~~Confidential~~

Helsinki, 06 September 2022

Addressees
Registrant(s) of TBC_201-071-2_JS as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
03 March 2022

Registered substance subject to this decision ("the Substance")
Substance name: Tributyl citrate
EC number: 201-071-2

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 ***12 December 2025***.

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).

Information required from all the Registrants subject to Annex VIII of REACH

2. In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.; test method: OECD TG 473) or In vitro micronucleus study (Annex VIII, Section 8.4.2.; test method: OECD TG 487);

3. If negative results are obtained in tests performed for the information requirement of Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2. then: In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490);

4. Justification for an adaptation of a Short-term repeated dose toxicity (28 days) based on the results of the Sub-chronic toxicity study (90 days) requested below (Annex VIII, Section 8.6.1.);

5. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) by oral route, in rats.

Information required from all the Registrants subject to Annex IX of REACH

6. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.; test method: OECD TG 408) by oral route, in rats;

7. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit);

8. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: EU C.20./OECD TG 211);

9. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: EU C.47./OECD TG 210).

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

0. Reasons common to several requests

0.1. Assessment of weight of evidence adaptations

1 You have adapted the following standard information requirements by applying weight of evidence (WoE) adaptation in accordance with Annex XI, section 1.2:

- *In vitro* gene mutation study in bacteria (Annex VII, Section 8.4.1.);
- *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.);
- Short-term repeated dose toxicity (28 day) (Annex VIII, Section 8.6.1.);
- Sub-chronic toxicity (90-day) (Annex IX, Section 8.6.2);
- Pre-natal developmental toxicity study, one species (Annex IX, Section 8.6.2).

2 Your weight of evidence adaptations are based on information obtained from the Substance itself and/or analogue substances structurally similar to the Substance.

3 Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information enabling, through a reasoned justification, a conclusion on the information requirement, while the information from each single source alone is insufficient to fulfil the information requirement.

4 The justification must have regard to the information that would otherwise be obtained from the study that must normally be performed for this information requirement.

5 According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude on the corresponding information requirement.

6 Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence approach. This documentation must include robust study summaries of the studies used as sources of information and a justification explaining why the sources of information together provide a conclusion on the information requirement.

7 You have not included a justification for your weight of evidence adaptation for each of the relevant information requirement, which would include an adequate and reliable (concise) documentation as to why the sources of information provide sufficient weight to conclude on the information requirements under consideration.

8 In spite of this critical deficiency, ECHA has nevertheless assessed the validity of your adaptation. Your weight of evidence approach has deficiencies that are common to all information requirements under consideration and also deficiencies that are specific for these information requirements individually.

9 The common deficiencies are set out here, while the specific ones are set out under the information requirement concerned in the Sections below.

0.1.1. Reliability of the information provided from analogue substances

10 ECHA understands that you use data obtained with the following analogue substances in a read-across approach as part of your weight of evidence adaptation:

- tributyl 2-acetoxypropane-1,2,3-tricarboxylate (ATBC), EC 201-067-0, for the endpoints *in vitro* gene mutation study in bacteria, *in vitro* gene mutation in mammalian cells, short-term repeated dose toxicity (28 days), sub-chronic toxicity (90 days), pre-natal developmental toxicity;
- tris(2-ethylhexyl) 2-acetoxypropane-1,2,3-tricarboxylate (ATEHC), EC 205-617-0, for the endpoint *in vitro* gene mutation study in bacteria.

11 For this information to reliably contribute to the weight of evidence approaches, it would have to meet the requirements for Grouping of substances and read-across approaches.

12 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.

13 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).

14 You provide a read-across justification document in IUCLID Section 13.2.

15 Within this document you state that "*it is proposed to group the chemicals into a category and perform read-across for the endpoints where data is lacking*", referring to the substances tributyl citrate (EC 201-071-2), triethyl citrate (EC 201-070-7), tris(2-ethylhexyl)-Oacetylcitrate (ATEHC) (EC 205-617-0), tributyl-O-acetylcitrate (ATBC) (EC 201-067-0) and triethyl-O-acetylcitrate (ATEC) (EC 201-066-5).

16 However, for the endpoints listed above, you only refer to one "*near analogue*" in the endpoint study records in your dossier. You provide the following reasoning for the prediction of toxicological properties: *"It can be assumed that the same [toxicological property] applies to tributyl citrate as it is a near analogue to the test substance acetyl tributyl citrate" or "acetyltri-2-ethylhexylcitrat (ATEHC)"*.

17 Therefore ECHA understands that you are using an analogue approach for predicting the toxicological properties of the Substance from the analogue substances ATBC or ATEHC for the endpoints listed above, using the reasoning described in the read-across justification document.

18 You reason that the substances have common structural moieties which determine a common functionality, and that they are similar by closely related breakdown/metabolite products. You state that they are expected to have similar biological activity and behave in a comparable way in living organisms.

19 ECHA understands that your read-across hypothesis assumes that different compounds have the same type of effects. You predict the properties of your Substance to be quantitatively equal to those of the source substance.

20 We have identified the following issue(s) with the prediction(s) of toxicological properties:

0.1.1.1. Missing supporting information

21 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 supporting 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.).

22 As indicated above, your read-across hypothesis is based on the assumption that the structurally similar substances cause the same type of effect(s). In this context, relevant, reliable and adequate information allowing to compare the properties of the Substance and of the source substance(s) is necessary to confirm that both substances cause the same type of effects and that structural differences would not affect the predicted properties of the substances. Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and of the source substance(s).

23 You have provided the following information on the Substance and analogue substances to support your hypothesis:

- structural information
- information on structural alerts and estimation of metabolic fate using the software Toxtree v2.5.0
- similarity indices obtained using the Toxmatch tool (v.1.07)
- structural characteristics and mechanistic alerts obtained from the OECD QSAR Toolbox v2.2
- information on physicochemical, and absorption, distribution, metabolism, excretion properties
- data on acute toxicity on the Substance and analogue substances, as well as skin irritation, eye irritation, skin sensitisation, *in vitro* gene mutation in bacteria for both analogue substances.

24 ECHA has assessed the provided supporting information and identified the following issues:

25 The Substance and source substance have a triester backbone as common structural element, i.e. tricarboxylic acid with three short-chain alkyl esters. However, the substances differ structurally in the chain length and branching of the alkyl groups (linear butyl for the Substance vs. branched 2-ethylhexyl for the analogue substance ATEHC) as well as by having either a hydroxyl moiety in the Substance or acetyl moiety in the source substances, respectively.

26 You have assessed the impact of these structural differences using a set of physico-chemical and (abiotic and biotic) degradation properties, structural characteristics and mechanistic alerts obtained from Toxtree v2.5.0 and the OECD QSAR Toolbox v2.2 for the Substance and the source substances.

Similarity indices, structural and mechanistic alerts

27 Regarding the structural and descriptor based similarity (Toxmatch), you have identified a "*little change in the similarity […] accounted by acetyl group*". You state that TBC and ATEHC have a "*moderate level of similarity*".

28 You state that the results obtained with the OECD QSAR Toolbox v2.2 show that "*endpoint specific mechanisms/modes of action, structural alerts, functional groups etc. are very similar*". The profiles of structural alerts for the Substance and the analogue substance are consistent, except a difference, that you have also identified, between the Substance and the analogue substance for the structural alert for DNA binding: the non-acetylated Substance is indicated as non-binder to DNA, whereas the acetylated analogue substances might bind to DNA (structural alert for "acetoxy compounds"), pointing at different chemical mechanisms. This is related to the the acetyl group, which might increase the electrophilicity of the ester's moiety for ATBC and ATEHC. This difference in the structural alerts for DNA binding indicates that the substances may have different reactivity, which is directly relevant for gene mutation. Therefore the information on the structural alerts

provided does not support the similar toxicological profile for the Substance and analogue substance.

29 Furthermore, ECHA notes that while the similarity in presence or absence of structural alerts may indicate that the differences do not influence the reactivity of the substance e.g. on proteins, this information does not confirm, on its own, that the Substance and the source substances have similar toxicological properties such as repeated dose toxicity and reproductive toxicity. The complexity of the systemic interactions and the reproductive process and the large number of targets/mechanisms associated with those broad areas of toxicity is not covered by computational tools.

30 Therefore, the structural alerts reported in the justification document do not represent adequate information on the above-mentioned properties of the Substance and the source substance, e.g. bridging studies of comparable design and duration.

Physicochemical and toxicokinetic properties

31 You also state that the "*acetyl group implicates a certain influence of physicochemical properties of citrates*" although you claim that "*the differences between non-acetylated and acetylated citrates follow a predictable pattern of changes*" and "*the toxicity level of non-acetylated and acetylated chemicals would not deviate significantly from each other*".

32 The physicochemical properties such as water solubility, hydrophobicity and vapour pressure differ depending on the length and nature (linear/branching) of the alkyl rests and acetylation. Tributyl citrate is moderately water soluble, whereas ATBC is slightly soluble and ATEHC is practically insoluble in water. ATBC and ATEHC are "*expected to be fairly or poorly absorbed*".

33 Hydrophobicity increases with the length of the alkyl chain; acetylated citrates are more hydrophobic than non-acetylated citrates.

34 Therefore the physicochemical properties indicate differences in the toxicokinetics behaviour of the substances in the organism which could have an impact on toxicological effects.

Hydrolysis and breakdown products

35 The substances are likely to be hydrolysed in the stomach. Despite potentially having similar hydrolysis properties, with the common hydrolysis product citric acid, they form non-common hydrolysis products. The non-common hydrolysis products are, amongst others, butanol for the Substance and 2-ethylhexanol for the analogue substance ATEHC, which differ structurally, in analogy to the substances. Further non-common hydrolysis products for both analogue substances include acetyl citrate and acetic acid; acetyl mono (di) butyl citrate, butyric acid for ATBC as well as acetyl mono (di) ethylhexyl citrate for ATHEC.

36 Furthermore, in your justification document it is stated that ATBC "*was hydrolysed relatively slowly in human serum (half-life ca. 7 hours)*".

37 Therefore, the contribution of the parent substance has also to be taken into account. There is no other information on the hydrolysis rate of the Substance and analogue substance ATEHC. However you state that "*the ability to hydrolyse [is] graded: tributylcitrate > tributyl-O-acetylcitrate > tris(2-ethylhexyl)-O-acetylcitrate*".

Comparison of toxicological properties

38 You only provide experimental data for oral acute toxicity for the Substance and the analogue substances, in order to compare the toxicological profiles and justify the prediction of mutagenicity, repeated dose and reproductive toxicity from the analogue substances.

39 ECHA notes that studies on acute toxicity, as well as skin irritation, eye irritation, skin sensitisation do not inform on the mutagenicity, repeated dose and developmental toxicity properties of the Substance and of the source substance. Accordingly, this information is not considered as relevant to support your read-across hypothesis.

40 In conclusion, you have not provided adequate supporting information demonstrating that the structural differences between the Substance and the analogue substances do not influence the toxicological properties and have no impact on the read-across prediction between these two substances.

41 In the absence of such information, you have not established that the Substance and the source substances are likely to have similar properties. Therefore you have not provided sufficient supporting information to strengthen the rationale for the read-across.

42 In the absence of reliable read-across from the analogue substances, the properties of your Substance cannot be predicted from the data on the analogue substances.

43 Therefore the information from the analogue substances cannot reliably contribute to your weight of evidence adaptations.

Reasons related to the information under Annex VII of REACH

1. In vitro gene mutation study in bacteria

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

1.1. Information provided

45 You have adapted this information requirement by using weight of evidence based on the following experimental data:

(i) an *in vitro* gene mutation study in bacteria (2000) with the analogue substance tris(2-ethylhexyl) 2-acetoxypropane-1,2,3-tricarboxylate (ATEHC), EC 205-617-0;

(ii) an *in vivo* chromosomal aberration study in rats (2002) with the analogue substance tributyl 2-acetoxypropane-1,2,3-tricarboxylate (ATBC), EC 201-067-0;

(iii) an *in vitro* gene mutation study in mammalian cells (1991) with the analogue substance ATBC, EC 201-067-0.

46 You justify the weight of evidence as follows: "*Based on all pieces of weight of evidence it is clear that tributyl citrate is not mutagenic*."

1.2. Assessment of the information provided

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

48 As explained under Section 0.1. of the Reasons common to several requests, the weight of evidence adaptation must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude on the information requirement under consideration.

49 As explained in Section 0.1. of the Reasons common to several requests, your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

50 Relevant information that can be used to support weight of evidence adaptation for the information requirement of Annex VII, Section 8.4.1 includes similar information that is produced by the OECD TG 471. The following aspects are covered:

- Detection and quantification of gene mutations (base pairs, substitution or frame shift) in cultured bacteria including data on the number of revertant colonies; and
- Data provided on 5 bacterial strains: four strains of *S. typhimurium* (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101).

51 We have assessed the individual sources of information with regard to relevance and identified the following issue(s):

52 Sources of information (ii) and (iii) do not provide relevant information on the detection and quantification of gene mutations (base pairs, substitution or frame shift) in cultured bacteria. More specifically, study (ii) provides information on the detection and quantification of structural or numerical chromosomal aberrations in cultured mammalian cells and study (iii) provides information on the detection and quantification of gene mutations in cultured mammalian cells. Consequently, these studies do not provide relevant information for this information requirement.

53 The source of information (i) provides relevant information on detection and quantification of gene mutations in bacteria.

54 However, the reliability of the source of information (i) is significantly affected by the following deficiency:

1.2.1. Reliability of the contribution of the information on the analogue substance

55 For the reasons explained in the Section 0.1.1. of the Reasons common to several requests above, you have not established that the information from study (i) can reliably contribute to your weight of evidence adaptation.

1.3. Conclusion on the weight of evidence

56 Taken together, only the source of information (i) provides relevant information.

57 However, the reliability of the contribution of the information is hampered by the deficiency identified related to the use of information on the analogue substance.

58 Therefore, it is not possible to conclude, based on any source of information alone or considered together, on the information requirement for gene mutations in bacteria. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

1.4. Specification of the study design

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

Reasons related to the information under Annex VIII of REACH

2. In vitro cytogenicity study in mammalian cells or In vitro micronucleus study

60 An *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is an information requirement under Annex VIII, Section 8.4.2..

2.1. Information provided

61 You have adapted this information requirement by using Column 2 of Annex VIII, Section 8.4.2. To support the adaptation, you have provided the following information:

(i) a justification stating that *"in accordance with Column 2 adaptation statement of REACH Annex VIII, the study does not need to be conducted if adequate data from a reliable in vivo mammalian gene mutation test are available. From a valid in vivo study […] according to OECD Guideline 475 as well as a valid carcinogenicity study it can be concluded, that adverse effects concerning mutagenicity/genotoxicity are not to be expected."*;

(ii) an *in vivo* chromosomal aberration study in rats (2002) with the analogue substance tributyl 2-acetoxypropane-1,2,3-tricarboxylate (ATBC), EC 201-067-0.

2.2. Assessment of the information provided

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

2.2.1. The provided adaptation does not meet the criteria of Annex VIII, Section 8.4.2., Column 2

63 Under Annex VIII, Section 8.4.2., Column 2, the study usually does not need to be conducted "if adequate data from an in vivo cytogenicity test are available". The Guidance on IRs and CSA, Section R.7.7.6.3 and Table R.7.7–3 clarifies that the in vivo somatic cell cytogenicity test must be either a micronucleus test or a chromosomal aberration test, performed according to the OECD TG 474 or 475, respectively.

64 The carcinogenicity study mentioned in your justification above is neither a micronucleus test nor a chromosomal aberration test. Therefore, it does not meet the requirements of Column 2.

65 The study (ii) provided is described as a mammalian bone marrow chromosome aberration test, performed with the analogue substance ATBC.

66 Therefore, ECHA has evaluated the study as read-across adaptation under Annex XI, Section 1.5. of REACH and identified the following issues.

2.2.2. Assessment of the read-across approach

67 For this information (study (ii)) to reliably contribute to the Coumn 2 adaptation, it would have to meet the requirements for Grouping of substances and read-across approaches.

68 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.

69 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).

70 You use data obtained with the analogue substance ATBC to predict *in vivo* mammalian bone marrow chromosome aberration for the Substance.

71 You provide a read-across justification document in IUCLID Section 13.2.

72 Within this document you state that "*it is proposed to group the chemicals into a category and perform read-across for the endpoints where data is lacking*", referring to the substances triethyl citrate (EC 201-070-7), tributyl citrate (EC 201-071-2), tris(2-ethylhexyl)-Oacetylcitrate (ATEHC) (EC 205-617-0), tributyl-O-acetylcitrate (ATBC) (EC 201-067-0) and triethyl-O-acetylcitrate (ATEC) (EC 201-066-5).

73 However, for this information requirement, among other, you only refer to one "near analogue" in the endpoint study records in your dossier. You provide the following reasoning for the prediction of toxicological properties: "*It can be assumed that the same [toxicological property] applies to tribuyl citrate as it is a near analogue to the test substance acetyl tributyl citrate*."

74 Therefore ECHA understands that you are using an analogue approach for predicting the toxicological properties of the Substance from the analogue substance ATBC for this information requirement, using the reasoning described in the read-across justification document.

75 You reason that the substances have common structural moieties which determine a common functionality, and that they are similar by closely related breakdown/metabolite products. You state that they are expected to have similar biological activity and behave in a comparable way in living organisms.

76 ECHA understands that your read-across hypothesis assumes that different compounds have the same type of effects. You predict the properties of your Substance to be quantitatively equal to those of the source substance.

77 As already explained in more detail in Section 0.1.1. of the Reasons common to several requests, you have not provided supporting information to strengthen the rationale for the read-across and you have not established that the Substance and the source substance are likely to have similar properties. The shortcomings identified and explained under Section 0.1.1. equally apply to the read-across approach submitted under your Column 2 adaptation. Your adaptation based on grouping of substances and read-across under Annex XI, Section 1.5 is rejected.

78 Based on this, you have not established that *in vivo* mammalian bone marrow chromosome aberration properties of the Substance can be predicted from data on the source substance ATBC. Therefore the information from the analogue substance cannot contribute to your Column 2 adaptation.

79 The Column 2 criteria are not met.

80 Therefore, your adaptation is rejected.

81 On this basis, the information requirement is not fulfilled.

2.3. Specification of the study design

82 To fulfil the information requirement for the Substance, either in vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2., test method OECD TG 487) are considered suitable.

3. In vitro gene mutation study in mammalian cells

83 An in vitro gene mutation study in mammalian cells is an information requirement under Annex VIII, Section 8.4.3., in case of a negative result in the in vitro gene mutation test in bacteria and the in vitro cytogenicity test.

3.1. Triggering of the information requirement

84 Your dossier contains an adaptation for an *in vitro* gene mutation study in bacteria, and an adaptation for an *in vitro* cytogenicity study in mammalian cells or in vitro micronucleus study.

85 The information for the *in vitro* gene mutation study in bacteria and for the *in vitro* cytogenicity study in mammalian cells or in vitro micronucleus study provided in the dossier are rejected for the reasons provided in requests 1 and 2.

86 The result of the requests for an *in vitro* gene mutation study in bacteria and for an *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study will determine whether the present requirement for an in vitro mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3 is triggered.

~~87~~ Consequently, you are required to provide information for this information requirement, if the *in vitro* gene mutation study in bacteria and the *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study provide a negative result.

3.2. Information provided

88 You have adapted this information requirement by using weight of evidence based on the following experimental data:

(i) an *in vitro* gene mutation study in bacteria (2000) with the analogue substance tris(2-ethylhexyl) 2-acetoxypropane-1,2,3-tricarboxylate (ATEHC), EC 205-617-0;

(ii) an *in vivo* chromosomal aberration study in rats (2002) with the analogue substance tributyl 2-acetoxypropane-1,2,3-tricarboxylate (ATBC), EC 201-067-0;

(iii) an *in vitro* gene mutation study in mammalian cells (1991) with the analogue substance ATBC, EC 201-067-0.

89 You justify the weight of evidence as follows: "*Based on all pieces of weight of evidence it is clear that tributyl citrate is not mutagenic*."

3.3. Assessment of the information provided

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

91 As explained under Section 0.1. of the Reasons common to several requests, the weight of evidence adaptation must fulfil the information requirement based on relevant and reliable

sources of information. These sources of information must provide sufficient weight to conclude on the information requirement under consideration.

92 As explained in Section 0.1. of the Reasons common to several requests, your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

93 Relevant information that can be used to support weight of evidence adaptation for information requirement of Section 8.4.3 at Annex VIII includes similar information that is produced by the OECD TG 476/490 and OECD TG 488. This includes:

- Detection and quantification of gene mutations (point mutations, frame-shift mutations, small deletions, etc.) including data on the frequency of mutant colonies in cultured mammalian cells (*in vitro*) or mutant frequency for each tissue in mammals (*in vivo*).

94 We have assessed the individual sources of information with regard to relevance and identified the following issue(s):

95 Sources of information (i) and (ii) do not provide relevant information on the detection and quantification of gene mutations (point mutations, frame-shift mutations, small deletions, etc.) in cultured mammalian cells (*in vitro*) or mutant frequency for each tissue in mammals (*in vivo*). More specifically, study (i) provides information on the detection and quantification of gene mutations in bacteria and study (ii) provides information on the detection and quantification of structural or numerical chromosomal aberrations in cultured mammalian cells. Consequently, these studies do not provide relevant information for this information requirement.

96 The source of information (iii) provides relevant information on gene mutation in mammalian cells.

97 However, there are deficiencies affecting its reliability:

3.3.1. Reliability of the contribution of the information on the analogue substance

98 For the reasons explained in the section 0.1.1. of the Reasons common to several requests above, you have not established that the information from study (iii) can reliably contribute to your weight of evidence adaptation.

3.3.2. Reliability of the contribution of the study (iii)

99 The evaluation of the reliability of the contribution of each relevant line of information to the weight of evidence approach includes an assessment of each source of information against the specifications of the test guideline followed.

100 The study (iii) was conducted following the OECD TG 476. This test guideline requires that:

- at least 4 concentrations are evaluated, in absence and in presence of metabolic activation;
- the maximum concentration tested induces 80-90% of cytotoxicity compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test concentration corresponds to 10 mM, 2 mg/mL or 2 µL/mL, whichever is the lowest;
- data on the cytotoxicity and the mutation frequency for the treated and control cultures are reported.

101 In the source of information (iii), the following investigation/specification is not to the requirements of OECD TG 476:

- no information on the number and level of concentrations used is reported
- no data on the cytotoxicity and the mutation frequency for the treated and control cultures is provided.

102 Based on the above, the reliability of the contribution of the results obtained from this study to the weight of evidence is limited. The unclarity regarding how the results were obtained introduces uncertainty in the results which must be considered.

3.4. Conclusion on the weight of evidence

103 Taken together, only one source of information (study (iii)) provides relevant information on gene mutation in mammalian cells.

104 However, the reliability of this information is hampered by:

- the deficiency identified related to the use of information on the analogue substance and
- issues related to how the results were obtained in the studies which increases the uncertainty of the conclusion for the Substance.

105 Therefore, it is not possible to conclude, based on any source of information alone or considered together, on the information requirement for gene mutations in mammalian cells. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

3.5. Specification of the study design

106 To fulfil the information requirement for the Substance, either the in vitro mammalian cell gene mutation tests using the hprt and xprt genes (OECD TG 476) or the thymidine kinase gene (OECD TG 490) are considered suitable.

4. Justification for an adaptation of a Short-term repeated dose toxicity (28 days) based on the results of the Sub-chronic toxicity study (90 days)

107 A short-term repeated dose toxicity study (28 days) is an information requirement under Annex VIII, Section 8.6.1. This information may take the form of a study record or a valid adaptation in accordance with either a specific adaptation rule under Column 2 of Annex VIII or a general adaptation rule under Annex XI.

4.1. Information provided

108 You have adapted this information requirement by using weight of evidence based on the following experimental data:

(i) a repeated dose 90-day oral toxicity study in rats (2003) with the analogue substance tributyl 2-acetoxypropane-1,2,3-tricarboxylate, EC 201-067-0;

(ii) an 8-week-study in rat (1959) with the Substance;

(iii) an 8-week-study in cat (1959) with the Substance.

109 You justify the weight of evidence as follows: "*Based on all pieces of weight of evidence it is clear that tributyl citrate is of very low toxicity after repeated administrations*."

110 You consider that the information you have provided on the Substance itself and on the analogue substance, when taken together, is adequate to fulfil the information requirement under consideration.

4.2. Assessment of the information provided

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

112 As explained under Section 0.1. Reasons common to several requests, the weight of evidence adaptation must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude on the information requirement under consideration.

113 As explained in Section 0.1. of the Reasons common to several requests, your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

114 Relevant information that can be used to support weight of evidence adaptation for the information requirement of Annex VIII, Section 8.6.1 includes similar information that is produced by the OECD TG 407. The following aspects of systemic toxicity in intact, non-pregnant and young adult males and females are covered: 1) in-life observations, 2) blood chemistry, 3) organ and tissue toxicity.

115 We have assessed the individual sources of information with regard to relevance and reliability and identified the following issues:

4.2.1. Aspect 1) in-life observations

116 In-life observations must include information on survival, body weight development, clinical signs, functional observations, food/water consumption and other potential aspects of in life observations on the relevant physiological systems (circulatory, digestive/excretory, integumentary, musculoskeletal, nervous, renal/urinary, and respiratory).

117 The sources of information (i) to (iii) provide some relevant information, however they do not cover all of the key elements of this aspect. More specifically, based on the information reported in your dossier, these sources of information do not inform on functional observations.

118 Consequently, the sources of information (i) to (iii) only provide partially relevant information on aspect 1).

119 In addition, these sources of information have deficiencies affecting their reliability:

4.2.1.1. Reliability of the contribution of the information on the analogue substance (study (i))

120 For the reasons explained in the Section 0.1.1. of the Reasons common to several requests above, you have not established that the information from study (i) can reliably contribute to your weight of evidence adaptation.

4.2.2. Aspect 2) blood chemistry

121 Information on blood chemistry must include haematological (full-scale) and clinical chemistry analysis (full-scale), and other potential aspects related to blood chemistry to address relevant physiological systems (circulatory digestive/excretory, endocrine, immune, musculoskeletal, and renal/urinary.)

122 The sources of information (i) to (iii) provide relevant information on some of the elements of aspect 2). However, they do not provide information on the following aspects to address relevant physiological systems: circulatory digestive/excretory, endocrine, immune and musculoskeletal systems.

123 Consequently, the sources of information (i) to (iii) provide only partially relevant information on aspect 2).

124 In addition, the sources of information have deficiencies affecting their reliability:

4.2.2.1. Reliability of the contribution of the information on the analogue substance (study (i))

125 For the reasons explained in the Section 0.1.1. of the Reasons common to several requests above, you have not established that the information from study (i) can reliably contribute to your weight of evidence adaptation.

4.2.3. Aspect 3) organ and tissue toxicity

126 Organ and tissue toxicity must include information on terminal observations on organ weights, gross pathology and histopathology (full-scale) and other potential aspects related to organ and tissue toxicity to address relevant physiological systems (circulatory, digestive/excretory, endocrine, immune, integumentary, musculoskeletal, nervous, renal/urinary system, reproductive, and respiratory).

127 The source of information (i) provides relevant information on organ weight and gross pathology. However, not all required information on histopathology is covered, as also stated in your dossier: "*only limited histopathology performed and no special neurotoxicity examination included*." Based on the information provided in the study record, the histopathology of the following organs was not investigated: heart, gastrointestinal tract, spleen, brain, spinal cord, pituitary, adrenal gland, trachea and lungs, uterus, cervix vagina, epididymides, prostate, seminal vesicle, coagulation glands, mammary glands, urinary bladder, lymph nodes, peripheral nerves, skeletal muscle, bone, bone marrow.

128 The source of information (ii) provides relevant information on some of the elements of aspect 3) but do not cover all the required information on gross pathology and full histopathology. Based on the information provided in the study record, the following organs were not investigated: brain, spinal cord, pituitary, adrenal gland, stomach, trachea, ovaries, uterus, cervix vagina, epididymides, prostate, testes, seminal vesicle, coagulation glands, mammary glands, urinary bladder, lymph nodes, peripheral nerves, skeletal muscle, bone, bone marrow.

129 Therefore, the studies (i) and (ii) do not cover all the necessary information on gross pathology and full histopathology, as specified in the OECD TG 407.

130 The study record for the source of information (iii) does not mention any organs and tissues investigated, therefore the study does not provide relevant information for aspect 3).

131 Consequently, only the sources of information (i) and (ii) provide partially relevant information on aspect 3).

132 In addition, the sources of information have deficiencies affecting their reliability:

4.2.3.1. Reliability of the contribution of the information on analogue substances (study (i))

133 In general, for the reasons in the Section 0.1.1. of the Reasons common to several requests above, you have not established that the information from study (i) can reliably contribute to your weight of evidence adaptation.

4.2.4. Conclusion on the weight of evidence

134 Taken together, the sources of information (i) and (ii) provide partially relevant information on some elements of aspects 1) in-life observations, 2) blood chemistry and 3) organ and tissue toxicity, and the source of information (iii) provides partially relevant information on some elements of aspects 1) and 2). However, they do not cover the entire set of elements expected to be obtained from the OECD TG 407 for all aspects 1) to 3), as described above.

135 Furthermore, any robust conclusion on any of the 3 aspects is hampered by the reliability issue related to the use of information on the analogue substance (study (i)).

136 Therefore, it is not possible to conclude, based on any source of information alone or considered together, on the information requirement for short-term repeated toxicity (28 days). Therefore, your adaptation is rejected and the information requirement is not fulfilled.

137 Annex VIII, Section 8.6.1., Column 2 provides that an experimental study for this information requirement is not needed if a reliable sub-chronic (90 days) or chronic toxicity study is available.

138 The present decision requests the registrants concerned to generate and submit a reliable sub-chronic toxicity study (90 days) (see request 6). According to Annex VIII, Section 8.6.1., Column 2 and to prevent unnecessary animal testing, a short-term toxicity study (28 days) does not therefore need to be conducted.

139 Because you still must comply with the information requirement in Annex VIII, Section 8.6.1., you are requested to submit a justification for the adaptation provided in Column 2 of that provision.

5. Screening for reproductive/developmental toxicity

140 A screening for reproductive/developmental toxicity (OECD 421 or OECD 422) is an information requirement under Annex VIII, Section 8.7.1.. This information may take the form of a study record or a valid adaptation in accordance with either a specific adaptation rule under Annex VIII, Section 8.7.1., Column 2 or a general adaptation rule under Annex XI, Section 8.7.1., Column 2.

141 You state that "*This endpoint is waived due to the low toxicity potential of tributyl citrate*."

142 To support the data waiving, you have provided statements on low toxicity referring to several studies with the Substance on skin and eye irritation, skin sensitisation, mutagenicity and systemic toxixicty; as well as on the good oral absorption, limited dermal absorption and poor inhalation absorption of the Substance; on the metabolism of the Substance to non hazardous constituents of the body. You conclude that "*adverse effects concerning toxicity to reproduction are not to be expected and there is no scientific justification for planning further animal tests to investigate this endpoint*".

143 You may adapt the standard information requirement according to the specific rules outlined in Annex VIII, Section 8.7.1., Column 2 and/or according to the general rules contained in Annex XI to the REACH Regulation, provided you fulfil the specific criteria, and submit a scientifically-supported justification.

144 However, you do not further clarify to which adaptation you are referring to.

145 The information you provide does not correspond to any specific adaptation rule under Annex VIII, Section 8.7.1., Column 2 or general adaptation rule under Annex XI, Section 8.7.1., Column 2 for the endpoint information requirement.

146 Based on the above, you have not provided any information or any valid adaptation to fulfil the information requirement.

147 Therefore, the information requirement is not fulfilled.

5.1. Specification of the study design

148 A study according to the test method EU B.63/OECD TG 421 or EU B.64/OECD TG 422 must be performed in rats.

149 The study must be conducted with oral administration of the Substance (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

Reasons related to the information under Annex IX of REACH

6. Sub-chronic toxicity study (90-day)

150 A sub-chronic toxicity study (90 day) is an information requirement under Annex IX, Section 8.6.2.

6.1. Information provided

151 You have adapted this information requirement by using weight of evidence based on the following experimental data:

(i) a repeated dose 90-day oral toxicity study in rats (2003) with the analogue substance tributyl 2-acetoxypropane-1,2,3-tricarboxylate (ATBC), EC 201-067-0;

(ii) an 8-week-study in rat (1959) with the Substance

(iii) an 8-week-study in cat (1959) with the Substance.

152 You justify the weight of evidence as follows: "*Based on all pieces of weight of evidence it is clear that tributyl citrate is of very low toxicity after repeated administrations*."

153 You consider that the information you have provided on the Substance itself and on the analogue substance, when taken together, is adequate to fulfil the information requirement under consideration.

6.2. Assessment of the information provided

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

155 As explained under Section 0.1. Reasons common to several requests, the weight of evidence adaptation must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude on the information requirement under consideration.

156 As explained in Section 0.1. of the Reasons common to several requests, your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

157 Relevant information that can be used to support weight of evidence adaptation for the information requirement of Annex IX, Section 8.6.2 includes similar information that is produced by the OECD TG 408. The following aspects of systemic toxicity in intact, non-pregnant and young adult males and females are covered: 1) in-life observations, 2) blood chemistry, 3) organ and tissue toxicity.

158 We have assessed the individual sources of information with regard to relevance and reliability and identified the following issues:

6.2.1. General consideration

159 According to Column 1 of Annex IX, Section 8.6.2., a sub-chronic toxicity (90-day) study has to be performed in one species, rodent, via most appropriate route of administration.

The source of information (iii) provides information on another species than rodent, more specifically cat.

160 Therefore, the source of information (iii) does not provide relevant information.

161 In the following the relevance of the information provided by studies (i) and (ii) regarding the aspects 1) in-life observations, 2) blood chemistry and 3) organ and tissue toxicity is assessed.

6.2.2. Aspect 1) in-life observations

162 In-life observations must include information on survival, body weight development, clinical signs, functional observations, food/water consumption and other potential aspects of in life observations on the relevant physiological systems (circulatory, digestive/excretory, integumentary, musculoskeletal, nervous, renal/urinary, and respiratory).

163 The sources of information (i) and (ii) provide some relevant information, however they do not cover all of the key elements of this aspect. More specifically, based on the information reported in your dossier, these sources of information do not inform on functional observations.

164 Consequently, the sources of information (i) and (ii) only provide partially relevant information on aspect 1).

165 In addition, these sources of information have deficiencies affecting their reliability:

6.2.2.1. Reliability of the contribution of the information on the analogue substance (study (i))

166 For the reasons explained in the Section 0.1.1. of the Reasons common to several requests above, you have not established that the information from study (i) can reliably contribute to your weight of evidence adaptation.

6.2.2.2. Reliability of the contribution of the study (ii)

167 For a sub-chronic toxicity study, OECD TG 408 requires dosing of the Substance daily for a minimum of 90 days, i.e. 13 weeks.

168 In study (ii), the following specifications are not according to the requirements of the OECD TG 408 since the exposure duration is of 8 weeks.

169 Therefore, the actual exposure period in study (ii) is shorter than the minimum exposure duration expected from a study conducted according to the OECD TG 408. This condition of exposure is essential because the effects observed over the required period of exposure of 90-days might be considerably more pronounced than over a shorter study duration.

170 Therefore, the the reliability of the contribution of the results obtained from the studies (i) and (ii) to the weight of evidence is limited. The specifications according to which the results were obtained introduce uncertainty in the results which must be considered.

6.2.3. Aspect 2) blood chemistry

171 Information on blood chemistry must include haematological (full-scale) and clinical chemistry analysis (full-scale), and other potential aspects related to blood chemistry to address relevant physiological systems (circulatory digestive/excretory, endocrine, immune, musculoskeletal, and renal/urinary.)

172 The sources of information (i) and (ii) provide relevant information on some of the elements of aspect 2). However, they do not provide information on the following aspects to address

relevant physiological systems: circulatory digestive/excretory, endocrine, immune and musculoskeletal systems.

173 Consequently, the sources of information (i) and (ii) provide only partially relevant information on aspect 2).

174 In addition, the sources of information have deficiencies affecting their reliability:

6.2.3.1. Reliability of the contribution of the information on the analogue substance (study (i))

175 For the reasons explained in the Section 0.1.1. of the Reasons common to several requests above, you have not established that the information from study (i) can reliably contribute to your weight of evidence adaptation.

6.2.3.2. Reliability of the contribution of the study (ii)

176 The reliability issues identified in section 6.2.2.2. above, related to exposure duration (study ii)), equally apply to the aspect 2).

177 As a result, the reliability of the contribution of the results obtained from the studies (i) and (ii) to the weight of evidence is limited. The specifications according to which the results were obtained introduce uncertainty in the results which must be considered.

6.2.4. Aspect 3) organ and tissue toxicity

178 Organ and tissue toxicity must include information on terminal observations on organ weights, gross pathology and histopathology (full-scale) and other potential aspects related to organ and tissue toxicity to address relevant physiological systems (circulatory, digestive/excretory, endocrine, immune, integumentary, musculoskeletal, nervous, renal/urinary system, reproductive, and respiratory).

179 The source of information (i) provides relevant information on organ weight and gross pathology. However, not all required information on histopathology is covered, as also stated in your dossier: *"only limited histopathology performed and no special neurotoxicity examination included."* Based on the information provided in the study record, the histopathology of the following organs was not investigated: heart, gastrointestinal tract, pancreas, spleen, brain, spinal cord, pituitary, adrenal gland, thyroid, parathyroid, oesophagus, salivary glands, trachea and lungs, aorta, uterus, cervix vagina, epididymides, prostate, seminal vesicle, coagulation glands, mammary glands, urinary bladder, gall bladder, lymph nodes, peripheral nerves, skeletal muscle, bone, bone marrow.

180 The source of information (ii) provides relevant information on some of the elements of aspect 3) but do not cover all the required information on gross pathology and full histopathology. Based on the information provided in the study record, the following organs were not investigated: brain, spinal cord, pituitary, adrenal gland, thyroid, parathyroid, oesophagus, salivary glands, stomach, trachea, aorta, ovaries, uterus, cervix vagina, epididymides, prostate, testes, seminal vesicle, coagulation glands, mammary glands, urinary bladder, gall bladder, lymph nodes, peripheral nerves, skeletal muscle, bone, bone marrow.

181 Therefore, the studies (i) and (ii) do not cover all the necessary information on gross pathology and full histopathology, as specified in the OECD TG 408.

182 Consequently, the sources of information (i) and (ii) only provide partially relevant information on aspect 3).

183 In addition, the sources of information have deficiencies affecting their reliability:

6.2.4.1. Reliability of the contribution of the information on analogue substances (study (i))

184 In general, for the reasons in the Section 0.1.1. of the Reasons common to several requests above, you have not established that the information from study (i) can reliably contribute to your weight of evidence adaptation.

6.2.4.2. Reliability of the contribution of the study (ii)

185 The reliability issues identified in section 6.2.2.2. above, related to exposure duration (study ii)), equally apply to the aspect 3).

186 As a result, the the reliability of the contribution of the results obtained from the studies (i) and (ii) to the weight of evidence is limited. The specifications according to which the results were obtained introduce uncertainty in the results which must be considered.

6.2.5. Conclusion on the weight of evidence

187 Taken together, the sources of information (i) and (ii) provide partially relevant information on some elements of aspects 1) in-life observations, 2) blood chemistry and 3) organ and tissue toxicity. However, the two studies do not cover the entire set of elements expected to be obtained from the OECD TG 408 for all aspects 1) to 3), as described above.

188 Furthermore, any robust conclusion on any of the 3 aspects is hampered by the following reliability issues:

- the deficiency identified related to the use of information on the analogue substance (study (i)) and
- issues related to how the results were obtained in the studies which increases the uncertainty of the conclusion for the Substance (study (ii)).

189 Therefore, it is not possible to conclude, based on any source of information alone or considered together, on the information requirement for sub-chronic toxicity (90 days). Therefore, your adaptation is rejected and the information requirement is not fulfilled.

6.3. Specification of the study design

190 Following the criteria provided in Annex IX, Section 8.6.2, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity of the Substance; Guidance on IRs and CSA, Section R.7.5.6.3.2.

191 According to the OECD TG 408, the rat is the preferred species.

192 Therefore, the study must be performed in rats according to the OECD TG 408 with oral administration of the Substance.

7. Pre-natal developmental toxicity study in one species

193 A pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is an information requirement under Annex IX, Section 8.7.2.

7.1. Information provided

194 You have adapted this information requirement by using weight of evidence based on the following experimental data:

(i) 12-month toxicity study in rats via diet with pairing of animals in the ninth month (1977) with the analogue substance tributyl 2-acetoxypropane-1,2,3-tricarboxylate, EC 201-067-0

(ii) 12-month toxicity study in mice via diet with pairing of animals in the ninth month (1977) with the analogue substance tributyl 2-acetoxypropane-1,2,3-tricarboxylate, EC 201-067-0.

7.2. Assessment of the information provided

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

196 As explained under Section 0.1. of the Reasons common to several requests, the weight of evidence adaptation must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude on the information requirement under consideration.

197 As explained in Section 0.1. of the Reasons common to several requests, your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

198 Relevant information that can be used to support weight of evidence adaptation for the information requirement of Annex IX, Section 8.7.2 includes similar information that is produced by the OECD TG 414 with a design as specified in this decision. OECD TG 414 requires the study to investigate the following key elements: 1) pre-natal developmental toxicity, 2) maternal toxicity, 3) maintenance of pregnancy.

199 We have assessed the individual sources of information with regard to relevance and reliability and identified the following issues:

7.2.1. Aspect 1) Pre-natal developmental toxicity

200 Pre-natal developmental toxicity includes information after pre-natal exposure on embryonic/foetal survivial (number of live foetuses; number of resorptions and dead foetuses, postimplantation loss), growth (body weights and size) and structural malformations and variations (external, visceral and skeletal).

201 The sources of information (i) and (ii) are described as 12-month toxicity studies, via diet, with pairing of animals in the ninth month and subsequent examination of reproductive parameters and offspring. ECHA understands from the information in your dossier that the provided studies investigate post-natal effects on the offspring after natural delivery instead of caesarean section. The studies provide limited relevant information on embryonic survival (early and late embryonic death). However, the studies do not provide relevant information on foetal survival (number of live foetuses), growth (body weights and size) and structural malformations and variations (external, visceral and skeletal). Consequently, the studies provide partially relevant information on aspect 1).

202 In addition, the reliability of these sources of information is significantly affected by the following deficiencies:

7.2.1.1. Reliability of the contribution of the information on the analogue substance

203 For the reasons explained in the section 0.1.1. of the Reasons common to several requests, you have not established that the information from studies (i) and (ii) can reliably contribute to your weight of evidence adaptation.

7.2.1.2. Reliability of the contribution of the studies (i) and (ii)

204 Investigations/specifications in a developmental toxicity study (OECD TG 414) include at least 20 female animals with implantation sites for each test and control group.

205 In your dossier no information on the numbers of animals is reported. Therefore, it cannot be concluded whether the statistical power of the information from the studies (i) and (ii) is equivalent to the OECD TG 414.

206 Based on the above, the reliability of the contribution of the results obtained from these studies to the weight of evidence is limited. The unclarity regarding how the results were obtained introduces uncertainty in the results which must be considered.

7.2.2. Aspect 2) Maternal toxicity

207 Maternal toxicity includes information after gestational exposure on maternal survival, body weight and clinical signs and other potential aspects of maternal toxicity in dams.

208 The sources of information (i) and (ii) provide relevant information on maternal toxicity.

209 However, the reliability of these sources of information is significantly affected by the following deficiencies:

7.2.2.1. Reliability of the contribution of the information on the analogue substance

210 For the reasons explained in the section 0.1.1. of the Reasons common to several requests, you have not established that the information from studies (i) and (ii) can reliably contribute to your weight of evidence adaptation.

7.2.2.2. Reliability of the contribution of the studies (i) and (ii)

211 The reliability issue identified in section 7.2.1.2. above related to statistical power equally applies to the aspect 2).

212 Based on the above, the reliability of the contribution of the results obtained from these studies to the weight of evidence is limited. The unclarity regarding how the results were obtained introduces uncertainty in the results which must be considered.

7.2.3. Aspect 3) Maintenance of pregnancy

213 Maintenance of pregnancy includes information on abortions and/or early delivery as a consequence of gestational exposure and other potential aspects of maintenance of pregnancy.

214 The sources of information (i) and (ii) provide relevant information on maintenance of pregnancy, according to the examinations that are stated in your dossier as performed for the studies, i.e. placental weight, number of normal, resorptive and deformed tissues.

215 However, the reliability of these sources of information is significantly affected by the following deficiencies:

7.2.3.1. Reliability of the contribution of the information on the analogue substance

216 For the reasons explained in the section 0.1.1. of the Reasons common to several requests, you have not established that the information from studies (i) and (ii) can reliably contribute to your weight of evidence adaptation.

7.2.3.2. Reliability of the contribution of the studies (i) and (ii)

217 The reliability issue identified in section 7.2.1.2. above related to statistical power equally applies to the aspect 3).

218 Based on the above, the reliability of the contribution of the results obtained from these studies to the weight of evidence is limited. The unclarity regarding how the results were obtained introduces uncertainty in the results which must be considered.

7.3. Conclusion on the weight of evidence

219 Taken together, the sources of information provide relevant information on maternal toxicity and maintenance of pregnancy. However, they provide only partially relevant information on pre-natal developmental toxicity. More specifically, they do not provide information on foetal survival, growth and on external, skeletal and visceral malformations/ variations/ abnormalities.

220 Furthermore, any robust conclusion on any of the 3 aspects is hampered by reliability issues affecting all sources of information (studies (i) and (ii)):

- related to the use of information on the analogue substance
- related to how the results were obtained in the studies which increases the uncertainty of the conclusion.

221 Therefore, it is not possible to conclude, based on any source of information alone or considered together, on the information requirement for pre-natal developmental toxicity in one species. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

7.4. Specification of the study design

222 A PNDT study according to the test method OECD TG 414 should be performed in rat or rabbit as preferred species.

223 The study must be performed with oral administration of the Substance (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

224 Therefore, the study must be conducted in rats or rabbits with oral administration of the Substance.

8. Long-term toxicity testing on aquatic invertebrates

225 Long-term toxicity testing on aquatic invertebrates is an information requirement under Annex IX to REACH (Section 9.1.5.).

8.1. Information provided

226 You have adapted this information requirement by using Column 2 of Annex IX, Section 9.1. To support the adaptation, you have provided the following information:

(i) A justification for data waiving claiming that: "*In accordance with Column 2 of REACH Annex IX, the long-term aquatic toxicity to invertebrates study (required in Section 9.1.5) does not need to be conducted as the chemical safety assessment according to Annex I indicates that this is not necessary.*

In Annex IX of Regulation (EC) No 1907/2006, it is laid down that chronic toxicity tests with invertebrates shall be proposed by the registrant if the chemical safety

assessment indicates the need to investigate further the effects on invertebrates. According to Annex I of this regulation, the chemical safety assessment triggers further action when the substance or the preparation meets the criteria for classification as dangerous according to Directive 67/548/EEC or Directive 1999/45/EC or is assessed to be a PBT or vPvB. The hazard assessment of tributyl citrate reveals neither a need to classify the substance as dangerous to the environment, nor is it a PBT or vPvB substance. According to reliable study results, tributyl citrate is considered to be rapidly biodegradable in aquatic compartments; the bioaccumulation potential is regarded to be low (BCF 94.7 traditional method/6.54 Arnot-Gobas method). Furthermore, from metabolism studies in animals no metabolites are expected to occur that pose a significant risk to aquatic organisms. Therefore, a chronic invertebrate study is assumed to be not justifiable."

8.2. Assessment of the information provided

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

8.2.1. Annex IX, Section 9.1., Column 2 is not a valid basis to omit the study

228 Annex IX, Section 9.1., Column 2 does not allow omitting the need to submit information on long-term toxicity to aquatic invertebrates under Column 1. It must be understood as a trigger for providing further information on aquatic invertebrates if the chemical safety assessment according to Annex I indicates the need (Decision of the Board of Appeal in case A-011-2018).

229 Your adaptation is therefore rejected and the information requirement is not fulfilled.

9. Long-term toxicity testing on fish

230 Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.).

9.1. Information provided

231 You have adapted this information requirement by using Column 2 of Annex IX, Section 9.1. To support the adaptation, you have provided the following information:

(i) A justification for data waiving claiming that: "*In accordance with Column 2 of REACH Annex IX, the long-term aquatic toxicity to fish study (required in Section 9.1.6) does not need to be conducted as the chemical safety assessment according to Annex I indicates that this is not necessary.*

In Annex IX of Regulation (EC) No 1907/2006, it is laid down that chronic fish toxicity tests shall be proposed by the registrant if the chemical safety assessment indicates the need to investigate further the effects on fish. According to Annex I of this regulation, the chemical safety assessment triggers further action when the substance or the preparation meets the criteria for classification as dangerous according to Directive 67/548/EEC or Directive 1999/45/EC or is assessed to be a PBT or vPvB. The hazard assessment of tributyl citrate reveals neither a need to classify the substance as dangerous to the environment, nor is it a PBT or vPvB substance. According to reliable study results, tributyl citrate is considered to be rapidly biodegradable in aquatic compartments; the bioaccumulation potential is

regarded to be low (BCF 94.7 traditional method/6.54 Arnot-Gobas method). Furthermore, from metabolism studies in animals no metabolites are expected to occur that pose a significant risk to aquatic organisms. Therefore, with respect to animal welfare the performance of a chronic fish study is assumed to be not justifiable."

9.2. Assessment of the information provided

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

9.2.1. Annex IX, Section 9.1., Column 2 is not a valid basis to omit the study

233 Annex IX, Section 9.1., Column 2 does not allow omitting the need to submit information on long-term toxicity to fish under Column 1. It must be understood as a trigger for providing further information on long-term toxicity to fish if the chemical safety assessment according to Annex I indicates the need (Decision of the Board of Appeal in case A-011-2018).

234 In addition, minimisation of vertebrate animal testing is not on its own a legal ground for adaptation under the general rules of Annex XI.

235 Your adaptation is therefore rejected and the information requirement is not fulfilled.

9.3. Study design and test specifications

236 To fulfil the information requirement for the Substance, the Fish, Early-life Stage Toxicity Test (test method OECD TG 210) is the most appropriate (Guidance on IRs and CSA, Section R.7.8.2.).

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 27 September 2021.

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

ECHA did not receive any comments within the commenting period.

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.

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.

Appendix 3: Addressees 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]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[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[2].

(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

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

(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 variation in compositions reported by all members of the joint submission,
- 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 and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[3].

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