~~Confidential~~

Helsinki, 05 October 2023

Addressee(s)
Registrant(s) of JS_cobalt_cobalt sulphide as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
11/10/2021

Registered substance subject to this decision ("the Substance")
Substance name: Cobalt sulphide
EC/List number: 215-273-3

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

DECISION ON TESTING PROPOSAL(S)

Under Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***10 January 2028.***

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

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

1. Transgenic rodent somatic and germ cell gene mutation assays (Annex I, Section 0.5.; test method: OECD TG 488 from 2022) with the analogue substance cobalt sulphate, EC number 233-334-2, in transgenic rats, inhalation route, specified as follows:
   (i) The following tissues must be analysed: lung, liver, bone marrow, and kidney; and if technically possible also adrenals and pancreas.
   (ii) The study must include measurements of cobalt concentrations in whole blood in all animals of all dose groups at 7, 14 and 28 days; the measurements must be conducted directly after the inhalation exposure period in a standardised manner.

2. In vivo mammalian alkaline comet assay (Annex I, Section 0.5.; test method: OECD TG 489) with the analogue substance cobalt sulphate, EC number 233-334-2, in F344 (Fisher) rats, inhalation route, specified as follows:
   (i) The following tissues must be analysed: adrenals, lung, liver, bone marrow, kidney, and pancreas.
   (ii) The study must have a duration of 28 days.
   (iii) The study must include measurements of cobalt concentrations in whole blood in all animals of all dose groups at 7, 14 and 28 days; the measurements must be conducted directly after the inhalation exposure period in a standardised manner.
   (iii) The number of control animals per control group must be justified with a power calculation; ECHA recommends at least 15 control animals per control group.

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

3. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.; test method: OECD TG 413) by inhalation route, in rats, specified as follows:
   (i) The testing scheme in option B for poorly soluble solid aerosols specified in the OECD TG 413 must be followed. The study must include two satellite groups at 28 and 90 days post-exposure.
   (ii) The study must include measurements of cobalt concentrations in whole blood in all animals of all dose groups at 7, 14, 28 and 90 days of exposure and at the termination for the satellite groups; the measurements must be conducted directly after the inhalation exposure period in a standardised manner.

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

4. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) with the analogue substance tricobalt tetraoxide (EC No. 215-157-2) by oral route (diet), in rats, specified as follows:
   (i) Ten weeks premating exposure duration for the parental (P0) generation;
   (ii) The highest dose level in P0 animals must be determined based on clear evidence of an adverse effect on sexual function and fertility without severe suffering or deaths in P0 animals as specified further in Appendix 1, or follow the limit dose concept.

   The reporting of the study must provide the justification for the setting of the dose levels;
   (iii) Cohort 1A and 1B (Reproductive toxicity);
   (iv) Cohort 3 (Developmental immunotoxicity); and
   (v) The study must include measurements of cobalt concentrations in whole blood in P animals of all dose groups at 7, 14, 28 and 90 days of exposure.

   In addition, cobalt concentrations in whole blood in all F1 animals must be conducted at the time of termination.

   The measurements must be conducted in a standardised manner and animals may not be fasted.

   You must report the study performed according to the above specifications. Any expansion of the study must be scientifically justified.

Your originally proposed test using the analogue substance tricobalt tetraoxide, EC No. 215-157-2 is rejected, according to Article 40(3)(d):

- Sub-chronic toxicity study (90-day), inhalation route (OECD TG 413)

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 addressee(s) of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

The information requested under numbers 1, 2 and 4 of this decision is also requested from other registrants of the same category. Before performing the requested test(s), you are collectively required to make every effort to reach an agreement as to who is to generate that information on behalf of the other registrants. Under Article 53(1), you must inform ECHA within 90 days of the receipt of the adopted decision who will perform the

studies. You may already inform ECHA using the web form above. Once the current draft decision becomes adopted following procedure of Art. 50 and Art. 51, obligations and rights expressed in Article 53 will apply to you. Under Article 53(2 and 3) of the REACH Regulation if a registrant performs a test on behalf of other registrants, they shall all share the cost of that study equally and the registrant performing the test shall provide each of the others concerned with a copy/copies of the full study report(s).

In relation to the request for an Extended one-generation reproductive toxicity study, the requested design varies between the registrants with some for which a ten-week pre-mating exposure is required but no extension to mate the Cohort 1B animals to produce the F2 generation while, for other, a two-week pre-mating exposure is required with extension to mate the Cohort 1B animals to produce the F2 generation.

To avoid unnecessary animal testing, only one Extended one-generation reproductive toxicity study on tricobalt tetraoxide must be conducted.

In relation to the pre-mating exposure, the pre-mating exposure of ten weeks required in this decision can be reduced to two weeks in connection with the extension of cohort 1B to generate the F2 generation in order to permit a single study to be conducted.

You are only required to share the costs of information that you must submit to fulfil your information requirements (i.e., not for the F2 extension).

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 the read-across approach

1 You have used a read-across approach and grouped the Substance into a category and have identified the additional information which is considered necessary to produce the chemical safety report (CSR). You have proposed the following additional tests:

- Transgenic rodent somatic and germ cell gene mutation assays (Annex I, Section 0.5.)
- *In vivo* mammalian alkaline comet assay (Annex I, Section 0.5.)
- Sub-chronic toxicity (90 days; Annex IX, Section 8.6.2)

2 ECHA has considered the scientific and regulatory validity of your read-across approach(es) in general before assessing the specific testing proposals.

3 Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used.

4 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.

5 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.

0.1.1. Scope of the grouping of substances (category)

6 You provide a read-across justification documents in the CSR.

7 For read-across by the oral route, you have grouped cobalt substances into three groups: 'Bioavailable Co substances', 'Inorganic poorly soluble substances' and 'Poorly soluble organic ligand' with the following members:

8 Group 1: 'Bioavailable Co substances'

- Cobalt (EC No. 231-158-0)
- Cobalt bis(2-ethylhexanoate) (EC No. 205-250-6)
- Cobalt carbonate (EC No. 208-169-4)
- Cobalt di(acetate) (EC No. 200-755-8)
- Cobalt dichloride (EC No. 231-589-4)
- Cobalt dinitrate (EC No. 233-402-1)
- Cobalt oxalate (EC No. 212-409-3)
- Cobalt oxide (EC No. 215-154-6)
- Cobalt sulfate (EC No. 233-334-2)
- Cobalt(2+)propionate (EC No. 216-333-1)
- Cobalt(II) 4-oxopent-2-en-2-olate (EC No. 237-855-6)
- Cobalt, borate 2-ethylhexanoate complexes (EC No. 295-032-7)
- Cobalt dihydroxide (EC No. 244-166-4)

- Cobalt lithium dioxide (EC No. 235-362-0)

9 Group 2: 'Inorganic poorly soluble substance'

- Cobalt hydroxide oxide (EC No. 234-614-7)
- Cobalt sulphide (EC No. 215-273-3)
- Tricobalt tetraoxide (EC No. 215-157-2)

10 Group 3: 'Poorly soluble with an organic ligand'

- Cobalt, borate neodecanoate complexes (EC No. 270-601-2)
- Naphthenic acids, cobalt salts (EC No. 263-064-0)
- Neodecanoic acid, cobalt salt (EC No. 248-373-0)
- Resin acids and Rosin acids, cobalt salts (EC No. 273-321-9)
- Stearic acid, cobalt salt (EC No. 237-016-4)

11 For mutagenicity read-across, you have grouped all cobalt substances listed above into the same group.

12 For read-across by the inhalation route, you have grouped cobalt substances into two groups: 'Reactive Co substances' and 'Poorly soluble / poorly reactive Co substances' with the following members:

13 Group A: 'Reactive Co substances

- Cobalt (EC No. 231-158-0)
- Cobalt sulfate (EC No. 233-334-2)
- Cobalt dichloride (EC No. 231-589-4)
- Cobalt dinitrate (EC No. 233-402-1)
- Cobalt carbonate (EC No. 208-169-4)
- Cobalt di(acetate) (EC No. 200-755-8)
- Cobalt dihydroxide (EC No. 244-166-4)
- Cobalt oxide (EC No. 215-154-6)

14 Group B: 'Inorganic poorly soluble substance'

- Cobalt hydroxide oxide (EC No. 234-614-7)
- Cobalt sulphide (EC No. 215-273-3)
- Tricobalt tetraoxide (EC No. 215-157-2)
- Cobalt lithium dioxide (EC No. 235-362-0)

15 You justify the grouping of substances by the fact that all substances liberate the same toxic entity, i.e. the cobalt cation, upon dissolution in aqueous biological media. You consider that the toxicity resulting from the cobalt ion will be the same in qualitative terms while there may be differences in quantitative terms due to differences in dissolution rates between the groups.

16 You have based the grouping primarily on the dissolution in artificial gastric fluid. To support your grouping, you refer to differences in the toxicity profile between members of the different groups. available repeated dose toxicity studies within the groups.

17 ECHA notes that your grouping is based on expected differences in toxicity based on cobalt ion release and that you intend to use the same grouping for both the oral and inhalation routes of exposure.

18 The grouping clearly and unambiguously defines the applicability domain of each group

19 ECHA understands that this is the applicability domain of the groupings and your predictions within each group are assessed on this basis.

20 However, we emphasise that any final determination on the validity of your read-across adaptation will only be possible when the information on requested studies will be available in the dossier after assessing whether it confirms or undermines the read-across hypothesis.

0.1.2. Prediction (category)

21 The assessment of the proposed predictions of toxicological properties are assessed in the endpoint specific sections below.

Reasons for the decision(s) related to the information under Annex VIII of REACH

1. Transgenic rodent somatic and germ cell gene mutation assays; and

2. *In vivo* mammalian alkaline comet assay

22 Under Annex I, Section 0.5. to REACH, additional tests listed in Annex IX or X to may be proposed if the information obtained from these tests are considered necessary to produce the Chemical Safety Report (CSR).

23 In such cases, a testing strategy explaining why the additional information is necessary shall be submitted.

2.1. Further in vivo mutagenicity testing

24 You have provided a testing strategy which aims to further explore the potential for *in vivo* mutagenicity following inhalation exposure.

25 As part of this testing strategy, you have submitted testing proposals for

(i) Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488) by inhalation with cobalt sulphate; and

(ii) *In vivo* mammalian alkaline comet assay (OECD TG 489) by inhalation with cobalt sulphate.

26 In addition, the following information is relevant for the testing proposal examination:

(i) Toxicology and carcinogenesis studies of cobalt sulphate heptahydrate in F344/N rats and B6C3F1 mice (inhalation studies; EC No. 233-334-2; [redacted] 1998).

(ii) Toxicology studies of cobalt metal in F344/N rats and B6C3F1/N mice and toxicology and carcinogenesis studies of cobalt metal in F344/NTac rats and B6C3F1/N mice (inhalation studies; EC No. 213-158-0; [redacted], 2014);

(iii) Oral Sub-chronic toxicity study on the Substance ([redacted], 2015);

(iv) Oral Sub-acute toxicity study on the Substance ([redacted], 2015);

(v) Toxicological Profile for Cobalt (ASTDR, 2004); and

(vi) RAC Opinion on cobalt metal (CLH-O-0000001412-86-172/F; ECHA, 2017)

27 ECHA understands, that you have proposed a testing strategy which intends to provide further information in support of your hypothesis that the cobalt-related cancers are not caused by a genotoxic mode of action but a secondary (indirect) consequence of a non-genotoxic mode of action, i.e. persistent inflammation resulting in meta-, hyper- and ultimately neoplasia in the respiratory tract.

28 In the sections below, ECHA has assessed the testing proposals in relation to the aims of the testing strategy.

29 Cobalt metal, cobalt sulphate, cobalt dichloride, cobalt dinitrate, cobalt carbonate and cobalt di(acetate) have harmonised classifications which include Muta. 2:H341 'Suspected to cause genetic defects'; Index No. 027-001-00-9. 027-005-00-0, 027-004-00-5, 027-009-00-2, 027-010-00-8, and 027-006-00-6, respectively.

30 The genotoxicity of cobalt metal has been reviewed in detail by RAC and can be summarised as follows: "Cobalt metal and cobalt salts can cause DNA damage measured by Comet assay and chromosomal aberrations and micronuclei in vitro, although they do not cause direct

mutagenic effects."; and "Overall, the critical issue is whether the available in vivo data gathered via physiological exposure routes can provide enough evidence to conclude that genotoxicity in vivo is not relevant via these routes. If not, classification as Muta. 2 is warranted based on ip [intraperitoneal] data and in vitro data. At present, although the recent studies using oral or inhalation routes suggest that genotoxicity may be below the detection limit of these test assays, it is difficult to exclude relevant systemic genotoxicity, especially when there are additionally some indications from earlier – although less reliable - studies on the genotoxic effects via physiological routes." (RAC Opinion on cobalt metal, 2017).

31 Currently local (direct) genotoxicity at the port-of-entry cannot be excluded due to lack of data.

32 Therefore, further information is needed to produce the CSR.

2.2. Information provided

33 You have submitted testing proposals for a Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488); and an *in vivo* mammalian alkaline comet assay (OECD TG 489) both studies are proposed to be conducted with the analogue substance cobalt sulphate, EC No. 233-334-2.

34 ECHA requested your considerations for alternative methods to fulfil the information requirement for *in vivo* mutagenicity. You provided your considerations and you applied read-across to fulfil the respective information requirement, and no other alternative methods were available. ECHA has taken these considerations into account.

35 ECHA agrees that the proposed studies are necessary to produce the chemical safety reports for the Substance.

2.3. Grouping of substances and read-across approach

36 You have provided a read-across justification document in the CSR and IUCLID.

37 As explained in Section 0.1. above you have grouped all cobalt substances into the same group.

38 To generate additional information needed for the CSR, you propose to test cobalt sulphate (EC No. 233-334-2) for in vivo mutagenicity. The selection of the test material is based on a 'worst case' approach.

39 ECHA understands that you read-across hypothesis assumes that different compounds have the same type of effects. The properties of the Substance are predicted based on a worst-case approach.

40 Cobalt sulphate belongs to the 'Bioavailable Co substances' and is soluble and fully dissociated in water (and biological media). Following oral or inhalation administration, at toxicologically relevant dose levels, the cobalt sulphate can be assumed to be fully dissociated based on the water solubility of the substance, toxicokinetic information and available repeated dose toxicity studies.

41 Furthermore, the toxicity profile of the counter-ion is already known and does not require further investigation.

42 Therefore, cobalt sulphate can be considered as a worst-case in terms of exposure to the cobalt ion for all groups of cobalt substances.

43 As explained above, you have established that the properties of the Substance can be predicted from data on the analogue substance.

44 ECHA agrees with your read-across hypothesis.

45 However, ECHA emphasises that any final determination on the validity of your read-across adaptation will only be possible when the information on requested studies will be available in the dossier and after assessing whether it confirms or undermines the read-across hypothesis.

2.4. Test selection

46 You have proposed to conduct a Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488); and an *in vivo* mammalian alkaline comet assay (OECD TG 489).

47 The proposed tests explore different aspects of mutagenicity, i.e. gene mutations and chromosomal aberrations. The comet assay "can detect single and double stranded breaks, resulting, for example, from direct interactions with DNA, alkali labile sites or as a consequence of transient DNA strand breaks resulting from DNA excision repair. These strand breaks may be repaired, resulting in no persistent effect, may be lethal to the cell, or may be fixed into a mutation resulting in a permanent viable change".

48 Therefore, the in vivo comet assay is regarded as indicator assay for general DNA damage, but not as an assay to detect specific mutations.

49 In contrast, the transgenic rodent will evaluate gene mutations only.

50 Therefore, to be able to differentiate between gene mutations and chromosomal aberrations following inhalation exposure both tests are needed.

51 In addition, the tests may provide support for a non-genotoxic mode of action for the cancers observed following inhalation exposure.

52 Therefore, ECHA considers that both tests will provide important information needed to further explore genotoxicity following inhalation exposure.

53 However, a significant amount of information is required to demonstrate an alternative non-genotoxic mode of action. This will require a side-by-side comparison of the key events in the different modes of action in terms of time and dose concordance for both for systemic and port-of-entry effects. Any conclusion with regard to potential for *in vivo* genotoxicity is dependent on the outcome of the proposed test.

54 On this basis, a transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488) and an *in vivo* mammalian alkaline comet assay (OECD TG 489) are needed to develop the CSR for all cobalt substances in Groups 1-3.

2.5. Specification of the study design for the transgenic rodent somatic and germ cell gene mutation assays

55 Based on the recent update of the OECD TG 488, you are requested to follow the new 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals.

2.5.1. Specification of test species

56 You proposed testing in transgenic rats.

57 According to the OECD TG 488, the test may be performed in transgenic mice or rats.

58 The aim of the testing strategy is to exclude local (port-of-entry) genotoxicity as a mode of action for the tumours observed in the carcinogenicity studies with cobalt sulphate and cobalt metal ([redacted], 1998; [redacted]; 2014). An additional aim is to identify threshold values for both secondary (indirect) genotoxic effects and inflammation at the site of contact.

59 The [redacted] studies were conducted in F344 (Fisher) rats.

60 Ideally, the test should be performed in F344 (Fisher) rats because this was the strain in which the concern was identified.

61 However, this is a transgenic model and changing the genetic background of the model would require a significant breeding effort.

2.5.2. Specification of the route of exposure

62 You proposed testing by the inhalation route.

63 According to the OECD TG 488, test substance is usually administered orally.

64 However, having considered the aim of the testing strategy (investigate site-of-contact mutagenicity following inhalation exposure), the anticipated routes of human exposure, and adequate exposure of the target tissue(s), performance of the test by the inhalation route is appropriate.

65 You propose to use dust as the form of dispersion.

66 According to the OECD TG 488, test chemicals can be administered as gas, vapour, or a solid/liquid aerosol, depending on their physicochemical properties.

67 In the previous inhalation studies with the cobalt sulphate ([redacted], 1998), "cobalt sulfate heptahydrate in deionized water (approx. 400 g/L) was siphoned from the bulk reservoir to the nebulizer reservoir and then aspirated into the nebulizer chamber and expelled as a stream through the larger orifice. Shear forces broke the stream into droplets that were evaporated to leave dry particles of cobalt sulfate heptahydrate."

68 This dispersion method is demonstrated to be technically feasible and using a similar method of dispersion will facilitate result comparison.

69 Therefore, cobalt sulphate must be dispersed as previously described by [redacted].

2.5.3. Specification of target tissues

70 You proposed to analyse tissues from bone marrow and kidney in addition to liver and lung.

71 According to the OECD TG 488 "the selection of tissues to be collected should be based upon the reason for conducting the study and any existing mutagenicity, carcinogenicity or toxicity data for the test chemical under investigation".

72 The aim of the testing strategy is to determine local (port-of-entry) genotoxicity as a mode of action for the tumours observed in the carcinogenicity studies with cobalt sulphate and cobalt metal ([redacted], 1998; [redacted]; 2014).

73 Based on measured cobalt tissue organs content/concentration from available toxicity studies ([redacted], 2014; ASTDR, 2004), the following tissues/organs may be target organs for cobalt ion: adrenals, bone marrow, brain, heart, kidney, liver, lung, pancreas and testis.

74 ECHA agrees that analysis of bone marrow and kidney should be included in the study because they are cobalt target organs.

75 However, in the inhalation carcinogenicity studies ([redacted] 1998; [redacted]; 2014) systemic tumours were also observed in the adrenals, pancreas and liver.

76 To confirm or exclude the hypothesis of the testing strategy, tissues were tumours have been observed must be investigated in the study. This is because you have not demonstrated the representativeness of the target organs of bone marrow and kidneys, taking into account the fact that the mechanism of tumour formation is unknown.

77 In your comments on the draft decision, you agree to analyse tissues in the TGR animals that are technically feasible (i.e. of sufficient size/weight) and qualified (i.e. historical

control database, positive control data). You state that based upon discussions with the testing laboratory, that both the adrenal glands and pancreas are not qualified tissues and the adrenals may not be technically feasible to analyse in the TGR study and that further discussion with the laboratory is needed.

78 ECHA considers that it is important to investigate adrenals and pancreas because these tissues are identified target organs in the [redacted] carcinogenicity studies. You are to make every effort in investigating these tissues if technically feasible.

79 Based on the above, the following tissues should be analysed in the study: lung, liver, bone marrow and kidney; and if technically feasible adrenals and pancreas.

2.5.4. Germ cells

80 You should collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA to decide on the need for assessment of mutation frequency in the collected germ cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

2.5.5. Measurements of cobalt levels in the blood

81 Where a test method offers flexibility in the study design, the chosen test design must ensure that the data generated are adequate for hazard identification and risk assessment (by analogy, REACH Annexes VII-X, introductory paragraphs).

82 In this case, the objective of testing is to generate adequate information for hazard identification, in particular to confirm or invalidate the hypothesis of your testing strategy, and risk assessment, in particular to assess which route(s) of human exposure may require or not specific risk management measures.

83 According to the OECD TG 488, blood measurement may be considered to demonstrate tissue exposure. The OECD TG 488 does not prohibit, and thus leave flexibility, to consider such measurement in light of the testing objective.

84 In this case, the objective for testing is to confirm or exclude a hypothesis based on existing data as well as with other data to be generated for the same purpose.

85 The measurements are required to demonstrate tissue exposure as well as to be able to compare the effects observed in these studies with the previously conducted carcinogenicity studies via inhalation route.

86 The fact that blood measurement has been done in the past in the [redacted] studies confirms that this is technically feasible.

87 Therefore, you must include measurements of cobalt concentrations in whole blood in the study design after 7 days, 14 days and at 28 days of exposure. The cobalt blood measurements can be done in either as part of the main study or in a satellite group with identical exposure conditions.

88 In your comments on the draft decision, you propose to measure cobalt levels in the TGR animal tissues if technically feasible. ECHA considers that you may include tissue measurements in the study at your own discretion as long as it does not interfere with the objectives of the study.

89 In addition, this is an inhalation study. Therefore, measurements of cobalt levels in the blood must be conducted immediately after the inhalation exposure in a standardised manner.

2.6. *Specification of the study design for the In vivo mammalian alkaline comet assay*

2.6.1. *Specification of rat strain*

90 You proposed testing in the rat.

91 According to the OECD TG 489, rats are the preferred species.

92 The aim of the testing strategy is to exclude local (port-of-entry) genotoxicity as a mode of action for the tumours observed in the carcinogenicity studies with cobalt sulphate and cobalt metal ([redacted], 1998; [redacted]; 2014). These studies were conducted in F344 (Fisher) rats.

93 Therefore, the study must be conducted using F344 (Fisher) rats.

94 In your comments on the on the draft decision, you agree to conduct the study in F344 (Fisher) rats.

95 However, you raise the issue that there may be problems with having an adequate historical control as many laboratories stopped using Fisher rats 10 years ago. To accommodate this and the variation in the Comet assay you propose to add more concurrent control animals in the study.

96 Normally, there are 5 animals in each control group of the OECD TG 489. However, the lack of adequate historical controls must be compensated by a higher number to ensure the reliability of the study. In this situation, the study results must be interpreted solely based on the concurrent controls. A reliable method to determine such number is the power calculation. Based on a preliminary assessment, considering the results of other comet assays, ECHA recommends using at least 15 control animals per control group must be included to facilitate the interpretation of the results. A higher number may be required under the power calculation on the basis of more detailed information that are available to a laboratory.

2.6.2. *Specification of the route of exposure*

97 You proposed testing by the inhalation route.

98 According to the OECD TG 489, test substance is usually administered orally.

99 For the same reasons as explained in Section 2.5.2., the study must be performed with dispersion of cobalt sulphate as previously described by [redacted].

2.6.3. *Specification of the study duration*

100 According to the OECD TG 489, animals should be given daily treatments over 2 or more days and extended dose regimens, e.g. 28-day daily dosing are acceptable.

101 You have proposed a duration of 28 days for this study.

102 The proposed test is proposed as part of a testing strategy. This strategy also includes a Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488) to be conducted with the same substance.

103 To facilitate interpretation of the results ECHA considers that the duration of both studies should be identical.

104 According to the OECD TG 488, the study duration must be at least 28 days.

105 Therefore, the duration of this study must 28 days.

2.6.4. *Specification of target tissues*

106 You did not specify which tissues are to be investigated in the study.

107 To be able to achieve the goals of the testing strategy and allow a side-by-side comparison of the results. ECHA considers that the same tissues should be analysed in both the OECD TG 488 and OECD TG 489. For reasons for selection of target organs, see Section 2.5.3.

108 In your comments on the draft decision, you highlight that although technically feasible to collect the adrenals has not been measured in the past and there are no historical controls.

109 ECHA notes that to compensate for the lack of adequate historical controls for the Fisher strain you propose to increase the number of concurrent controls. ECHA considers that with an increased number of concurrent controls, there is no reason not to investigate also the adrenals.

110 Therefore, the following tissues must be analysed in the study: adrenals, lung, liver, bone marrow, kidney, and pancreas.

2.6.5. Measurements of cobalt levels in the blood

111 Measurements of cobalt levels in the blood must be included in the study as explained in Section 2.5.5.

2.6.6. Germ cells

112 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other afore mentioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, you should consider analysing the slides prepared with gonadal cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

2.6.7. Additional investigations

113 You propose additional analyses for cytotoxicity and other parameters to assess potential secondary effects are foreseen (such as: 8-OH-dG lesions, hypoxia upregulation, inflammatory markers, cell infiltration, cytotoxicity, 8-oxoguanine DNA glycosylase, poly ADP ribose and gamma H2AX). Your justification is that the additional analyses are needed to correlate cytotoxicity to comet assay results, due to the sensitivity and lack of specificity of the comet assay.

114 It is at your discretion whether to include these as part of the study as long as inclusion of these additional parameters does not compromise the integrity of the OECD TG 489 study design, or the additional investigations specified in this decision.

2.7. Outcome

115 Under Article 40(3)(b) your testing proposals for a transgenic rodent somatic and germ cell gene mutation assays; and an *in vivo* mammalian alkaline comet assay are accepted under modified conditions and you are requested to conduct the test with the analogue substance cobalt sulphate, EC No. 233-334-2, as specified above.

Reasons for the decision(s) related to the information under Annex IX of REACH

3. Sub-chronic toxicity study (90-days)

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

3.1. Information provided to fulfil the information requirement

117 You have submitted a testing proposal for a Sub-chronic toxicity study (90 day) according to OECD TG 413 with the analogue substance tricobalt tetraoxide, EC No. 215-157-2.

118 Your dossier contains a sub-acute inhalation toxicity: 28-Day Study (2019) conducted with tricobalt tetraoxide. No sub-chronic inhalation studies are provided.

119 ECHA requested your considerations for alternative methods to fulfil the information requirement for Repeated dose toxicity. You provided your considerations and you applied read-across to fulfil the respective information requirement, and no other alternative methods were available. ECHA has taken these considerations into account.

120 ECHA agrees that a 90-day study is necessary.

3.2. Grouping of substances and read-across approach

121 You have provided a read-across justification document in the CSR and IUCLID.

122 As explained in Section 0.1. above you have grouped the Substance into a category of 'Inorganic poorly soluble' cobalt compounds.

123 You provide the following reasoning for the grouping the substances: "There are quantitative differences in the dissolution rate in different aqueous biological media, thus an assumed difference in systemic toxicity which is predicted to correlate with the ability of the substance to release cobalt cations (dissolution kinetics)" (RAAF, Scenario 3).

124 ECHA understands that your read-across hypothesis assumes that different compounds have the same type of effects. You predict the properties of your Substance based on an identified trend within the group.

3.2.1. Prediction of toxicological properties

125 From your Strategy to address read-across and grouping of cobalt and cobalt compounds for chronic inhalation toxicity in your CSR, ECHA understands that your intention is to demonstrate that the sub-group B of 'Poorly soluble / poorly reactive Co substances' cobalt compounds do not cause lung cancer.

126 The 'Poorly soluble / poorly reactive Co substances ' cobalt substances […] are insoluble in pH neutral fluids and poorly soluble in lysosomal fluid and do not produce 'persistent inflammation' or metaplasia in the respiratory tract. This group also includes substances that are highly soluble in pH neutral fluids as complexes- thereby not releasing the Co^{2+} ion.

127 You postulate that "Members of the non-reactive group, such as tricobalt tetraoxide or cobalt sulphide, do not show test-item related (persistent) local inflammation. The effects of the non-reactive substances is best compared with the effects seen with other poorly-soluble low-toxicity particles (PSLT), leading to a minimal or mild inflammatory response only at the maximum tolerated concentration in repeated dose toxicity studies via inhalation." You describe a multi-tier testing plan, which may result in evidence to substantiate this.

128 More notably, your hypothesis on the mode of action of carcinogenicity is: "The common compound formed by all substances within this category is the Co^{2+} cation. It is assumed that liberation of the common compound leads to the following key events:

- Upregulation of in vitro biomarkers
- 'Persistent' inflammation and/or upper respiratory tract reactivity upon acute exposure

129 These key events are hypothesised to lead to inhalation carcinogenicity. Absence of these key events is hypothesised to be associated with lack of inhalation carcinogenicity."

130 Your testing strategy consists of six tiers:

Tier 1:	Bioaccessibility (artificial lung fluids)
Tier 2:	In vitro biomarkers (hypoxia and cytotoxicity) and gene reporter assay (p53, protein damage, oxidative stress, DNA damage, hypoxia)
Tier 3:	In vivo persistent inflammation or upper respiratory tract meta- and hyperplasia (acute inhalation testing)
Tier 4:	28-day RTD inhalation testing (tricobalt tetraoxide)
Tier 5:	90-day RDT inhalation testing (tricobalt tetraoxide)
Tier 6:	Chronic inhalation testing (depending on the results of Tier 5)

131 ECHA as assessed your testing strategy and identified the following issues:

3.2.1.1. Tier 1, 2 and 4: Read-across hypothesis contradicted by existing data

132 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 must 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.).

133 Your read-across hypothesis is based on an observed trend in increasing toxicity with correlated with the increasing release of the cobalt ion.

134 To support your hypothesis you provide a tiered testing strategy.

135 **For Tier 1** of your testing strategy: Bioelution testing in artificial interstitial (pH 7.4), alveolar (pH 7.4 with phosphatidyl choline) and lysosomal (pH 4.5-5) fluid for cobalt substances over 5 hours (Stopford et al, 2003)

Substance name	Exposure duration [h]	cobalt release concentration [µg Co/mL]		
		Interstitial	*Alveolar*	*Lysosomal*
Co_3O_4	5	0.05	0.08	22
CoS	5	15	15	7.6
$CoLiO_2$	5	0.05	0.05	15
CoOOH	5	0.2	0.1	68

136 You conclude that "no robust correlation is observed between the bioelution profile of a substance and its acute inhalation toxicity".

137 ECHA agrees with this conclusion, which is not in line with your hypothesis.

138 In addition, ECHA would like to highlight the limitations of the method applied. *In vitro* bioelution is a static model which estimates the cobalt dissolution under the given conditions of the test, i.e. at equilibrium.

139 The model provides an estimate of cobalt release under the specified conditions.

140 However, this test system does not consider the fact that *in vivo* equilibrium is never reached because the released cobalt ions are removed by the blood flow in the lung.

141 Consequently, the model may underestimate the release *in vivo*.

142 ECHA concludes that the cobalt ion will be released to by all substances in the group and you have not demonstrated that tricobalt tetraoxide would be the 'worst case' within the group.

143 In addition, it is not possible to identify a trend within the group because it varies between the different compartments simulated.

144 **For Tier 2** of your testing strategy: *In vitro* biomarkers (hypoxia and cytotoxicity) and gene reporter assay (p53, protein damage, oxidative stress, DNA damage, hypoxia).

145 However, the mode of action of cobalt carcinogenicity is unknown and the selected markers are based on a hypothesised mode of action yet to be demonstrated.

146 Regardless the results obtained are identical within the category of 'Poorly soluble inorganic' cobalt substances. This contradicts your read-across hypothesis that it is the cobalt ion which drives toxicity because they fail to identify a trend within the group despite an approximate 10-30 fold difference in the in Tier 1 estimated cobalt release of cobalt sulphide, cobalt lithium dioxide and cobalt hydroxioxide when compared to tricobalt tetraoxide.

147 **For Tier 4** of your strategy: 28-day RDT inhalation testing (tricobalt tetraoxide), you compare the effects observed with those observed in sub-chronic inhalation studies with a 'Bioavailable cobalt substance' (cobalt sulphate) and a poorly soluble particle (titanium dioxide). You conclude that the effects observed via 28 days inhalation toxicity study with tricobalt tetraoxide were toxicologically similar to effects observed for titanium dioxide.

148 ECHA fails to understand how this observation supports the grouping.

149 Firstly, According to Annex XI section 1.5 predictions must be made within the defined group and both substances are outside the defined group of 'Poorly soluble / poorly reactive Co substances ' cobalt substances. There is only one data point within the group for which you have not demonstrated that it allows to define a 'worst case' yet alone a trend within the group.

150 In addition, ECHA notes that both cobalt sulphate and titanium dioxide have a harmonised classification for carcinogenicity.

151 Your read-across hypothesis state that it is the cobalt ion that drives toxicity. By comparing the tricobalt tetraoxide with titanium dioxide you introduce a second mode of action based on a poorly soluble particle effect.

152 The substances within the group of poorly soluble inorganic cobalt substances both release the cobalt ion and exhibit toxicity which you attribute as a poorly soluble particle effect.

153 ECHA concludes that the substances in the group are likely to have more than one mode of action this is in contradiction with your read-across hypothesis.

154 ECHA concludes that the results of Tier 1, 2 and 4 contradict your read-across hypothesis.

3.2.1.2. Tiers 4 and 5: Insufficient data density to confirm a trend

155 Annex XI, Section 1.5. provides that "substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances".

156 According to the Guidance on IRs and CSA, Section R.6.2.1.5., one of the factors in determining the robustness of a category is the density and distribution of the available data across the category. To identify a regular pattern and/or to derive reliable prediction of the properties of the members of the category, adequate and reliable information covering the range of structural variations identified among the category members needs to be available.

157 As indicated above, read-across hypothesis is based on an observed trend in increasing toxicity with correlated with the increasing release of the cobalt ion.

158 In **Tier 3** of your testing strategy, you present results of acute inhalation testing.

159 You have presented preliminary data that supports your hypothesis that there may be a trend within the group. The normalized severity scores for tricobalt tetraoxide, cobalt sulphide and cobalt lithium dioxide are 0.056; 0.07; and 0.252, respectively (cobalt hydroxide oxide not tested).

160 The normalized severity after acute exposure differs by up to a factor of 4.5 (0.252/0.056) between the tested substances in the group and tricobalt tetraoxide. With increasing exposure duration more significant differences can be expected because of the longer study duration. The data suggest that tricobalt tetraoxide may constitute one of the borders of the category; however the information provided does not demonstrate that it constitute the 'worst case' within the group.

161 In **Tier 4** of your testing strategy, you present the results of one 28-day RDT inhalation testing with tricobalt tetraoxide.

162 In **Tier 5** of your testing strategy, you propose test tricobalt tetraoxide for one sub-chronic inhalation toxicity.

163 Information for one category member does not establish a trend across the category.

164 Therefore, the information provided is not sufficient to conclude that toxicological properties are likely to follow a regular pattern (trend).

3.2.2. Conclusion on the read-across approach

165 Based on the above, you have not established that relevant properties of the Substance can be predicted from data on the source substance(s). Your read-across approach under Annex XI, Section 1.5. is rejected.

3.3. Specification of the study design

3.3.1. Specification of test species

166 You proposed testing in the rat. ECHA agrees with your proposal because the rat is the preferred species according to the OECD TG 413. Therefore, the study must be conducted in the rat.

3.3.1. Specification of route of exposure

167 You proposed testing by the inhalation route. ECHA agrees with your proposal because the criteria in Column 2 of Annex IX, Section 8.6.2. as to when testing via the inhalation route is appropriate are met. Exposure of humans via inhalation is likely taking into account the possibility of exposure to particles of an inhalable size.

3.3.2. Satellite groups

168 You proposed to include a 90-day satellite (recovery) group in the study.

169 As described in the OECD TG 413, recovery group(s) may be needed to address lung clearance kinetics. Because the substances in the group are poorly soluble, low clearance may influence the mode of actions and the toxic effects observed. The OECD TG 413 recommends more than one satellite groups, see study design Option B.

170 Therefore, satellite groups at 28 and 90 days post-exposure must be included in the study as outlined in the study design Option B for poorly soluble aerosols in OECD TG 413.

171 In your comments on the draft decision, you agree that satellite groups at 28- and 90-days are required.

3.3.3. Measurements of cobalt levels in the blood

172 Where a test method offers flexibility in the study design, the chosen test design must ensure that the data generated are adequate for hazard identification and risk assessment (by analogy, REACH Annexes VII-X, introductory paragraphs).

173 The objective of testing is to generate adequate information for hazard identification, in particular to confirm or exclude the hypothesis of your testing strategy, and to assess which route(s) of human exposure may require specific risk management measures.

174 The OECD TG 413 leave flexibility to consider additional investigations in light of the testing objective.

175 In this case, the objective for testing is to confirm or exclude a hypothesis based on existing data as well as with other data to be generated for the same purpose.

176 The aim of your testing strategy is to demonstrate that the group of "Poorly soluble / poorly reactive Co substances" do not cause lung cancer which is the case for the "bioavailable cobalt substances". Your read-across hypothesis assumes that it is the cobalt ion which drive toxicity.

177 Therefore, determination of the of cobalt levels in the blood is necessary to confirm the hypothesis; measurements must be conducted after 7 days, 14 days, 28 days and 90 days of exposure and at the end of the recovery period. The fact that blood measurement has been done in the past confirms that this is technically feasible.

178 In addition, this is an inhalation study. Therefore, measurements of cobalt levels in the blood must be conducted immediately after the inhalation exposure in a standardised manner.

179 In your comments on the draft decision, you agree to measure cobalt levels in blood and propose to do so by adding satellite animals to all dose groups.

180 ECHA considers that adding satellite animals is at your discretion.

3.3.4. Specification of the additional investigations

181 You proposed to extend the sub-chronic toxicity study (90 day) by including the following additional examinations/parameters: haematology (because of known effect caused by systemic availability of the cobalt cation), histopathology (with a focus on the assumed target organs), immunohistochemistry (investigations for oxidative DNA lesions in the lung by scoring 8-OH-dG) and bronchoalveolar lavage (for the analysis of markers relevant for PSLT and cobalt exposure: total cell count, differential cell count, β-glucuronidase, total protein, LDH, HIF-1α, IL-8, MCP-1).

182 ECHA considers that it is at your discretion to perform the intended additional examinations, as long as they do not interfere with the examinations prescribed by the OECD TG 413 or specified above.

3.4. Outcome

183 Your testing proposal is rejected under Article 40(3)(d) of REACH. Under Article 40(3)(c) you are requested to carry out the additional test with the Substance, as specified above.

Reasons for the decision(s) related to the information under Annex X of REACH

4. Extended one-generation reproductive toxicity study

184 The basic test design of an extended one-generation reproductive toxicity study (EOGRTS) is a standard information requirement under Annex X to the REACH Regulation. Furthermore, column 2 of Section 8.7.3. defines when the study design needs to be expanded.

4.1. Information provided to fulfil the information requirement

185 You have submitted a testing proposal for an EOGRTS according to OECD TG 443 with the Substance.

186 Your dossier contains combined repeated dose toxicity study with the reproduction / developmental toxicity screening tests with tricobalt tetraoxide (2012; OECD TG 422) and cobalt sulphide (2012; OECD TG 422). No EOGRTS is available.

187 ECHA requested your considerations for alternative methods to fulfil the information requirement for Toxicity to reproduction. You provided your considerations and you applied read-across to fulfil the respective information requirement, and no other alternative methods were available. ECHA has taken these considerations into account.

188 ECHA agrees that an EOGRTS is necessary.

4.2. Grouping of substances and read-across approach

189 You have provided a read-across justification document in the CSR and IUCLID.

190 As explained in Section 0.1. above you have grouped the Substance into a category of 'Inorganic poorly soluble' cobalt compounds.

191 You provide the following reasoning for the grouping the substances: "There are quantitative differences in the dissolution rate in different aqueous biological media, thus an assumed difference in systemic toxicity which is predicted to correlate with the ability of the substance to release cobalt cations (dissolution kinetics)"

192 ECHA understands that your read-across hypothesis assumes that different compounds have the same type of effects. You predict the properties of your Substance based on a worst-case approach.

193 To support your read-across hypothesis you have provided in vitro bioaccessibility data in artificial gastric juice. The mean release rate [µg Co/cm²/h] is 0.023, 0.017 and 0.651 for tricobalt tetraoxide, cobalt hydroxioxide and cobalt sulphide, respectively.

194 The *in vitro* model is a static model which do not consider the fact that equilibrium likely is not reached in the gut because the absorption of cobalt ions is facilitated by the divalent metal-ion transporter-1 (DMT1) in the duodenum and proximal jejunum. Therefore, the *in vivo* absorption is likely higher than what the model predicts.

195 In your comments on the draft decision, you have provided *in vivo* toxicokinetic information (OECD TG 417) which estimates the relative oral bioavailability of cobalt dichloride, tricobalt tetraoxide and cobalt sulphide compared to an intravenous injection of cobalt dichloride. The studies show that both tricobalt tetraoxide and cobalt sulphide have a relative oral bioavailability of 0.1% and that the oral relative bioavailability of cobalt dichloride is 6.8-11.7%.

196 On this basis, ECHA considers your read across approach as plausible.

197 In your comments on the initial draft decision, you propose to change the test material from the Substance to the analogue substance tricobalt tetraoxide because the pure form of cobalt sulphide is no longer on the market. In your comments on the proposal for amendment, you re-iterate your proposal to use analogue substance tricobalt tetraoxide as the test material because the pure form of cobalt sulphide is no longer on the market. ECHA agrees with this proposal.

4.3. Specification of the study design

4.3.1. Species and route selection

198 You proposed testing in rats. ECHA agrees with your proposal.

199 As the Substance is a solid, the study must be conducted with oral administration of the Substance (Annex X, Section 8.7.3, Column 1).

200 You proposed testing via the oral route. However, you did not further specify the administration method.

201 The OECD TG 443 has been designed for administration of the test chemical through the diet although administration though gavage and drinking water way be considered.

202 Absorption of cobalt ions is facilitated by the divalent metal-ion transporter-1 (DMT1) in the duodenum and proximal jejunum. DMT1 is a H(+)-coupled metal-ion transporter which is responsible for the absorption of divalent metal ions including iron and zink. The selectivity of this DMT1 is Cd(2+) > Fe(2+) > Co(2+), Mn(2+) ≫ Zn(2+), Ni(2+) (Illing, 2012[2]).

203 Gavage administration will result in intermittently high concentrations of cobalt ions in the duodenum and proximal jejunum. These intermittent high concentrations of cobalt ions are likely to overload the facilitated transport mechanism, and thereby impair bioavailability. Impaired bioavailability may underestimate the hazard.

204 The substance is poorly soluble in water. Therefore, administration via drinking water is not an option.

205 Based on the above, the substance must be administered though the diet.

206 In your comments on the draft decision, you agree to conduct the study with administration of the test item through the diet.

207 However, you highlight the fact that the current database consists of gavage studies and that are required before a full EOGRTS is conducted. You propose a 14-day study and an abbreviated (in terms of animals per group) OECD TG 421 as palatability studies.

4.3.2. Pre-mating exposure duration

208 The length of pre-mating exposure period must be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

209 Ten weeks pre-mating exposure duration is required to obtain results adequate for classification and labelling and/or risk assessment. There is no substance specific information in the dossier supporting shorter premating exposure duration (Guidance on IRs and CSA, Section R.7.6.).

210 Therefore, the requested pre-mating exposure duration for the P0 animals is two weeks.

211 In your comments on the draft decision, you agree with the pre-mating exposure duration.

[2] Illing AC, Substrate profile and metal-ion selectivity of human divalent metal-ion transporter-1. *J Biol Chem.* 2012 Aug 31;287(36):30485-96. doi: 10.1074/jbc.M112.364208.

4.3.3. Dose-level setting

212 The aim of the requested test must be to demonstrate whether the classification criteria of the most severe hazard category for sexual function and fertility (Repr. 1B; H360F) and developmental toxicity (Repr. 1B; H360D) under the CLP Regulation apply for the Substance (OECD TG 443, para. 22; OECD GD 151, para. 28; Annex I Section 1.0.1. of REACH and Recital 7, Regulation 2015/282), and whether the Substance meets the criteria for a Substance of very high concern regarding endocrine disruption according to Art.57(f) of REACH as well as supporting the identification of appropriate risk management measures in the chemical safety assessment.

213 To investigate the properties of the Substance for these purposes, the highest dose level must be set on the basis of clear evidence of an adverse effect on sexual function and fertility, but no deaths (i.e., no more than 10% mortality; Section 3.7.2.4.4 of Annex I to the CLP Regulation) or severe suffering such as persistent pain and distress (OECD GD 19, para. 18) in the P0 animals.

214 In case there are no clear evidence of an adverse effect on sexual function and fertility, the limit dose of at least 1000 mg/kg bw/day or the highest possible dose level not causing severe suffering or deaths in P0 must be used as the highest dose level. A descending sequence of dose levels should be selected to demonstrate any dose-related effect and aiming to establish the lowest dose level as a NOAEL.

215 In summary: Unless limited by the physical/chemical nature of the Substance, the highest dose level in P0 animals must be as follows:

(1) in case of clear evidence of an adverse effect on sexual function and fertility without severe suffering or deaths in P0 animals, the highest dose level in P0 animals must be determined based on such clear evidence, or
(2) in the absence of such clear evidence, the highest dose level in P0 animals must be set to be the highest possible dose not causing severe suffering or death, or
(3) if there is such clear evidence but the highest dose level set on that basis would cause severe suffering or death, the highest dose level in P0 animals must be set to be the highest possible dose not causing severe suffering or death, or
(4) the highest dose level in P0 animals must follow the limit dose concept.

216 You have to provide a justification with your study results demonstrating that the dose level selection meets the conditions described above.

217 Numerical results (i.e. incidences and magnitudes) and description of the severity of effects at all dose levels from the dose range-finding study/ies must be reported to facilitate the assessment of the dose level section and interpretation of the results of the main study.

218 In your comments on the draft decision, you state that the intention is to test up to the limit dose; this may be reconsidered based on the results of the dose-range-finding studies.

219 In addition, you propose that the dose via feed is adjusted based on feed consumption and body weight data for the animals at each life stage.

220 ECHA agrees with this proposal.

4.3.4. Cohorts 1A and 1B

221 Cohorts 1A and 1B belong to the basic study design and must be included.

Histopathological investigations in Cohorts 1A and 1B

222 In addition to histopathological investigations of cohorts 1A, organs and tissues of Cohort 1B animals processed to block stage, including those of identified target organs, must be

subjected to histopathological investigations (according to OECD TG 443, para. 67 and 72) if:

- the results from Cohort 1A are equivocal,
- the test substance is a suspected reproductive toxicant or
- the test substance is a suspected endocrine toxicant.

Splenic lymphocyte subpopulation analysis

223 Splenic lymphocyte subpopulation analysis must be conducted in Cohort 1A (OECD TG 443, para. 66; OECD GD 151, Annex Table 1.3).

Investigations of sexual maturation

224 To improve the ability to detect rare or low-incidence effects, all F1 animals must be maintained until sexual maturation to ensure that sufficient animals (3/sex/litter/dose) are available for evaluation of balano-preputial separation or vaginal patency (OECD GD 151, para. 12 in conjunction with OECD TG 443, para. 47). For statistical analyses, data on sexual maturation from all evaluated animals/sex/dose must be combined to maximise the statistical power of the study.

4.3.5. Cohort 3

225 The developmental immunotoxicity Cohort 3 needs to be conducted in case of a particular concern on (developmental) immunotoxicity.

226 In your justification of the study design attached under the endpoint in IUCLID. You state that existing information on substance(s) structurally analogous to the Substance in animals and humans, i.e. cobalt sulphate and cobalt dichloride, show evidence of adverse effects on the haemapoetic system including increased red blood cell parameters, decreased reticulocytes, leucocytes and platelets. Furthermore, in 2-week and 13-week inhalation studies with cobalt sulfate, decreased absolute and relative thymus weights were reported in rats ([redacted] 1998).

227 The effects observed which are considered specific mechanism(s)/mode(s) of action with an association to developmental immunotoxicity because leucocytes and the thymus are integral part of the immune system.

228 You proposed to include Cohort 3.

229 ECHA agrees that inclusion of the developmental immunotoxicity Cohort 3 is necessary.

4.3.6. Additional measurements of cobalt levels in the blood

230 Where a test method offers flexibility in the study design, the chosen test design must ensure that the data generated are adequate for hazard identification and risk assessment (by analogy, REACH Annexes VII-X, introductory paragraphs).

231 In this case, the objective of testing is to generate adequate information for hazard identification, in particular to confirm or exclude the hypothesis of your testing strategy, and risk assessment, in particular to assess which route(s) of human exposure may require or not specific risk management measures.

232 The OECD TG 443 leaves flexibility to consider additional blood measurements in light of the testing objective.

233 In this case, the objective for testing is to confirm or exclude a hypothesis based on existing data as well as with other data to be generated for the same purpose.

234 Your grouping of substances is based on *in vitro* bioaccessibility in gastric juice which places the substance in the group poorly soluble inorganic cobalt substances.

235 Your read-across hypothesis assumes that it is the cobalt ion which drive toxicity.

236 To be able to confirm your read-across hypothesis that the substance in the group are poorly absorbed *in vivo* conformation of cobalt blood measurements is required; this is important also because red blood cells are a target organ for cobalt.

237 Without cobalt measurements in blood to confirm the hypothesis, the read-across hypothesis would need to be rejected and all members of the group would need to be tested for EOGRTS resulting in unnecessary animal testing for the target substances.

238 Based on the above, measurements of cobalt levels in the blood must be included in the study as specified below.

239 Sampling times in the P animals must be the same as in the sub-chronic toxicity study, see Section 3.1.1. above.

240 In addition, cobalt levels in blood must be measured in all F1 animals at termination.

241 The requested study is a dietary study and cobalt levels in whole blood is therefore highly dependent on when the animals last ate. To minimise variation these measurements must be conducted at the same time of the day in animals with ad libitum access to food and water. Animals must not be fasted.

242 Based on the above, measurements of cobalt concentrations in blood must be conducted (as specified above).

4.4. Outcome

243 Under Article 40(3)(b) your testing proposal is accepted under modified conditions, and you are requested to conduct the test with the analogue substance tricobalt tetraoxide, as specified above.

4.4.1. Further expansion of the study design

244 The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) were identified. However, you may expand the study by including the extension of Cohort 1B and/or Cohorts 2A and 2B if relevant information becomes available from other studies or during conduct of this study. Inclusion is justified if the available information meets the criteria and conditions which are described in Column 2, Section 8.7.3., Annex IX/X. You may also expand the study due to other scientific reasons in order to avoid a conduct of a new study. The study design, including any added expansions, must be fully justified and documented. Further detailed guidance on study design and triggers is provided in Guidance on IRs & CSA, Section R.7.6.

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).
Guidance for monomers and polymers; ECHA (2012).
Guidance on intermediates; ECHA (2010).

All guidance documents are 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

ECHA received your testing proposal(s) on 25 February 2019 and started the testing proposal evaluation in accordance with Article 40(1).

ECHA held a third-party consultation for the testing proposal(s) from 21 September 2020 until 5 November 2020. ECHA did not receive information from third parties.

ECHA followed the procedure detailed in Articles 50 and 51 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.

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

ECHA took into account your comments and amended the request(s).

In your comments on the draft decision, you requested an extension of the deadline to provide information from 36 to 72 months from the date of adoption of the decision.
You also propose that ECHA allows for the staggered conduct of the 5 testing proposal studies for the cobalt categories. You cite laboratory capacity, significant animal use and the significant resources needed for inhalation toxicity testing. You propose the following schedule:

- Oral combined chronic/carcinogenicity study – As soon as final decision received
- 90-day RDT inhalation study – As soon as final decision received
- In vivo TGR and COMET studies – 1 year after start of combined chronic/carcinogenicity study
- EOGRTS – 1.5 – 2 years after start of combined chronic/carcinogenicity study.

The deadlines set in the initial decision already considered the fact that some tests within a given decision are interrelated. ECHA recognises that this is a testing strategy for a large group of substances and that there are interrelations also between the different decisions.

ECHA has also reconsidered the time needed to conduct the combined chronic/carcinogenicity study including 14-day and 90-day dose-range finding studies prior to the main study and granted the request to extend the deadline to 72 months for the decisions concerned. The deadline was also extended for the mutagenicity studies to 48-months. Therefore, the deadline for this decision has also been extended to 48 months.

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

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s) and referred the modified draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee unanimously agreed on the draft decision in its MSC-83

written procedure. ECHA adopted the decision under Article 51(6) of REACH.

Appendix 3: Addressee(s) 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]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[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[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

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.

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

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[4].

[4] https://echa.europa.eu/manuals