~~Confidential~~

Helsinki, 16 January 2024

**Addressee**
Registrant of Reactive Blue 225 MC as listed in Appendix 3 of this decision

**Date of submission of the dossier subject to this decision**
27/05/2020

**Registered substance subject to this decision ("the Substance")**
Substance name: Reaction mass of lithium sodium hydrogen 4-amino-6-({5-[(5-chloro-2,6-difluoropyrimidin-4-yl)amino]-2-sulfonatophenyl}diazenyl)-5-hydroxy-3-[(4-{[2-(sulfonatooxy)ethyl]sulfonyl}phenyl)diazenyl]naphthalene-2,7-disulfonate and lithium sodium hydrogen 4-amino-6-({5-[(5-chloro-2,6-difluoropyrimidin-4-yl)amino]-2-sulfonatophenyl}diazenyl)-5-hydroxy-3-{[4-(vinylsulfonyl)phenyl]diazenyl}naphthalene-2,7-disulfonate
EC/list number: 941-533-7

**Decision number:** Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/F)

## DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***23 July 2026***.

Requested information must be generated using the Substance unless otherwise specified.

**Information required from all the Registrants subject to Annex VII of REACH**

1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: OECD TG 471, 2020)

2. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: EU C.2./OECD TG 202)

3. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: EU C.3./OECD TG 201 or EU C.26./OECD TG 221)

The reasons for the decision(s) are explained in Appendix 1.

**Information required depends on your tonnage band**

You must provide the information listed above for all REACH Annexes applicable to you in accordance with Articles 10(a) and 12(1) of REACH. The addressees of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

**How to comply with your information requirements**

To comply with your information requirements, you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also **update the chemical safety report, where** relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general requirements for testing and reporting new tests under REACH, see Appendix 4.

**Appeal**

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

**Failure to comply**

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the decision
Appendix 2: Procedure
Appendix 3: Addressees of the decision and their individual information requirements
Appendix 4: Conducting and reporting new tests under REACH

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

## Appendix 1: Reasons for the decision

### Contents

**Reasons related to the information under Annex VII of REACH**

## 1. In vitro gene mutation study in bacteria

1 In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: Bacterial reverse mutation test, OECD TG 471 (2020)) with Prival modification.

2 An in vitro gene mutation study in bacteria is an information requirement under Annex VII, Section 8.4.1.

### *1.1. Information provided*

3 You have initially provided, in your dossier:

(i) An in vitro gene mutation study (1986) in bacteria with the Substance.

4 In your comments on the draft decision, you have adapted this information requirement by using Annex XI, Section 1.5. (Grouping of substances and read-across approach) based on the following experimental data from the source substances:

(ii) An in vitro gene mutation study (2003) in bacteria, including the Prival modification, with the source substance *Reaktiv-Rot F 66813*, EC 405-900-0;
(iii) read-across justification document.

5 You provide the following reasoning for the prediction of this information requirement: "*The most defining common characteristic of both substances is that they do not become biologically available at a relevant amount as a consequence of their physico-chemical properties, and mainly due to the sizes of the molecules.*"

6 ECHA understands that you predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects. The properties of your Substance are predicted to be quantitatively equal to those of the source substance.

### *1.2. Assessment of the information provided*

7 We have assessed this information and identified the following issues:

#### *1.2.1. The provided study (i) does not meet the specifications of the test guideline(s)*

8 To fulfil the information requirement, a study must comply with the OECD TG 471 (Article 13(3) of REACH). Therefore, the following specifications must be met:

a) if the Substance is an azo-dye or a diazo-compound, the test in presence of metabolic activation is performed following the Prival modification.

9 In study (i) described as an in vitro gene mutation study in bacteria:

a) although the tested substance is an azo-dye, the test in presence of metabolic activation was not performed following the Prival modification.

10 The information provided does not cover the specification(s) required by the OECD TG 471.

#### *1.2.2. Read-across adaptation rejected*

11 Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

12 Additional information on what is necessary when justifying a read-across approach can be found in the Guidance on IRs and CSA, Chapter R.6. and related documents (RAAF, 2017; RAAF UVCB, 2017).

*1.2.2.1. Missing information on non-common variations*

13 Annex XI, Section 1.5. requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide information to scientifically justify the read-across explanation for prediction of properties. The set of (supporting) information should strengthen the rationale for the read-across in allowing to verify the crucial aspects of the read-across hypothesis and establishing that the properties of the Substance can be predicted from the data on the source substance(s) (Guidance on IRs and CSA R.6, Section R.6.2.2.1.f.).

14 Such information must cover the impact of non-common variations.

15 As indicated above, your read-across hypothesis assumes that different compounds have the same type of effects. In this context, exposure to the Substance may lead to exposure to other compounds than that of the source substance which shares structural commonalities. The impact of exposure to these non-common variations on the prediction of properties of the target needs to be assessed to ensure that a reliable prediction can be made.

16 In your justification document you have identified the source study ii. used as key study, which covers partially structural variations common to both target and this source substance. However, you have not provided information for another source substance to cover the structural variations which are currently unique to the target substance (2,4-fluoro-5-chloro-6-amino-1,3-pyrimidine).

17 You have not provided information characterising the exposure to the non-common variations resulting from exposure to the Substance. No experimental data or other adequate and reliable information addressing the impact of exposure to these non-common variations is included in the documentation of your read-across approach.

18 In the absence of such information, you have not established that a reliable prediction of the property under consideration of the Substance can be derived on the basis of your read-across hypothesis. Therefore, you have not provided sufficient supporting information to scientifically justify for the read-across.

*1.2.3. Conclusion*

19 Therefore, the information requirement is not fulfilled.

*1.3. Specification of the study design*

20 To fulfil the information requirement for the Substance, the in vitro gene mutation study in bacteria (OECD TG 471, 2020) is considered suitable.

21 Your Substance is an azo dye for which the standard procedure may not detect all mutations. Therefore, you are required to use the Prival modification (see Paragraph 10 of OECD TG 471).

## 2. Short-term toxicity testing on aquatic invertebrates

22 Short-term toxicity testing on aquatic invertebrates is an information requirement under Annex VII to REACH (Section 9.1.1.).

### *2.1. Information provided*

23 You have provided the following information:

(i) Non-guideline study on Daphnia magna (1986) with the Substance;

### *2.2. Assessment of the information provided*

24 We have assessed this information and identified the following issue:

#### *2.2.1. The provided study does not meet the specifications of the test guideline*

25 To fulfil the information requirement, a study must comply with OECD TG 202 (Article 13(3) of REACH). Therefore, the following specifications (among others) must be met:

26 Characterisation of exposure

a) analytical monitoring must be conducted. A reliable analytical method for the quantification of the test material in the test solutions with reported specificity, recovery efficiency, precision, limits of determination (i.e. detection and quantification) and working range must be available;

27 The study (i) described as short-term toxicity study on daphnids shows the following:

28 Characterisation of exposure

a) no analytical monitoring of exposure was conducted

29 Based on the above, there are critical methodological deficiencies resulting in the rejection of the study results. More, specifically analytical monitoring of exposure concentrations was not performed.

30 Therefore, the requirements of OECD TG 202 are not met.

### *2.3. Comments on the draft decision*

31 In your comments to the initial draft decision you intend to adapt this information requirement by using Annex XI, Section 1.5. (Grouping of substances and read-across approach) based on the following experimental data from the source substance:

(i) Reaction mass of tetrasodium 5-{[4-chloro-6-(4-{[2-(sulfonatooxy)ethyl]sulfonyl}anilino)- 1,3,5-triazin-2-yl]amino}-4-hydroxy-3-[(4-{[2-(sulfonatooxy)ethyl]sulfonyl}phenyl) diazenyl]naphthalene-2,7-disulfonate and trisodium 5-({4-chloro-6-[4-(vinylsulfonyl) anilino]- 1,3,5-triazin-2-yl}amino)-4-hydroxy-3-[(4-{[2-(sulfonatooxy)ethyl]sulfonyl}phenyl)diazenyl]naphthalene-2,7-disulfonate (EC/list number: 701-360-8);

32 You provide the following reasoning for the prediction of this information requirement:

*"(…) the source and the target substances consist of high molecular weight compounds with a similar structural formula and manufacturing process. Therefore, it is considered that source and target substances have the same type of toxicological effects based on common underlying mechanisms (…)."*

33 ECHA understands that you predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects. The properties of your Substance are predicted to be quantitatively equal to those of the source substance.

34 We have assessed this information and identified the following issue(s):

*2.3.1. Read-across adaptation rejected*

35 Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

36 Additional information on what is necessary when justifying a read-across approach can be found in the Guidance on IRs and CSA, Chapter R.6. and related documents (RAAF, 2017; RAAF UVCB, 2017).

*2.3.1.1. Inadequate read-across hypothesis*

37 Annex XI, Section 1.5. requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must include an explanation why the properties of the Substance may be predicted from other substances in the group, i.e. a read-across hypothesis. This hypothesis should be based on recognition of the structural similarities and differences between the substances (Guidance on IRs and CSA, Section R.6.). It should explain why the differences in the chemical structures should not influence the ecotoxicological properties or should do so in a regular pattern, taking into account that variations in chemical structure can affect both toxicokinetics (uptake and bioavailability) and toxicodynamics (e.g. interactions with receptors and enzymes) of substances (Guidance on IRs and CSA, Section R.6.2.1.3.).

38 Your read-across hypothesis is only based on the structural similarities between the target and source substance, which you consider a sufficient basis for predicting the properties of the Substance. However, your hypothesis does not explain why the structural differences between the substances (presence of pyrimidine ring, fluoride substituents and lithium counter-ion in the Substance in contrast to the source substance which lacks these functionalities but contains triazine ring not present in the Substance) do not influence the ecotoxicological properties or do so in a regular pattern.

39 While structural similarity is a prerequisite for applying the grouping and read-across approach, it does not necessarily lead to predictable or similar ecotoxicological properties. You have not provided a well-founded hypothesis to establish a reliable prediction for a ecotoxicological property, explaining why the structural differences do not influence toxicokinetics and toxicodynamics of the substances, and thus why the properties of the Substance may be predicted from information on the source substance.

40 As explained above, you have not established that relevant properties of the Substance can be predicted from data on the source substance. Therefore, your read-across approach under Annex XI, Section 1.5. is rejected and the information requirement is not fulfilled.

*2.3.2. Conclusion*

41 Therefore, the information requirement is not fulfilled.

*2.4. Study design and test specifications*

42 The Substance is difficult to test due to the ionisable properties (based on ACD Percepta estimation of the dissociation behaviour, the Substance is permanently ionised at environmental pH, with several sulphonate groups simultaneously). In addition, the Substance is difficult to test due to the colouring properties which are mentioned in section 6.1.5 of IUCLID dossier (Toxicity to aquatic algae and cyanobacteria). OECD TG 202 specifies that, for difficult to test substances, you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented. Due to the properties of Substance, it may be difficult to achieve and maintain the desired exposure concentrations. Therefore, you must monitor the test concentration(s) of the Substance throughout the exposure duration and report the results. If it is not possible to demonstrate the stability of exposure concentrations (i.e. measured concentration(s) not within 80-120% of the nominal concentration(s)), you must express the effect concentration based on measured values as described in OECD TG 202. In case a dose-response relationship cannot be established (no observed effects), you must demonstrate that the approach used to prepare test solutions was adequate to maximise the concentration of the Substance in the test solution.

## 3. Growth inhibition study aquatic plants

43 Growth inhibition study on aquatic plants is an information requirement under Annex VII to REACH (Section 9.1.2.).

### *3.1. Information provided*

44 You have provided the following information:

(i) Modified algal growth inhibition test, [redacted] (2001) according to EU Method C.3 with the Substance.

### *3.2. Assessment of the information provided*

45 We have assessed this information and identified the following issues:

#### *3.2.1. The provided study does not meet the specifications of the test guideline*

46 To fulfil the information requirement, a study must comply with OECD TG 201 and the requirements of OECD GD 23 if the substance is difficult to test (Article 13(3) of REACH). Therefore, the following specifications must be met:

47 Key parameter to be measured

a) the concentrations of the test material leading to a 50 % and 0% (or 10%) inhibition of growth at the end of the test are estimated. Growth must be expressed as the logarithmic increase in biomass (average specific growth rate) during the exposure period.

48 Validity criteria

b) exponential growth in the control cultures is observed over the entire duration of the test;
c) at least 16-fold increase in biomass is observed in the control cultures by the end of the test;
d) the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures is ≤ 35%;
e) the coefficient of variation of average specific growth rates during the whole test

period in replicate control cultures is ≤ 7% in tests with *Desmodesmus subspicatus*.

49 Additional specifications

f) results should be based on direct toxic effects. Guidance on IRs and CSA, Section R.7.b, Table R.7.8-3 explains: *"Since the amount of light absorbed will vary with solution concentration, effects seen at high concentration are not necessarily environmentally relevant. The endpoint for regulatory use should therefore be based on direct toxic effects. If the test has not been designed to indicate whether any observed effects are caused by light limitation, then the results cannot be used*." The ETAD[2] method attempted to compare direct and indirect contact of the test substance with algae, with the indirect contact used to evaluate light inhibition only is considered too simplistic for evaluation of aquatic toxicity to algae.

50 Additional requirements of OECD GD 23 (chapter 7.6.1, point 124) for coloured test substances:

g) the irradiation (light intensity) should be above 120 µE/m$^2$sec, which is the maximum level recommended in OECD TG 201;
h) the light path should be shortened by reduction of the volume of the test solutions from e.g. 100 to 5 - 25 mL or even 1 mL;
i) sufficient agitation (for example by moderate shaking) should be performed in order to obtain a high frequency of exposure of the algae to high irradiation at the surface of the test solution. However, agitation is not advised for algal species that tend to form clumps upon shaking.

51 Your registration dossier provides an EU Method C.3 study showing the following:

52 Key parameter measured

a) the concentrations of the test material leading to a 50 % and 0% (or 10%) inhibition of growth at the end of the test are not estimated.

53 Validity criteria

54 The information on the above validity criteria b)-e) is missing.

55 Additional specifications – results based on direct toxic effects

f) the ErC50 value for growth inhibition reported in the study is the highest tested concentration (50 mg/L). On that basis you conclude that the true algicidal effect of the Substance was not observed, as the growth inhibition results from algistatic light absorption by the discolouration of the test medium.

56 Additional requirements of OECD GD 23 (chapter 7.6.1, point 124) for coloured test substances:

57 The Substance has colouring properties. However, the above requirements g) – i) of OECD GD 23 have not been applied.

*3.2.2. Invalid adaptation*

58 Your cross-reference to other study (Determination of the growth of terrestrial plants, 2001) do not relate to any valid adaptation rule under Annex VII, Section 9.1.2.

59 Based on the above,

- the information provided does not cover the key parameter required by the OECD TG 201,

[2] Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers

- the validity criteria of OECD TG 201 are not met,
- the additional requirements of OECD GD 23 (chapter 7.6.1, point 124) for coloured test substances have not been applied.

60 In addition, there are critical methodological deficiencies resulting in the rejection of the study results. More specifically, the results of the test are not based on direct toxic effects and cannot be used for the regulatory purpose.

Therefore, the requirements of OECD TG 201 are not met.

*3.3. Comments on the draft decision*

61 In your comments to the draft decision you agree with the request.

*3.4. Study design and test specifications*

62 The Substance has colouring properties. While OECD TG 201 is the preferred method to fulfil the information requirement, OECD TG 221 can be an acceptable alternative for coloured substance (see Guidance on IRs and CSA, Chapter R.7b, Table R.7.8-3: Summary of difficult substance testing issues).

63 OECD TG 221 specifies that, for difficult to test substances, OECD GD 23 must be followed. As already explained above, the Substance is difficult to test. Therefore, you must fulfil the requirements described in 'Study design and test specifications' under section 2.3.

**References**

The following documents may have been cited in the decision.

***Guidance on information requirements and chemical safety assessment (Guidance on IRs & CSA)***

Chapter R.4 Evaluation of available information; ECHA (2011).
Chapter R.6 QSARs, read-across and grouping; ECHA (2008).
Appendix to Chapter R.6 for nanoforms; ECHA (2019).
Chapter R.7a Endpoint specific guidance, Sections R.7.1 – R.7.7; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Chapter R.7b Endpoint specific guidance, Sections R.7.8 – R.7.9; ECHA (2017).
Appendix to Chapter R.7b for nanomaterials; ECHA (2017).
Chapter R.7c Endpoint specific guidance, Sections R.7.10 – R.7.13; (ECHA 2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Appendix R.7.13-2 Environmental risk assessment for metals and metal compounds; ECHA (2008).
Chapter R.11 PBT/vPvB assessment; ECHA (2017).
Chapter R.16 Environmental exposure assessment; ECHA (2016).

***Guidance on data-sharing***; ECHA (2017).

All Guidance on REACH is available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

***Read-across assessment framework (RAAF)***

RAAF, 2017 Read-across assessment framework (RAAF), ECHA (2017)
RAAF UVCB, 2017 Read-across assessment framework (RAAF) – considerations on multi- constituent substances and UVCBs), ECHA (2017).

The RAAF and related documents are available online:
https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

***OECD Guidance documents* (OECD GDs)**

OECD GD 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures; No. 23 in the OECD series on testing and assessment, OECD (2019).
OECD GD 29 Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; No. 29 in the OECD series on testing and assessment, OECD (2002).
OECD GD 150 Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption; No. 150 in the OECD series on testing and assessment, OECD (2018).
OECD GD 151 Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test; No. 151 in the OECD series on testing and assessment, OECD (2013).

**Appendix 2: Procedure**

This decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 06 April 2022.

ECHA notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments and did not amend the request(s).

The substance name was corrected as raised in your comments.

As a result of one or more changes of registration tonnage band or registration type, the requests for In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.) or In vitro micronucleus study (Annex VIII, Section 8.4.2.), In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.), Simulation testing on ultimate degradation in surface water (triggered by Annex VIII, Section 9.2.), Adsorption/ desorption screening (Annex VIII, Section 9.3.1.), and Identification of degradation products (triggered by Annex VIII, Section 9.2.) were removed from the decision. The deadline was not changed.

The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account currently longer lead times in contract research organisations.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

**Appendix 3: Addressee of this decision and their corresponding information requirements**

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

| Registrant Name | Registration number | Highest REACH Annex applicable to you |
|---|---|---|
| [redacted] | [redacted] | [redacted] |

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.

## Appendix 4: Conducting and reporting new tests for REACH purposes

### 1. Requirements when conducting and reporting new tests for REACH purposes

### 1.1. Test methods, GLP requirements and reporting

(1) Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

(2) Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

(3) Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[3].

(4) Under the introductory part of Annexes VII/VIII/IX/X to REACH, where a test method offers flexibility in the study design, for example in relation to the choice of dose levels or concentrations, the chosen study design must ensure that the data generated are adequate for hazard identification and risk assessment.

### 1.2. Test material

(1) Selection of the Test material(s)
The Test Material used to generate the new data must be selected taking into account the following:
- the boundary composition(s) of the Substance,
- the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

(2) Information on the Test Material needed in the updated dossier
- You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
- The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[4].

[3] https://echa.europa.eu/practical-guides
[4] https://echa.europa.eu/manuals

## 2. General recommendations for conducting and reporting new tests

### 2.1. Environmental testing for substances containing multiple constituents

Your Substance contains multiple constituents and, as indicated in Guidance on IRs & CSA, Section R.11.4.2.2, you are advised to consider the following approaches for persistency, bioaccumulation and aquatic toxicity testing:

- the "known constituents approach" (by assessing specific constituents), or
- the "fraction/block approach, (performed on the basis of fractions/blocks of constituents), or
- the "whole substance approach", or
- various combinations of the approaches described above

Selection of the appropriate approach must take into account the possibility to characterise the Substance (i.e. knowledge of its constituents and/or fractions and any differences in their properties) and the possibility to isolate or synthesize its relevant constituents and/or fractions.

References to Guidance on REACH and other supporting documents can be found in Appendix 1.