substance of Very High Concern) chemicals, i.e. REACH Article 57 (see also section 2.1.1.3).

For the ease of reference a non-limitative list¹⁰ is compiled of almost 1500 substances complying to the ZZS criteria. ZZS substances are identified for this list if they are placed on one of the following authoritative lists:

- Substances which are classified as C, M, or R category 1A or 1B according to Regulation (EC) 1272/2008;

¹⁰ ZZS-list: <https://rvs.rivm.nl/zoeksysteem/ZZSlijst/Index>

- Substances on the SVHC Candidate list for REACH Annex XIV;
- Substances which are on the POPS regulation (EC) 850/2004;
- Priority Hazardous substances according to the Water Framework Directive 2000/60/EC;
- Substances on the OSPAR list for priority action.

Within the Netherlands the ZZS policy focuses on the substitution of these substances by less harmful alternatives or, if not possible, on the prevention or minimisation of exposure.

Recently, a list of potential ZZS has been published by RIVM that can serve as a guidance for the environmental permitting procedure. Aim is to enhance the alertness for potential hazardous substances that may be ZZS but have not been identified as such. This can be due to lack of data or the fact that data have not been evaluated to conclude whether the substance meets the REACH Article 57 hazard criteria. The potential ZZS list is composed from the following lists:

- Public Activities Coordination Tool (PACT);
- Community Rolling Action Plan (CoRAP);
- Registry of Intentions (RoI).

These three lists are the result of the REACH SVHC roadmap and/or concerns that are raised by member states.

The most recent potential ZZS list can be found on the RVS website, including more background information on the selection procedure. Currently (version August 2018) the potential ZZS list contains 323 entries [17].

2.2.3 *OECD eChemPortal*

The OECD eChemPortal contains links to a collection of chemical hazard and risk information sources prepared for governmental chemical review programmes at national, regional and international levels. In addition, eChemPortal provides also exposure and use information on chemicals. Currently compiled in eChemPortal are more than 30 databases from all over the world [18]. The list with the available datbes is shown in "Annex B - OECD eChemPortal databases".

2.3 **QSAR estimations**

Next to the screening in the public databases, QSAR models were applied to predict hazard properties for both human health and the environment. This was particularly important for chemicals lacking relevant experimental data. DEREK [19] and the OECD QSAR toolbox [20] were used to screen for possible human health hazard (CMR) properties of the selected thirteen substances. A PBT screening tool developed by RIVM was used for screening of potential PBT properties [21]. For the weighing of the QSAR results in drawing a conclusion on the hazard profile of the individual substances, we refer to section 2.4.

2.4 **Data analyses and our concluding remarks per substance**

We combined on our data analyses (experimental data, QSAR modeling) with expert judgement to estimate whether or not there may be a

concern. We included human health and environmental data available in the data base search. However, to estimate whether there is a concern for human health or the environment, we focus on the endpoints identified by REACH Article 57 a – e (see paragraph 2.2.1.3).

Please note that for with respect to the concluding remarks, we did not include article 57 f, which corresponds to equivalent level of concern endpoints (such as endocrine disrupting substances or highly respiratory sensitizing). Also, we did not validate the data or the studies available in the data bases, i.e. we used the studies present in the ECHA database as such.

We end up with a summary of the toxicity screening for each substance with respect to the REACH article 57 a-e endpoints only:

With respect to environmental hazards (REACH article 57 d and e):

- “Not likely a concern for PBT, vPvB”: environmental score was based on the PBT screening tools and can be supported with experimental data and expert judgement [21];
- “likely a concern for PBT, vPvB”: environmental score was based on the PBT screening tools and can be supported with experimental data and expert judgement [21];

With respect to human health hazards (CMR only; REACH article 57 a-c):

- “There is not likely a concern with respect to human health hazard properties”: (i) this is based on the fact that there are no QSAR alerts found*/ and there either are no experimental data available or the data support the absence of alerts or raise no concern (ii) there are QSAR alerts available but the experimental data do not support the alerts;
- “There is no conclusion possible for a particular toxicological endpoint”: due to no or very limited experimental data and/or QSARs not leading to an unequivocal conclusion;
- “There is a concern for a particular toxicological endpoint”: in cases where there are no/insufficient experimental data, the QSAR results can be strengthened by known and validated mechanistic/structural insights for the specific hazard endpoint at hand.

*If the text indicates that “no QSAR alerts were identified or found”, it means that QSAR tools were used and that no alerts were found (i.e. only checked for CMR properties).

3 Results of the toxicity screening

3.1 Introduction

In this chapter we will describe the relevant data that were found and analysed for each bio-based substance. The substances are dealt with according to the following subgroups:

- Various bio-based alternatives (section 3.2)
- Lactic acid derivatives (section 3.3)
- Levulinic acid derivatives (section 3.4)

"Annex D - Overview of the modelling results (toolboxes)" shows an overview of the QSAR results per substance.

3.2 Toxicity screening of various bio-based alternatives for polar aprotic solvents (PAS)

3.2.1 *Cyrene/ Dihydrolevoglucosenone*

Cyrene (CAS RN 53716-82-8) is registered under REACH at a tonnage level of 10 tpa and is classified as an eye irritant category 2A by the notifier. It has been tested for biodegradation, acute aquatic toxicity (algae, daphnia and microorganism) for the environment. Based on these data cyrene is not acutely toxic for aquatic species, with E(L)C50 values higher than 100 mg/L and it is readily biodegradable. Cyrene has been tested in the oral acute toxicity test (TG423) and in the in vitro Ames genotoxicity test (TG471). The results showed low acute oral toxicity, and were negative genotoxicity. The same results have also been reported in the OECD eChemPortal. No experimental data are available for repeated dose toxicity tests, developmental/reproductive toxicity tests, carcinogenicity tests and in vivo mutagenicity tests. PBT screening using the RIVM tool indicates that cyrene is not expected to be PBT or vPvB.

No QSAR alerts were identified for CMR in the OECD QSAR.

In summary, we conclude that cyrene is expected not to be PBT/vPvB and there is not likely a concern with respect to human health hazard properties (CMR).

3.2.2 *Isosorbide dimethyl ether*

Isosorbide dimethyl ether (DMI, CAS 5306-85-4) is registered at a tonnage level of 10 tpa and is self-classified as Eye irrit. 2 by the notifier. DMI has been tested for biodegradation, and acute aquatic toxicity (algae, Daphnia and microorganism) for the environment. Based on these data DMI is not acutely toxic, with E(L)C50 values higher than 100 mg/L and it is not readily biodegradable. DMI has been tested in the oral acute toxicity test (TG401), 90-day repeated dose toxicity test (TG408), in vitro Ames genotoxicity test (TG471) and the developmental toxicity test (TG414). The results showed low acute oral toxicity, and gave negative results genotoxicity. Additionally, results of the 90-day repeated dose toxicity test and the developmental toxicity tests showed that no DMI-induced toxicity was observed at the highest doses of 375

and 300 mg/kg bw/day for these two tests, respectively. No experimental data are available for reproductive toxicity test, carcinogenicity tests and in vivo mutagenicity tests.

RIVM PBT scoring indicates that DMI is not expected to be PBT. DMI is not readily biodegradable (thus meeting the P criterion), but it does not seem to meet the B and T criterion.

Both DEREK and the OECD QSAR toolbox showed no alerts. The QSAR model applied for Annex III of REACH indicated an alert for mutagenicity. These suspected mutagenic properties are not supported by the available experimental evidence according to the results from TG471 and TG 429.

In summary, we conclude that isosorbide dimethyl ether is not expected to be PBT/vPvB and there is not likely a concern with respect to human health hazard properties (CMR).

3.2.3 *Methyl tetrahydrofurfuryl ether*

Methyl tetrahydrofurfuryl ether (CAS RN 19354-27-9) is not registered under REACH. No hazard classification is provided on the ECHA website. No experimental data relevant for REACH article 57 criteria were found in the examined databases.

PBT scoring indicates that the substance is not expected to be PBT. The QSAR model applied for Annex III of REACH indicated a concern on mutagenicity, but no alerts are identified by the DEREK software or by the OECD QSAR toolbox. Without further substantiation of the reasons for the positive prediction by the QSAR models used for Annex III of REACH, it is not possible to say whether this positive prediction should be taken seriously. The lack of alerts from the other models seem to indicate there is no (mutagenicity or other) concern for this substance. In summary we conclude that methyl tetrahydrofurfuryl ether is not expected to be PBT/vPvB. With respect to human health (CMR) no conclusion can be drawn because of inconsistency between alerts for mutagenicity.

Table 2: Summary of publicly available data on human health and environmental properties of various bio-based potential alternatives for polar aprotic solvents (PAS) and QSAR estimations (CMR & PBT/vPvB only): cyrene, isosorbide dimethyl ether and methyl tetrahydrofurfuryl, For more information, refer to Annexes B and C.

Compound	CAS	Registration REACH	Notified ¹¹ self-classification CLP	Experimental data	Annex III Inventory – QSAR estimations (CMR) [22]	This study - QSAR estimations ¹²
Cyrene	53716-82-8	Registered, 10 tpa	Eye irrit. 2A (H319)	Readily biodegradable E(L)C50 > 100 mg/L (daphnia and algae) LD50 > 2000 mg/Kg (oral) Negative in Ames test	Not in inventory	Not PBT or vPvB, No alerts for CMR
Isosorbide dimethyl ether (DMI)	5306-85-4	Registered, 10 tpa	Eye irrit. 2 (H319)	Not readily biodegradable E(L)C50 > 100 mg/L (daphnia and algae) LD50 4565 mg/Kg (oral) negative 90-d NOAEL: 375 mg/kg bw NOAEL (TG414): 300 mg/kg bw Negative in Ames test	Mutagen (VEGA QSAR)	Not PBT or vPvB, No alerts for CMR
Methyl tetrahydrofurfuryl ether	19354-27-9	Not registered	Not classified	No	Mutagen (VEGA QSAR)	Not PBT or vPvB, No alerts for CMR

¹¹ Reported **notified** classifications, unless stated otherwise.

¹² Section 2.3 for more information on methods used
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3.3 Toxicity screening of lactic acid derivatives as alternatives for polar aprotic solvents (PAS)

The lactic acid derivatives are all esters of lactic acid or a lactic acid derivative. These esters can undergo rapid hydrolysis in the intestinal tract, or their conversion is catalyzed by liver enzymes after uptake.

3.3.1 *Butyl lactate*

Butyl lactate (CAS RN 138-22-7) is not registered under REACH. The notifier classified the substance as Skin irrit. 2; Eye dam. 1; Eye irrit. 2 and STOT SE 3. No experimental data relevant for REACH article 57 criteria were found in the examined databases.

PBT screening with RIVM tool indicates that butyl lactate is not expected to be PBT or vPvB.

Both DEREK and the OECD QSAR toolbox do not identify alerts for CMR for butyl lactate. The metabolic products (hydrolysis) of butyl lactate are lactic acid and butanol. Lactic acid does not have the ether functionality of methoxy- and ethoxy-acetic acid; both metabolites are therefore not expected to give rise to developmental/teratogenic toxicity.

In summary, we conclude that butyl lactate is not expected to be PBT/vPvB and there is not likely a concern with respect to human health hazard properties (CMR).

3.3.2 *Ethyl lactate*

Ethyl lactate (CAS 97-64-3) is not registered under REACH. This substance has a harmonised classification Eye dam. 1 and STOT SE 3.

According to the eChemPortal information, the substance has been tested in a developmental toxicity test and in two *in vitro* mutagenicity tests and the results were negative, showing no effects. No experimental data are available for reproductive toxicity.

PBT screening using RIVM scoring method indicates that ethyl lactate is not expected to be PBT or vPvB.

DEREK did not identify any alert. The OECD QSAR toolbox showed an alert on developmental toxicity, which is also indicated by the QSAR model applied for Annex III of REACH showing a reproductive toxicity alert. Evaluation of the Developmental And Reproductive Toxicity (DART) alert shows that it is related to the teratogenic effects seen by methyl- and ethyl-glycol ethers and their metabolites methoxy acetic acid and ethoxy acetic acid, see Figure 1. The metabolic products (produced by hydrolysis) of ethyl lactate are ethanol and lactic acid. Lactic acid does not have the ether functionality of methoxy- and ethoxy-acetic acid; both metabolites are therefore not expected to give rise to developmental/teratogenic toxicity.

In summary, we conclude that ethyl lactate is not expected to be PBT/vPvB and that there is not likely a concern with respect to human health hazard properties (CMR).

3.3.3 *Methyl DL-lactate*

Methyl DL-lactate (MLA, CAS RN 547-64-8) is not registered under REACH. This substance has a harmonised classification as Eye irrit. cat.2 and STOT SE 3. No additional experimental data were found in the searched databases for human health or the environment. PBT screening

using RIVM scoring methodology indicates that MLA is not expected to be PBT or vPvB.

An alert on developmental toxicity was identified by the OECD QSAR toolbox, but not by DEREK. The QSAR model applied for Annex III of REACH indicated an alert for reprotoxicity and this is also based on the the OECD QSAR Toolbox alert.

Evaluation of the DART alert (the same alert as for ethyl-lactate) shows that it is related to the teratogenic effects seen by methyl- and ethyl-glycol ethers and more specifically their metabolites methoxy acetic acid (MAA) and ethoxy acetic acid (EAA), see Figure 1 below. The metabolic products (hydrolysis) of ethyl lactate are methanol and lactic acid. Lactic acid does not have the ether functionality of methoxy- and ethoxy-acetic acid; both metabolites are therefore not expected to give rise to developmental/teratogenic toxicity.

The carcinogen alert in annex III is considered doubtful in view of the negative results for butyl and ethyl lactate for this endpoint and is supported by expert judgement.

In summary we conclude that methyl DL-lactate is not expected to be PBT/vPvB and there is not likely a concern with respect to human health hazard properties (CMR).

3.3.4 *Methyl 2-methoxypropionate*

Methyl 2-methoxypropionate (CAS RN 17639-76-8) is not registered under REACH. The notifier classified the substance as Skin irrit. Cat.2, H315; Eye irrit. Cat.2, H319; STOT SE 3, H335. No experimental data are available for human health or the environment.

PBT screening using RIVM scoring methodology indicates that methyl 2-methoxypropionate is not expected to be PBT or vPvB.

Alerts on developmental toxicity were raised both by DEREK and the OECD QSAR toolbox based, with both alerts based on the known teratogenicity of the small glycol ethers. The OECD QSAR Toolbox alert is also the reason for the alert on Annex III of REACH for reprotoxicity. Evaluation of the DART alert shows that it is related to the teratogenic effects seen by methyl- and ethyl-glycol ethers and more specifically their metabolites methoxy acetic acid (MAA) and ethoxy acetic acid (EAA), Figure 1. The hydrolysis products of methyl 2-methoxypropionate are methoxy 2-propionic acid and methanol. Methoxy 2-propionic acid can be considered a very close structural analogue of MAA, the teratogenic metabolite of EGME, which has a harmonised C&L H360 (may damage fertility or the unborn child). Overall, the QSAR model prediction and the alerts screening raise serious concerns on developmental toxicity. Due to lack of experimental data, these concerns cannot be removed at this stage and should therefore be investigated as early as possible using OECD TG 414 or similar.

In summary, we conclude that methyl 2-methoxypropionate is not expected to be PBT/vPvB. With respect to human health hazard properties there is a concern for developmental and/or reproductive toxicity.

3.3.5 *Ethyl 2-ethoxypropionate*

Ethyl 2-ethoxypropionate (CAS RN 7737-40-8) is not registered under REACH and not found in the toxicity databases. No experimental data are available for human health or the environment.

PBT screening using RIVM methodology indicates that this substance is not expected to be PBT or vPvB.

Alerts on developmental toxicity were raised both by DEREK and the OECD QSAR toolbox based, with both alerts based on the known teratogenicity of the small glycol ethers. Evaluation of the DART alert shows that it is related to the teratogenic effects seen by methyl- and ethyl-glycol ethers and more specifically the possibility to produce the teratogenic metabolites methoxy acetic acid (MAA) and ethoxy acetic acid (EAA), see Figure 1. The hydrolysis products of ethyl 2-ethoxypropionate are ethoxy 2-propionic acid and ethanol. Ethoxy 2-propionic acid can be considered a very close structural analogue of EAA, the teratogenic metabolite of EGEE, which has a harmonised C&L H360 (May damage fertility or the unborn child).

Overall, the QSAR model prediction and the alerts screening raise serious concerns on developmental toxicity. Due to lack of experimental data, it is not possible to draw definitive conclusions on the hazard properties of this substance, but the known teratogenicity of the small glycol ethers raises a substantial concern for this substance. The teratogenicity potential of ethyl 2-ethoxypropionate should be evaluated at the earliest possibility using developmental toxicity testing (OECD TG414) or similar.

In summary, we conclude that ethyl 2-ethoxypropionate is not expected to be PBT/vPvB. With respect to human health hazard properties (CMR) there is a concern for reprotoxicity.

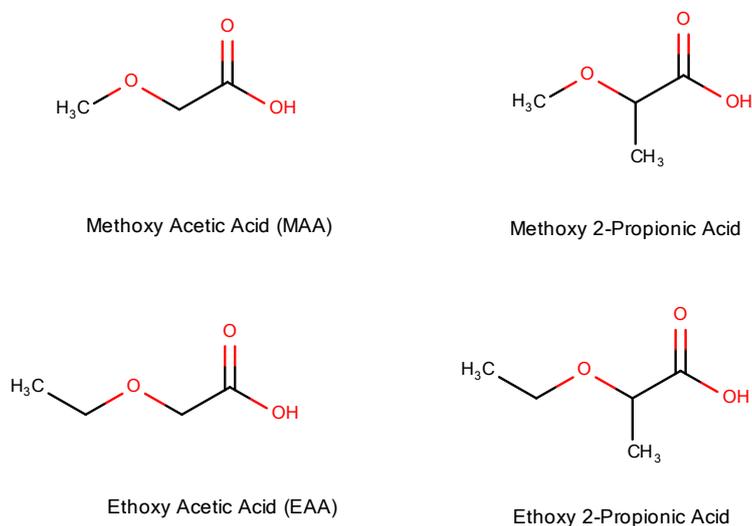


Figure 1: Schematic molecular structures of methoxy acetic acid (MAA), ethoxy acetic acid (EAA), methoxy 2-propionic acid and ethoxy 2-propionic acid, showing structural similarity (see sections 3.3.4 and 3.3.5).

Table 3: Summary of publicly available data on human health and environmental properties of various bio-based potential alternatives for polar aprotic solvents (PAS) and QSAR estimations (CMR & PBT/vPvB only) for lactic acid derivatives, for more information, refer to Annex C and D

Compound	CAS	Registration REACH	Notified ¹³ self-classification CLP	Experimental data	Annex III Inventory – QSAR estimations (CMR) ^[22]	This study - QSAR estimations ¹⁴
Butyl lactate	138-22-7	Not registered	Skin irrit. 2 (H315); Eye dam. 1 (H318); Eye irrit. 2 (H319); STOT SE 3, Resp. irrit. (H335); STOT SE 3, Narcosis (H336)	no	not listed	Not PBT or vPvB, No alerts on CMR
Ethyl lactate	97-64-3	Not registered	Harmonised (Index# 607-129-00-7): Eye Dam. 1 (H318); STOT SE 3, Resp. irrit. (H335)	no	Developmental/ Reprotoxic (VEGA QSAR & Dart scheme v1.0)	Not PBT or vPvB, Alert for Developmental toxicity, no alert in DEREK QSAR (see 3.3.2)
Methyl DL-lactate (MLA)	547-64-8	Not registered	Harmonised (Index#607-092-00-7): Eye irrit. 2 (H319); STOT SE 3, Resp. irrit. (H335)	no	Carcinogen, Developmental/ Reprotoxic (VEGA QSAR & Dart scheme v1.0)	Not PBT or vPvB, Alert for Developmental toxicity, no alert in DEREK QSAR (see 3.3.3)
Methyl 2-methoxypropionate	17639-76-8	Not registered	Skin irrit. 2 (H315); Eye irrit. 2 (H319); STOT SE 3, Resp. irrit. (H335)	no	Developmental/ Reprotoxic (VEGA QSAR & Dart scheme v1.0)	Not PBT or vPvB, Alerts for developmental toxicity
Ethyl 2-ethoxypropionate	7737-40-8	Not registered	No information	no	Not listed	Not PBT or vPvB Alerts for developmental toxicity

¹³ Reported **notified** classifications, unless stated otherwise.

¹⁴ Section 2.3 for more information on methods used
Toxicity screening of potential bio-based Polar Aprotic Solvents (PAS)
RIVM Memo - December 2018 v1.0

3.4 Toxicity screening of levulinic acid derivatives as alternatives for polar aprotic solvents (PAS)

3.4.1 *Methyl levulinate ethylene ketal*

Methyl levulinate ethylene ketal (CAS RN 35351-33-8) is not registered under REACH and not found in the toxicity databases. No experimental toxicology data are available for human health or the environment.

PBT screening using RIVM scoring methodology indicates that this substance is not expected to be PBT or vPvB.

Both DEREK and the OECD QSAR toolbox did not indicate the presence of structural alerts on CMR properties.

In summary, we conclude that methyl levulinate ethylene ketal is not expected to be PBT/vPvB and there is not likely a concern with respect to human health hazard properties (CMR), based on the absence of QSAR alerts.

3.4.2 *Levulinic ketal*

Levulinic ketal (CAS RN 42136-73-2) is not registered and not found in the toxicity databases. No experimental data are available for human health or the environment.

PBT screening using RIVM scoring methodology indicates that this substance is not expected to be PBT or vPvB.

Both DEREK and the OECD QSAR toolbox did not identify any alerts on CMR properties.

In summary, we conclude that levulinic ketal is not expected to be PBT/vPvB and there is not likely a concern with respect to human health hazard properties (CMR), based on the absence of QSAR alerts.

3.4.3 *Ethyl levulinate*

Ethyl levulinate (CAS 539-88-8) is registered under REACH at a tonnage level of 10-100 tpa. No hazard classification is provided on the ECHA website. An acute daphnid toxicity test was available on the ECHA website with a 24h EC50 of 982 mg/L. No biodegradation and other toxicity data were available on the the ECHA database.

One acute rat oral toxicity test showed a LD50 > 2000 mg/kg. Two in vitro genotoxicity tests (TG471 and 476) gave negative results. No extra information was provided in the eChemPortal.

PBT screening using RIVM scoring methodology indicates that ethyl levulinate is not expected to be PBT or vPvB.

Both DEREK and the OECD QSAR toolbox did not identify any alerts on CMR properties.

In summary, we conclude that ethyl levulinate is not expected to be PBT/vPvB and there is not likely a concern with respect to human health hazard (CMR).

3.4.4 *Gamma-valerolactone*

Gamma-valerolactone (CAS RN 108-29-2, GVL) is not registered under REACH. The notifier classifies the substance as Skin irrit. 2, H315; Eye irrit. 2, H319; STOT SE 3, H335. There was no additional experimental data found in the searched databases for human health or the environment.

PBT screening using RIVM scoring methodology indicates that GVL is not expected to be PBT or vPvB.

Alerts on mutagenicity and carcinogenicity are raised in the OECD QSAR toolbox. DEREK does not give any alerts. The QSAR models applied for Annex III of REACH indicate a positive prediction for mutagenicity and developmental toxicity, both from the CAESAR model in the VEGA QSAR platform.

The carcinogenicity alert found in the OECD QSAR toolbox is based on the EPA ONCOLOGIC primary classification scheme. The description of the alert (see Annex D - Overview of the modelling results (toolboxes)) indicates that gamma lactones are considerably weaker acylating agents than beta-lactones (4-ring lactones). Therefore, the carcinogenic potency of gamma-valerolactone is expected to be low.

The in vivo mutagenicity alert from the OECD QSAR toolbox is based on the gamma lactone (=tetrahydrofuran) substructure. The alert is based (a.o.) on the mutagenicity of cytarabine (a chemotherapeuticum) which can act as a pyrimidine analogue and be built into the DNA. It seems unlikely that g-valerolactone can have a mutagenic effect based on this mechanism of action as it lacks the structure to act as an analogue for DNA base pyrimidine. The QSAR prediction for developmental toxicity on Annex III is not evaluated in detail, but in the light of the absence of developmental alerts from the OECD QSAR toolbox and from DEREK, is disregarded. Hence the alerts on mutagenicity and carcinogenicity are not likely to indicate a concern with respect to human health.

In summary, we conclude that gamma-valerolactone is not expected to be PBT/vPvB and there is not likely a concern with respect to human health hazard properties CMR, based on absence of QSAR alerts.

3.4.5 *Methyl levulinate*

Methyl levulinate (ML, CAS RN 624-45-3) is not registered under REACH. The notifier classified the substance as Skin irrit. 2, H315; Eye irrit. 2, H319 and STOT SE 3, H335. There was no additional experimental data found in the searched databases for human health or the environment.

PBT screening using RIVM scoring methodology indicates that ML is not expected to be PBT or vPvB.

Both DEREK and the OECD QSAR toolbox did not indicate the presence of structural alerts on CMR properties.

In summary, we conclude that methyl levulinate is not expected to be PBT/vPvB and there is not likely a concern with respect to human health hazard properties (CMR), based on absence of QSAR alerts.

Table 4: Summary of publicly available data on human health and environmental properties of various bio-based potential alternatives for polar aprotic solvents (PAS) and QSAR estimations (CMR & PBT/ vPvB only) for levulinic acid derivatives, for more information, refer to Annexes B and C.

Compound	CAS	Registration REACH	Notified ¹⁵ self-classification CLP	Experimental data	Annex III Inventory – QSAR estimations(CMR) ^[22]	This study - QSAR estimations ¹⁶
Methyl levulinate ethylene ketal	35351-33-8	Not registered	No information	No	Not listed	Not PBT or vPvB, No alerts on CMR
Levulinic ketal	42136-73-2	Not registered	No information	No	Not listed	Not PBT or vPvB, No alerts on CMR
Ethyl levulinate	539-88-8	Registered, 10-100 tpa	Not classified	24EC50= 982 mg/L (Daphnia); LD50 >2000 mg/Kg (oral) Negative in in vitro mutagenicity tests (TG471, 476)	Not listed	Not PBT or vPvB No alerts on CMR
Gamma-valerolactone	108-29-2	Not registered	Acute Tox. 4 (dermal) (H312); Skin irrit. 2 (H315); Eye irrit. 2 (H319); STOT SE 3, Resp. irrit. (H335);	No	Mutagen, developmental (VEGA QSAR)	Not PBT or vPvB, Alerts for mutagenicity, carcinogenicity (OECD QSAR Toolbox)
Methyl levulinate	624-45-3	Not registered	Skin irrit. 2 (H315); Eye irrit. 2 (H319); STOT SE 3, Resp. irrit. (H335)	No	Not listed	Not PBT or vPvB No alerts on CMR

¹⁵ Reported **notified** classifications, unless stated otherwise.

¹⁶ Section 2.3 for more information on methods used

4 Discussion and conclusions

Wageningen Food and Bio-based Research (WFBR) recently identified a group of promising new bio-based alternatives to the currently disputed polar aprotic solvents (PAS): N-methyl pyrrolidone (NMP), dimethylacetamide (DMAc) and dimethylformamide (DMF). These compounds are classified as substances of very high concern (SVHC) under the REACH regulation because of their hazardous properties.

Several bio-based chemicals are (highly) polar, which increases the availability of 'new' bio-based substances with unique chemical structures and properties. This could be a solution to finding renewable alternatives to the disputed conventional PAS.

If designed in a proper way, bio-based compounds (non-food sources) may be safe and sustainable alternatives for substances of concern and so a 'win-win' is being created. In this way, bio-based safe PAS alternatives can also be of special policy interest when substituting SVHCs under REACH. This approach concurs with the 'Safe-by-Design' concept.

To avoid regrettable substitution of the disputed PAS, i.e. the replacement of PAS by a functional substitute, but not a safer alternative, RIVM carried out a toxicological screening on the environmental health and safety aspects of the WFBR candidates. We selected thirteen substances from the WFBR study (see annex E and Table 1 in section 2.1.1). Our screening was subsequently done based on several databases, QSAR (Quantitative Structure Activity Relationships) modeling and expert judgement. The focus was on the REACH Article 57 a-e endpoints only; i.e. carcinogenicity, mutagenicity and/or reprotoxicity (CMR), persistency, bioaccumulation and toxicity (PBT), very persistent and very bioaccumulative (vPvB). Since there was limited experimental data available in the databases, QSAR estimations and expert judgement provided extra information on whether a concern is expected; both on the human health as well as on the environmental hazard criteria. We weighed all the available results leading to overall concluding remarks based on the hazard profile of each substance. Note that this screening should be seen as the first steps in the 'Safe-by-Design' concept. Based on the screening results, potential bio-based PAS can be either further developed (next steps in looking at safety, applicability and upscaling) or discarded for further development.

The thirteen substances that we have screened on their toxicological properties, are not listed on both the current ZZS and potential ZZS lists [16, 17]. Only three of the thirteen bio-based substances were found to have a REACH registration. The substances also do not have classifications or notifications with respect to the REACH Article 57 hazard criteria¹⁷.

¹⁷ <https://echa.europa.eu/information-on-chemicals/cl-inventory-database>

Table 5 presents the summary of our toxicity screening with respect to the REACH article 57 a-e toxicological endpoints. As Table 5 shows, it seems unlikely that these substances are PBT/vPvB and for most of the selected bio-based substances there is not likely a concern for the other REACH Article 57 endpoints related to human health (CMR). However, for two substances (methyl 2-methoxypropionate and ethyl 2-ethoxypropionate) we conclude that there is a concern for reproductive toxicity and for one substance (methyl tetrahydrofurfuryl) we conclude there is uncertainty on mutagenicity.

Table 5: Summary of our estimations based on the toxicity screening (data screening and QSAR estimates and expert judgement) with a focus on the REACH article 57 a-e endpoints of the thirteen selected bio-based chemicals (see paragraph 2.3 for explanation), the colors used refer to the categories defined in section 2.4.

Compound (CAS no)	Human health endpoints (CMR)*	Environmental endpoints (PBT, vPvB)*
Cyrene (53716-82-8)	Not likely a concern	Not likely a concern
Isosorbide dimethyl ether (5306-85-4)	Not likely a concern	Not likely a concern
Methyl tetrahydrofurfuryl ether (19354-27-9)	There is no conclusion possible for mutagenicity	Not likely a concern
Butyl lactate (138-22-7)	Not likely a concern	Not likely a concern
Ethyl lactate (97-64-3)	Not likely a concern,	Not likely a concern
Methyl DL-lactate (547-64-8)	Not likely a concern	Not likely a concern
Methyl 2-methoxypropionate (17639-76-8)	A concern for developmental and/or reproductive toxicity	Not likely a concern
Ethyl 2-ethoxypropionate (7737-40-8)	A concern for reprotoxicity	Not likely a concern
Methyl levulinate ethylene ketal (35351-33-8)	Not likely a concern	Not likely a concern
Levulinic ketal (42136-73-2)	Not likely a concern	Not likely a concern
Ethyl levulinate (539-88-8)	Not likely a concern	Not likely a concern
Gamma-valerolactone (108-29-2)	Not likely a concern	Not likely a concern
Methyl levulinate (624-45-3)	Not likely a concern	Not likely a concern

*We stress that these concluding remarks, since they are based on currently (limited) available data, QSAR estimations and expert judgement, would need further study and validation to make a definitive conclusion.

We strongly emphasize that these results are the estimations based on currently available limited information in a selected number of databases and on a limited series of QSAR estimates and expert judgement (see section 2.3). Next to the database search performed (see section 2.2), we did not carry out additional literature searches nor did we validate the available data and studies reported in the databases that were searched. Note also that the information provided and our estimations cannot be seen as sufficient for registration for REACH/CLP, but must be seen as a first indication of expected results. Further, other toxicological endpoints, such as irritation, endocrine disruption or sensitisation, were not included in our screening and thus not in the overall summary shown in Table 5 above.

In some cases, the QSAR modeling applied for Annex III of REACH gave alerts that were not in line with the QSAR alerts as obtained in this study with DEREK and the OECD toolbox. It is out of scope to investigate the exact cause for these differences, though presumably one cause can be that different QSAR-tools have been used.

An additional note is that the REACH dossier for ethyl levulinate is not in compliance with the REACH requirements. Based on the tonnage of 10-100 tpa aquatic fish toxicity test, skin sensitisation, 28-day repeated toxicity test, reproductive and developmental screening toxicity test data should be provided. However, all these test results were not present in the registration dossier.

As described earlier, the selection of the new bio-based and potential PAS alternatives in this toxicity screening study was based on the potential new bio-based alternatives for PAS, as defined in the WFBR study [13]. It should be noted that the selection criteria for this WFBR list were mainly based on expert judgement and reflected in qualitative criteria, such as feedstock availability and the technical potential to serve as a PAS substitute. However, to prove the 'ultimate' potential of these bio-based substances as viable and safer alternatives, the technical and economic feasibility and safety should be further determined in various key solvent applications by the relevant stakeholders (industry, knowledge institutes and government). Our initial screening shows that for the environmental endpoints the thirteen selected bio-based substances are not expected to be Persistent, Bioaccumulative and Toxic (PBT) or very Persistent and very Bioaccumulative (vPvB). However, for three substances we concluded that there is a concern and/or uncertainty on either reprotoxicity or mutagenicity. If these three compounds are to be produced or imported in higher volumes and placed on the EEA market, experimental verification is necessary to further explore these concerns. For the remaining ten bio-based alternative PAS substances, there is no initial concern found on human health hazards (CMR). Therefore, these bio-based substances may indeed well fit within the above-mentioned Safe-by-Design concept. However, since our screening should be seen as a first step, we advise, parallel to the abovementioned technical feasibility studies, that further research should be done in line with the registration requirements for REACH (min. 1 tonne/year) to confirm the safety of these potential alternatives.

Finally, it should be noted that currently, under the partnership of the EU and the Bio-based Industries Consortium (“Resolve” project) identified potential bio-based alternatives (including some studied here) are being developed further towards safer bio-based solvents applicable in a wide range of solvent applications. We advise that those substances identified as potential safe PAS in our screening but not covered (yet) by Resolve, should also be the scope of further safety and feasibility research steps.

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31. <https://www.avantium.com/>.
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35. <http://www.reverdia.com/products/biosuccinium/>.
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Please note: references 26 to 38 are of Table 10 in Annex E, from [13].

Annex A - C&L Abbreviations

Acute Tox. 3 (inhal.) (H331) = acute toxicity (inhalation), hazard category 3, toxic if inhaled [23]
Acute Tox. 4 (dermal) (H312) = acute toxicity (dermal), hazard category 4, harmful in contact with skin [23]
Acute Tox. 4 (oral) (H302) = acute toxicity (oral), hazard category 4, harmful if swallowed [23]
Aquatic Acute 1 (H400) = hazardous to the aquatic environment, acute hazard, category 1, very toxic to aquatic life [23]
CLP = Classification, labelling and packaging [15]
Contains refrigerated gas (H281) = gases under pressure: contains refrigerated liquefied gas; may cause cryogenic burns or injury [23]
Dgr = danger, signal word code [15] (Annex 6)
Eye Dam. 1 (H318) = serious eye damage/eye irritation, hazard category 1, causes serious eye damage [23]
Eye Irrit. 2 (H319) = serious eye damage/eye irritation, hazard category 2, causes serious eye irritation [23]
Flam. Liq. 3 (H226) = flammable liquid and vapour, hazard category 3 [23]
GHS02 = symbol flame, physical hazard pictogram [24]
GHS05 = symbol corrosion, physical hazard pictogram [24]
GHS06 = symbol skull and crossbones, health hazard pictogram [24]
GHS07 = symbol exclamation mark, health hazard pictogram [24]
GHS08 = symbol health hazard, health hazard pictogram [24]
GHS09 = symbol environment, environmental hazard pictogram [24]
Met. Corr. 1 (H290) = corrosive to metals, hazard category 1, may be corrosive to metals [23]
Repr. 2 (H361) = reproductive toxicity, hazard category 2, suspected of damaging fertility or the unborn child [23]
Skin Corr. (H314) = skin corrosion/irritation, hazard category 1A, 1B, 1C, causes severe skin burns and eye damage [23]
Skin Irrit. 2 (H315) = skin corrosion/irritation, hazard category 2, causes skin irritation [23]
STOT SE 3, Narcosis (H336) = specific target organ toxicity, single exposure, hazard category 3, narcosis, may cause drowsiness or dizziness [23]
STOT SE 3, Resp. Irrit. (H335) = specific target organ toxicity, single exposure, hazard category 3, respiratory tract irritation, may cause respiratory irritation [23]
Wng = wng, signal word code [15] (Annex 6)

Annex B - OECD eChemPortal databases

Databases currently participating in eChemPortal compiled more than 30 databases from all over the world [18]. The list is shown below:

- [ACToR](#)
U.S. EPA Aggregated Computational Toxicology Resource
- [AGRITOX](#)
AGRITOX - Base de données sur les substances actives phytopharmaceutiques
- [APVMA-CR](#)
The Australian Pesticides and Veterinary Medicines Authority (APVMA) database of completed chemical reviews
- [CCR](#)
Canadian Categorization Results
- [CESAR](#)
Canada's Existing Substances Assessment Repository
- [Combined Exposures](#)
Collection of Case Studies on Risk Assessments of Combined Exposures to Multiple Chemicals
- [ECHA C&L inventory](#)
Public Classification and Labelling (C&L) Inventory according to the European Union (EU) CLP Regulation (EC) No 1272/2008
- [ECHA CHEM](#)
European Chemicals Agency's Dissemination portal with information on chemical substances registered under REACH.
- [EFSA Open Food Tox](#)
Chemical Hazards Database of the European Food Safety Authority
- [EnviChem](#)
Data Bank of Environmental Properties of Chemicals
- [EPA HHBP](#)
EPA Human Health Benchmarks for Pesticides
- [EPA OPPALB](#)
EPA Office of Pesticide Programs' Aquatic Life Benchmarks
- [GDL](#)
Gefahrstoffdatenbank der Länder (Germany)
- [GHS-J](#)
GHS Classification Results by the Japanese Government
- [GSBL](#)
Joint Substance Data Pool of the German Federal Government and the German Federal States
- [HPVIS](#)
High Production Volume Information System (HPVIS)
- [HSDB](#)
Hazardous Substance Data Bank
- [HSNO CCID](#)
New Zealand Hazardous Substances and New Organisms Chemical Classification Information Database
- [IGS](#)
IGS-Public Informationssystem für gefährliche Stoffe (Germany)

- [INCHEM](#)
Chemical Safety Information from Intergovernmental Organizations - INCHEM
- [INERIS-PSC](#)
INERIS-Portail Substances Chimiques
- [IPCHEM](#)
Information Platform for Chemical Monitoring
- [J-CHECK](#)
Japan CHEmicals Collaborative Knowledge database
- [JECDB](#)
Japan Existing Chemical Data Base
- [NICNAS IMAP](#)
Australia's National Industrial Chemicals Notification and Assessment Scheme's (NICNAS) Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework
- [NICNAS Other](#)
Australia's National Industrial Chemicals Notification and Assessment Scheme's (NICNAS) assessments of existing chemicals other than Priority Existing Chemical assessments
- [NICNAS PEC](#)
Australia's National Industrial Chemicals Notification and Assessment Scheme's (NICNAS) Priority Existing Chemical (PEC) Assessment Reports
- [OECD HPV](#)
Organisation for Economic Cooperation and Development (OECD) Existing Chemicals Database
- [OECD SIDS IUCLID](#)
OECD Existing Chemicals Screening Information Data Sets (SIDS) Database
- [SIDS UNEP](#)
OECD Initial Assessment Reports for HPV Chemicals including Screening Information Data Sets (SIDS) as maintained by United Nations Environment Programme (UNEP) Chemicals
- [SPIN](#)
Substances in Preparations In the Nordic countries
- [UK CCRMP Outputs](#)
UK Coordinated Chemicals Risk Management Programme Publications
- [US EPA IRIS](#)
United States Environmental Protection Agency Integrated Risk Information System
- [US EPA SRS](#)
United States Environmental Protection Agency Substance Registry Services

Annex D - Overview of the modelling results (toolboxes)

In this Annex the results of the quick-scan modelling are shown for the selection of potential bio-based PAS substances.

Table 6 shows the results of the PB (persistence and bioaccumulation) tool [21], Table 7 gives the results of the DEREK Nexus toolbox [19], and Table 8 show the results of the OECD QSAR toolbox [20].

Table 6: Results of the PB screening

Substance (CAS)	PB score screening		Result
	P-score	B-score	
cyrene (53716-82-8)	0.05	0	NOT PBT
isosorbide dimethyl ether (5306-85-4)	0.08	0.01	NOT PBT
butyl lactate (138-22-7)	0.01	0	NOT PBT
ethyl 2-ethoxypropionate (7737-40-8)	0.03	0	NOT PBT
ethyl lactate (97-64-3)	0.02	0	NOT PBT
methyl 2-methoxypropionate (17639-76-8)	0.03	0	NOT PBT
methyl DL-lactate (547-64-8)	0.02	0	NOT PBT
methyl levulinate ethylene ketal (35351-33-8)	0.06	0.02	NOT PBT
ethyl levulinate (539-88-8)	0.04	0	NOT PBT
Levulinic ketal (42136-73-2)	0.07	0.03	NOT PBT
gamma-valerolactone (108-29-2)	0.05	0	NOT PBT
methyl levulinate (624-45-3)	0.04	0	NOT PBT
methyl tetrahydrofurfuryl ether (19354-27-9)	0.04	0	NOT PBT

Table 7: Results of the DEREK toolbox screening

Substance (CAS)	Mutagenicity ¹⁸	Developmental
cyrene (53716-82-8)	INACTIVE	no alert
isosorbide dimethyl ether (5306-85-4)	INACTIVE	no alert
butyl lactate (138-22-7)	INACTIVE	no alert
ethyl 2-ethoxypropionate (7737-40-8)	INACTIVE	PLAUSIBLE: Glycol mono alkyl ether
ethyl lactate (97-64-3)	INACTIVE	no alert
methyl 2-methoxypropionate (17639-76-8)	INACTIVE	PLAUSIBLE: Glycol mono alkyl ether
methyl DL-lactate (547-64-8)	INACTIVE	no alert
methyl levulinate ethylene ketal (35351-33-8)	INACTIVE	no alert
ethyl levulinate (539-88-8)	INACTIVE	no alert
gamma-valerolactone (108-29-2)	INACTIVE	no alert
Levulinic ketal (42136-73-2)	INACTIVE	no alert
methyl levulinate (624-45-3)	INACTIVE	no alert
methyl tetrahydrofurfuryl ether (19354-27-9)	INACTIVE	no alert

¹⁸ In vitro (Ames) mutagenicity test modelled
Toxicity screening of potential bio-based Polar Aprotic Solvents (PAS)
RIVM Memo - December 2018 v1.0

Table 8: Results of the OECD toolbox screening

Substance (CAS)	IRR/CORR	MUT	CARC	REPRO
	Skin irritaton/ corrosion alerts (BfR)	DNA-binding by OASIS	ONCOlogic (EPA)	DART scheme
cyrene (53716-82-8)	Ketones	no alert	no alert	no alert
isosorbide dimethyl ether (5306-85-4)	no alert	no alert	no alert	no alert
butyl lactate (138-22-7)	no alert	no alert	no alert	no alert
ethyl 2- ethoxypropionate (7737-40-8)	no alert	no alert	no alert	a-hydroxy and alkoxy acetic acid derivative (22b)
ethyl lactate (97-64-3)	no alert	no alert	no alert	a-hydroxy and alkoxy acetic acid derivative (22b)
methyl 2- methoxypropionate (17639-76-8)	no alert	no alert	no alert	a-hydroxy and alkoxy acetic acid derivative (22b)
Leuvulinic ketal (42136-73-2)	no alert	no alert	no alert	no alert
methyl DL-lactate (547-64-8)	no alert	no alert	no alert	a-hydroxy and alkoxy acetic acid derivative (22b)
methyl levulinate ethylene ketal (35351-33-8)	no alert	no alert	no alert	no alert
ethyl levulinate (539-88-8)	Ketones	no alert	no alert	no alert
gamma- valerolactone (108-29-2)	Lactones	four- and five membered Lactones	Lactone type ¹⁹	no alert
methyl levulinate (624-45-3)	Ketones	no alert	no alert	no alert
methyl tetrahydrofurfuryl ether (19354-27-9)	no alert	no alert	no alert	no alert

¹⁹ Lactone type = lactone type reactive functional groups

Annex D - Overview of the information (from the REACH registration, ECHA database)

Table 9 below a short summary of the studies on human health and environmental effects are shown of cyrene (10 tons/annum), isosorbide dimethyl ether (10 tons/annum) and ethyl levulinate (10-100 tons/annum). For more detailed information (test specifics and results, please refer to the ECHA database [25], search by CAS number).

Substance (CAS)	Biodegradation	Acute fish (mg/L)	Chronic fish (mg/L)	Acute Daphnia (mg/L)	Algae (mg/L)	Micro-organisms (mg/L)	Acute, oral (mg/Kg bw)	Acute dermal (mg/Kg)	Acute inhalation (mg/L)	Dermal Irritation	Eye irritation	Sensitisation	Repeated dose, oral (mg/kg bw/day)	Repeated dose, dermal	Genetic toxicity (in vitro)	Carcinogenicity	Reprotoxicity	Develop-mental toxicity (mg/kg bw/day)
Cyrene (53716-82-8) ²⁰	readily biodegradable	-	-	48h EC50 > = 100	72h EC50 > 100	3h NOE C 500	LD50 > 2000	-	-	Neg.	No prediction can be made	Neg.	-	-	Neg.	-	-	-
DMI (5306-85-4) ²¹	not readily biodegradable	-	-	48h EC50 > 1000	72h EC50 > 100	6h EC10 > 100	LD50 = 6565	-	-	Neg.	No irritation	Neg.	NOAEL 375	-	Neg.	-	-	NOEL 300
EL (539-88-8) ²²	-	-	-	24h EC50 = 982 ± 66	-	-	-	LD50 > 2000 ²³	-	-	-	-	-	-	Neg. ²²	-	-	-

'-' = no data, Neg. = negative

²⁰ Cyrene, registration dossier, publicly available at <https://echa.europa.eu/registration-dossier/-/registered-dossier/16252/1>

²¹ Dimethyl Isosorbide, registration dossier, publicly available at <https://echa.europa.eu/registration-dossier/-/registered-dossier/21446/1>

²² Ethyl 4-oxovalerate registration dossier, publicly available at <https://echa.europa.eu/registration-dossier/-/registered-dossier/23920>

²³ Study categorised as reliable with restrictions (see for more information: <https://echa.europa.eu/registration-dossier/-/registered-dossier/23920>)

Annex E – Overview of emerging bio-based substances (and derivatives)

In this annex, an overview of emerging bio-based substances (and derivatives) is shown (Table 10), taken from van Es, 2017 [13], on which the selection of compounds used for this toxicity screening is based.

Table 10, Taken from van Es, 2017 [13] Overview of emerging bio-based substances (and derivatives), ranked according to their potential for substitution of polar aprotic solvents (PAS).^a

Substance ^b	PAS substitute ^c	(potential) Availability ^d	Industrial scale ^e	Commercial production	Remarks
High polarity					
Cyrene	+++	+++	+	+	^h [26]
Isohexide derivatives	++	+++	++	++	^h [27, 28]
<i>Isosorbide dimethyl ether</i>	++	+++	++	+++	^h [29]
Levulinic acid derivatives	++	+++	+	+	^h
<i>Methyl levulinate</i>	++	+++	+	+/-	[30, 31]
<i>Ethyl levulinate</i>	+	+++	+	+/-	[30]
<i>GVL</i>	++	+++	+/-	+/-	
Levulinic ketals	++	+++	+	+	[30]
Lactic acid derivatives	++	+++	++	++	[32]
THfurfuryl alcohol ethers	++	++	+	+/-	[33]
Succinic acid amides	++	++	-	-	^h
<i>1,2,3-TMP</i>	++	++	-	-	
HMF derivatives	+	+++	+	+	^h
3-HPA derivatives	+	+++	+	+	
1,3-PDO derivatives	+	+++	++	++	
Itaconic acid derivatives	+	++	+	+	^h [34]
1,4-PDO derivatives	+	++	+/-	-	
BHMTHF derivatives	+	+++	-	-	^h [33]
THFDCA esters	+	++	-	-	^h

Medium polarity					
Succinic acid esters	+/-	+++	++	++	[35, 36]
Isobutanol derivatives	+/-	+++	+	+/-	
2-MethylTHF	+/-	++	++	+	[33]
FDCA esters ^f	+/-	+++	+	+	^h
Limonene derivatives	+/-	+	+	+	
(iso) Amyl alcohol derivatives	+/-	++	+/-	+/-	
Methylsuccinic acid esters	+/-	++	+/-	-	^h
Difuran derivatives	+/-	++	+/-	-	
Muconic acid derivatives	+/-	+++	-	-	
2,3-BDO derivatives	+/-	++	-	-	
2,5-HDO derivatives	+/-	++	-	-	
Hexaric acid derivatives	+/-	+	-	-	^h [37]
Hexuronic acid derivatives	+/-	+	-	-	[37]
Aldaric ketals	+/-	+/-	-	-	[37]
Guaicols and syringols	+/-	+	-	-	
Low polarity					
Farnesene	-	++	+++	+	[38]
DMTHF	-	++	+/-	+/-	
2-Methylfuran ^g	-	++	+/-	+/-	
1,5-PDO derivatives	-	+	+/-	-	
2,5-DMF ^g	-	++	-	-	
Near drop-in bio aromatics	-	+	-	-	

^a +++ = good fit with criterion, ++ = fits with criterion, + = potential fit with criterion, +/- unclear fit with criterion, - = no fit with criterion; ^b Specific representatives for substance classes in italics; ^c Based on criteria described in Methods section [13]; ^d Based on feedstock availability and process feasibility; see Methods section [13]; ^e Demo scale and higher, > TRL 7; ^f Most polar FDCA derivatives are solids, while liquid diesters are relatively apolar; ^g High toxicity [39]; ^h Also covered in BBI project Resolve.