~~Confidential~~

Helsinki, 05 October 2023

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

Date of submission of the dossier subject to this decision
27/07/2021

Registered substance subject to this decision ("the Substance")
Substance name: Cobalt(II) 4-oxopent-2-en-2-olate
EC/List number: 237-855-6

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 information under request 1 and 2 below by ***10 January 2028*** and all other information listed below by ***10 January 2030***.

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.
   (iv) 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.

3. Combined chronic toxicity and carcinogenicity study (Annex I, Section 0.5.; test method: EU B.33./OECD TG 453) with the analogue substance cobalt dichloride, EC

number 231-589-4, by oral route (diet), in CD® / Crl:CD (Sprague Dawley) rats, specified as follows:

(i) A dose-range-finding study OECD TG 408 must be conducted prior to the main study, specified as follows:

a. The study must be conducted by oral route (diet) in CD® / Crl:CD (Sprague Dawley) rats.

b. The top dose in the dose-range-finding study must demonstrate the highest toxicity possible (maximum tolerable dose or MTD) without severe suffering such as persistent pain and distress (OECD GD 19, para. 18) or deaths (i.e., no more than 10% mortality).

The reporting of the study must provide the justification for setting the dose levels and that the MTD was reached.

c. The study must include measurements of cobalt concentrations in whole blood in all animals of all dose groups at 14, 28 and 90 days; animals must not be fasted prior to this investigation, and the measurements must be conducted at the same time of the day in a standardised manner.

(ii) The selection of doses for the main study must be based on the dose-range-finding study and meet the following criteria:

a. The top dose in the main study must provide signs of toxicity such as slight depression of body weight gain (aiming at not more than 10%), without causing e.g., tissue necrosis or metabolic saturation and without substantially altering normal life span due to effects other than tumours.

The reporting of the study must provide the justification for the setting of the dose levels.

b. The study must include at least 3 dose-groups and dose-spacing may not exceed a factor of 2 to 3.

(iii) The chronic toxicity phase of the study must include measurements in a standardised manner of:

a. cobalt concentrations in whole blood; in all animals of all dose groups at 3, 6, 12 months; as well as at study termination (if longer than 12 months); animals must not be fasted prior to this investigation.

b. erythropoietin (EPO) concentration in plasma (or serum); in all animals of all dose groups at 3, 6, 12 months; as well as at study termination (if longer than 12 months); animals must not be fasted prior to this investigation.

c. urinalysis which includes measurements of cobalt concentrations in urine, in all animals of all dose groups at 3, 6, 12 months; as well as at study termination (if longer than 12 months).

d. cobalt levels in the following tissues at study termination: adrenal medulla, bone marrow, brain, heart, kidney, liver, pancreas and testis.

(iv) The carcinogenicity phase of the study must include measurements in a standardised manner of:

a. cobalt concentrations in whole blood in all animals of all dose groups at study termination; animals must not be fasted prior to this investigation.

b. cobalt levels in the following tissues at study termination: adrenal medulla, bone marrow, brain, heart, kidney, liver, pancreas, and testis.

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.

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 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.)
- Combined chronic toxicity and carcinogenicity study (Annex I, Section 0.5.)

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 oral carcinogenicity and repeated dose toxicity, you have grouped cobalt substances into three groups for the oral read-across approach: '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 substances'

- 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, you have grouped all cobalt substances listed above into the same group.

12 ECHA understands that this is the applicability domain of the grouping and your predictions are assessed on this basis.

13 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.

14 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

15 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).

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

2.1. Further in vivo mutagenicity testing

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

18 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 route with cobalt sulphate; and

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

19 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)

20 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.

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

22 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.

23 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 i.p. [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).

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

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

2.2. Information provided

26 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.

27 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.

28 ECHA agrees that the proposed studies are necessary to produce the chemical safety report for the Substance.

2.3. Grouping of substances and read-across approach

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

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

31 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.

32 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.

33 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.

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

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

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

37 ECHA agrees with your read-across hypothesis.

38 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

39 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).

40 The proposed tests explore different aspects of mutagenicity, i.e. gene mutations and chromosomal aberrations. According to the OECD TG 489, 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".

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

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

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

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

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

46 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.

47 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

48 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

49 You proposed testing in transgenic rats.

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

51 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.

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

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

54 However, this is a transgenic model and changing the genetic background of the model would require a significant number of animals to back-cross the transgenic rats onto the preferred genetic background.

2.5.2. Specification of the route of exposure

55 You proposed testing by the inhalation route.

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

57 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.

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

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

60 In the previous inhalation studies with the cobalt sulphate ([redacted] 1998), "cobalt sulphate 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 sulphate heptahydrate."

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

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

2.5.3. Specification of target tissues

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

64 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".

65 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).

66 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.

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

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

69 To confirm or exclude the hypothesis of the testing strategy, tissues where 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.

70 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.

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

72 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

73 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

74 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).

75 In this case, the objective of testing is to generate adequate information for hazard identification, 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.

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

77 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.

78 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.

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

80 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.

81 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.

82 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

83 You proposed testing in the rat.

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

85 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.

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

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

88 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.

89 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

90 You proposed testing by the inhalation route.

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

92 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

93 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.

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

95 The 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.

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

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

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

2.6.4. Specification of target tissues

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

100 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.

101 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.

102 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.

103 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

104 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

105 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

106 You propose additional analyses for cytotoxicity and other parameters to assess potential secondary effects 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.

107 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

108 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.

3. Combined chronic toxicity and carcinogenicity study

109 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).

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

3.1. Further carcinogenicity testing

111 You have provided a testing strategy which aims to further explore the potential for carcinogenicity following oral exposure.

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

(i) Combined chronic toxicity and carcinogenicity study (OECD TG 453) by the oral route with cobalt dichloride.

113 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)

114 You provide the following justification as to why additional tests are needed to produce the CSR: "Hazard as well as robust risk assessment for cancer following oral and dermal exposure is crucial based on the multitude of consumer uses of cobalt containing mixtures, i.e. alloys and materials containing cobalt as an impurity. Affected sectors and uses include the use of stainless steel in food contact surfaces, where oral (pots and pans; use of stainless steel as material for water pipes) as well as dermal (cutlery) exposure to cobalt may occur on a daily, chronic time scale. Further included is the use of stainless steel and cobalt-chrome alloys in medical and dental applications, where there are numerous systemic or oral exposure pathways, e.g. through the use of hypodermic needles, surgical devices and medical and dental implants. Another potential source of exposure stems from the use of cobalt containing alloys as touch surfaces (door handles and pads), as are common in many public spaces, e.g. in public transport. All these areas require a robust risk assessment for oral, dermal and systemic exposure routes for effects that occur in the absence of the portal of entry effects following inhalation."

115 ECHA understands, that you intend to provide evidence supporting your hypothesis that cobalt substances do not cause cancer via the oral route. To provide evidence on the lack of cancer via the oral routes of exposure, you have proposed an oral combined chronic toxicity/carcinogenicity study to be conducted in rats with cobalt dichloride as a 'worst case' in terms of oral exposure of the cobalt ion.

116 Cobalt metal and cobalt sulphate cause increase in the alveolar adenomas and carcinomas in the [redacted] 2-year inhalation carcinogenicity studies in both F344/N or F344/NTac rats and B6C3F1/N mice in both sexes ([redacted] 1998; [redacted], 2014). There is also a concern for systemic

tumours, mainly pheochromocytomas and pancreatic cancers, however these systemic cancers occurred mainly at the highest dose levels.

117 According to the CLP regulation the route of exposure should only be stated if it is conclusively proven that no other routes of exposure cause the hazard.

118 Cobalt metal, cobalt sulphate, cobalt dichloride, cobalt dinitrate, cobalt carbonate and cobalt di(acetate) have harmonised classification which include Carc. 1B.

119 There are no oral carcinogenicity studies available on cobalt compounds, which could provide evidence on the possibility or lack of cancer via the oral route of exposure.

120 Therefore, it is not possible to confirm or exclude the possibility of induction of cancers via other routes of exposure.

121 To resolve this lack of information, you propose to conduct a carcinogenicity study by the oral route. You consider that the information is needed to be able to conclude on the classification of all cobalt substances.

122 ECHA agrees that information is needed to further investigate possible concerns for carcinogenicity via the oral route of exposure to produce the CSR.

3.2. Information provided

123 You have submitted a testing proposal for a Combined chronic toxicity and carcinogenicity study according to the OECD TG 453 with the analogue substance cobalt dichloride, EC number 231-589-4.

124 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.

125 ECHA agrees that the proposed study is necessary to produce the chemical safety reports for the Substance.

3.3. Grouping of substances and read-across approach

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

127 As explained in Section 0.1. above you have grouped the Substance into a category of 'Bioavailable Co substances'.

128 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.

129 To generate additional information needed for the CSR, you propose to test cobalt dichloride (EC No. 231-589-4) for carcinogenicity. The selection of the test material is based on a 'worst case' approach.

130 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.

131 Cobalt dichloride belongs to the 'Bioavailable cobalt substances' and is fully dissociated in water (and biological media). Following oral administration, at toxicologically relevant dose levels the substance can be assumed to be fully dissociated because the water solubility of the substance is magnitudes higher than the foreseen doses in any toxicological studies.

132 Therefore, cobalt dichloride can be considered as a worst-case in terms of exposure to the cobalt ion.

133 As explained above, you have established that the properties of the Substance can be predicted from data on the analogue substance. ECHA agrees with your read-across hypothesis.

134 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.

3.4. Test selection

135 The study must be appropriately designed to enable meaningful comparison with the results of existing inhalation studies ([redacted] 2014, 1998).

136 You have proposed to combine this carcinogenicity study with a chronic toxicity study. ECHA understands that the aim of the chronic toxicity data is to refine the point of departure for risk assessment following exposure via the oral route.

137 ECHA considers that refining the point of departure is justified given the extensive uses of cobalt compounds. A point of departure from a chronic study would be a more robust starting for risk management.

138 On this basis, a combined chronic toxicity/carcinogenicity study (OECD TG 453) is needed to develop the CSR.

3.5. Specification of the study design

3.5.1. Specification of rat strain

139 The OECD TG 453 primarily covers assessment and evaluation of chronic toxicity and carcinogenicity in rodents. The preferred rodent species is the rat. The choice of species should be justified.

140 You propose to conduct the study using CD® / Crl:CD (Sprague Dawley) rats.

141 ECHA agrees with the proposal.

142 Based on the above, the study must be conducted using CD® / Crl:CD (Sprague Dawley) rats.

3.5.2. Specification of the route of administration

143 According to the OECD TG 453, "the test chemical is normally administered orally, via the diet or drinking water, or by gavage. […] A rationale should be provided for the chosen route and method of administration." Additional guidance on route selection is provided by OECD GD 116.

144 According to the OECD GD 116, each method of oral administration has advantages and disadvantages, and it should in particular be kept in mind that the toxicokinetics of the test chemical may be affected by the method of oral administration. The kinetics of absorption will determine the internal exposure achieved.

145 In addition, the OECD TG 453 also provides that "in the interests of animal welfare, oral gavage should normally be selected only for those agents for which this route and method of administration reasonably represent potential human exposure, e.g. pharmaceuticals.

For dietary or environmental chemicals including pesticides, administration is typically via the diet or drinking water".

146 You propose to administer the Substance via oral gavage. You have not provided any reasoning as to why oral gavage is most appropriate route of administration for the Substance.

147 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 zinc. The selectivity of this DMT1 is $Cd^{2+} > Fe^{2+} > Co^{2+}$, $Mn^{2+} \gg Zn^{2+}$, Ni^{2+} (Illing, 2012[2]).

148 Gavage administration result in intermittently high concentrations of cobalt ions in the duodenum and proximal jejunum. These intermittent high concentrations of cobalt ions may overload the facilitated transport mechanism, and thereby impair bioavailability.

149 For this reason, bioavailability is expected to be higher in dietary or drinking water study compared to a gavage study; i.e. gavage administration may underestimate the hazard.

150 Further, based on the uses and exposure scenarios in the CSR, the potential human exposure to the Substance is not reasonably represented via a single bolus dose. The substances in the group of have wide dispersive uses by professionals.

151 For this reason, the study must be performed using diet or drinking water as the route of administration.

152 Administration by water is not appropriate because the Substance is likely to have a "salty" taste which may deter water intake.

153 Based on the above, ECHA concludes that the most appropriate oral route of administration is diet. Therefore, the study must be conducted using diet as the route of administration for the Substance.

154 In your comments, to the draft decision you agree to conduct the study with administration in the diet. You propose to administer the test substance in the diet as a constant dose level in terms of the animal's body weight. Based on laboratory input, diet concentrations are adjusted using measured food consumption, body weight and body weight change data from the previous week. For the 90-day (dose-range finding study), weekly adjustments would be made based on sex and group. For the chronic and carcinogenicity study, adjustments are performed weekly for a specified amount of time (either 3 or 6 months) and then change to every 2 or 4 weeks. This is in line with one of the two recommendations in OECD GD 116 for oral administration via diet, so ECHA agrees to this proposal.

3.5.3. Dose-range-finding study required

155 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).

156 The purpose of a long-term bioassay (chronic toxicity and/or carcinogenicity studies) is the detection of biological evidence of any toxic and/or carcinogenic potential of the substance being investigated for hazard identification. Protocols should therefore maximise the sensitivity of the test without significantly altering the accuracy and interpretability of the biological data obtained. The dose regimen has a critical bearing on these two critical elements (OECD GD 116).

[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.

157 The OECD TG 453 and OECD GD 116 state that a carcinogenicity study should only be carried out after initial information on toxicity has been obtained from studies of shorter duration. In addition, further (mechanistic) studies should be performed on the same species and strain as previous cancer/chronic toxicity studies.

158 Specific cobalt salts are carcinogenic after administration by the inhalation route and your objective is to determine if the same hazard (carcinogenicity) is manifest after oral administration. The study design should maximise the sensitivity of the test. To achieve this objective the study has to be conducted at as high doses as possible without compromising the usefulness of the study, e.g. due to high mortality.

159 ECHA understands that you intend to rely on an oral Sub-chronic toxicity study conducted with the cobalt dichloride (OECD TG 408; 2015) via oral gavage using Crl:CD rats for the dose setting of the carcinogenicity study.

160 However, as explained in Section 3.5.1., the combined study must be conducted in the same rat strain which have raised a concern for systemic cancers, i.e. F344 (Fisher) rats.

161 Furthermore, as explained in Section 3.5.2., dietary exposure is the most appropriate route considering likely human exposure for the combined study.

162 Finally, the Sub-chronic toxicity study (2015; gavage) with cobalt dichloride observed haematological effects and 10% reduced body weight at the highest dose tested. This study reached the maximally tolerated dose (MTD) which is needed for the dose-setting of a carcinogenicity study; however, the study was conducted via oral gavage and not via dietary administration.

163 There may be differences in toxicity consequent to different toxicokinetics and toxicodynamics arising from both (i) differences in rat strain, e.g. Crl:CD vs. F344 (Fisher) rats and (ii) change in mode of administration, such as between bolus administration by gavage and dietary administration. These differences can lead to large variation in the MTD which is to be used as the basis of the dose-setting in a carcinogenicity study.

164 Based on the above, the information from available studies is inadequate as a basis for dose-level setting of a carcinogenicity study as it may result in underestimation of toxicity.

165 Therefore, an appropriate dose-range finding study is required to ensure that adequate information is available for dose-setting of the carcinogenicity study.

166 In your comments to the draft decision, you agreed to conduct the dose-range finding study.

167 In addition, you propose to conduct a 14-day RDT oral palatability study prior to conducting the dose-range finding study. It is your responsibility whether to conduct a 14-day palatability study at your own discretion.

3.5.3.1. Specification of the dose-range-finding study

168 First, the dose-range-finding study will serve as the basis for dose-level setting for the proposed main (Combined chronic toxicity/carcinogenicity) study.

169 A study according to the OECD TG 408 is an appropriate basis for dose-level setting of the main study.

170 Second, the dose-range-finding study must use the same route of administration and the same rat strain as in the main study, i.e. dietary exposure of F344 (Fisher) rats.

171 Third, to be adequate as a dose-range-finding study the doses used must aim at the highest toxicity possible (maximal tolerable dose or MTD) without severe suffering such as persistent pain and distress (OECD GD 19, para. 18) or deaths (i.e., no more than 10% mortality).

172 You must provide a justification with your study results demonstrating that the dose-level selection meets the conditions described above.

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

174 Fourth, you have proposed to measure cobalt levels in blood in the main study to support the objective of the overall testing strategy, it must be possible to compare the systemic exposure of cobalt ions in the oral studies with the levels observed in the previous inhalation studies (see section 3.5.5.).

175 ECHA considers that measurements of cobalt blood levels are also required in the dose-range finding study because otherwise the objective of setting the doses in the combined test cannot be achieved.

176 Blood, specifically red blood cells (RBCs), is a target organ for cobalt.

177 The requested study is a dietary study and cobalt levels in in blood are therefore 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.

178 Therefore, you must include measurements of cobalt concentrations in whole blood, in all animals of all dose groups, as part of the haematology and clinical biochemistry investigations.

179 Sampling times must be 7, 14, and 28 days and at the termination of the dose-range finding study.

180 In your comments on the draft decision, you agree to include the whole blood measurements of cobalt in the dose-range study as specified above.

181 In addition, you propose to include measurements of cobalt in plasma/serum and whole blood in the 14-day palatability study because this was also done in the [redacted] Co metal powder 14-day study. ECHA agrees to this proposal.

3.5.4. Dose-level setting and number of dose groups in the combined study

182 The main objective of the proposed combined study is to investigate if there is a cancer hazard following oral administration of cobalt substances, for comparison with the known inhalation carcinogenicity of the substance.

183 The robustness of a carcinogenicity study is dependent on a demonstration that the dose levels selected in the study are adequate to show an effect or effects of the test substance.

184 The top dose must provide signs of toxicity such as slight depression of body weight gain (aiming at not more than 10%), without causing e.g., tissue necrosis or metabolic saturation and without substantially altering normal life span due to effects other than tumours.

185 Therefore, dose-levels for the proposed Combined chronic toxicity/carcinogenicity study must be based on the Sub-chronic toxicity (90 days) range finding-study also requested, see Section 3.5.3.

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

187 You have proposed to include 3 or 4 dose groups in the study.

188 The dual aims of a Combined chronic toxicity/carcinogenicity study targets opposite ends of the dose-response curve. This may be difficult to accommodate using the three dose-group.

189 Based on the above, the study must include at least 3 dose groups and dose spacing may not exceed a factor of two to three. You may include additional dose-groups if required to capture both ends of the dose-response curve.

190 In your comments on the draft decision, you agreed to include at least three dose-groups.

3.5.5. Additional measurements of cobalt levels in blood

191 ECHA ensures that the data generated are adequate for hazard identification and risk assessment (by analogy, REACH Annexes VII-X, introductory paragraphs).

192 In this case, the objective of testing is to generate adequate information for hazard identification, 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.

193 The OECD TG 453 leaves flexibility to consider additional investigations in light of the testing objective.

194 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.

195 You proposed to include measurements of cobalt concentrations in blood, at four timepoints throughout the study, and at sacrifice.

196 The aim of the proposed oral carcinogenicity is to investigate whether a concern for carcinogenicity stemming from the previous inhalation studies also applies to the oral route.

197 To support such an objective, the systemic exposure of cobalt ions must be demonstrated to be equal to or higher than that which caused systemic tumours in the inhalation carcinogenicity studies ([redacted] 1998; [redacted], 2014).

198 ECHA agrees with your proposal to include measurements of cobalt levels in blood.

199 However, red blood cells (RBCs) are a target organ for cobalt.

200 The requested study is a dietary study and cobalt levels in 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.

201 Therefore, you must include measurements of cobalt concentrations in whole blood, in all animals of all dose groups, as part of the haematology and clinical biochemistry investigations.

202 For the chronic toxicity phase of the study, sampling times must be 3, 6, and 12 months, as well as at study termination (if longer than 12 months); see para. 43 of the OECD TG 453 for details; animals must not be fasted.

203 For the carcinogenicity phase of the study, measurements of cobalt concentrations must be conducted at study termination; animals must not be fasted.

204 In the comments on the draft decision, you agreed to measure cobalt in whole blood.

3.5.6. Additional measurements of cobalt levels in urine in the chronic toxicity phase of the study

205 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).

206 In all repeated dose toxicity studies, investigations may need to be modified depending on the observed/expected effects from a given substance.

207 You proposed to include measurements of cobalt concentrations in urine.

208 Measurements of cobalt concentrations in urine provide information regarding cobalt excretion.

209 In addition, together with the rest of the urinalysis, this provides information on kidney function which is an important consideration in long-term studies of ionic substances.

210 ECHA agrees with your proposal to include measurements of cobalt levels in urine.

211 Therefore, you must include measurements of cobalt concentration in the urine, in all animals of all dose groups, as part of the urinalysis investigations.

212 For the chronic toxicity phase of the study, sampling times must be 3, 6, and 12 months, as well as at study termination (if longer than 12 months); see para. 46 of the OECD TG 453 for details.

213 These investigations of are not required for the carcinogenicity phase of the study.

214 In the comments on the draft decision, you agreed to measure cobalt in urine.

3.5.7. Additional measurements of haematological markers, including erythropoietin in the chronic toxicity phase of the study

215 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).

216 In all repeated dose toxicity studies, investigations may need to be modified depending on the observed/expected effects from a given substance.

217 You proposed to include determinations of haematological markers, including erythropoietin (EPO), at four timepoints throughout the study, and at sacrifice. You have not specified which additional haematological markers other than EPO you propose to include.

218 ECHA agrees with your proposal to include determination of EPO. Increased number of RBCs have been observed in the sub-chronic toxicity study ([redacted] 2015) conducted with the Substance. EPO plays a key role in the production of RBCs.

219 Therefore, you must include measurements of EPO, in all animals of all dose groups, as part of the haematology and clinical biochemistry investigations; see para. 43 of the OECD TG 453 for details.

220 For the chronic toxicity phase of the study, sampling times must be the same as those specified for cobalt blood measurements, see Section 3.4.6); animals must not be fasted.

221 These investigations of EPO are not required for the carcinogenicity phase of the study.

222 Regarding other haematological markers, you may include these as part of the haematology and clinical biochemistry investigations of the chronic toxicity and carcinogenicity phases of the study as long as inclusion of these additional parameters do not compromise the integrity of the OECD TG 453 study design, or the additional investigations specified in this decision. Ideally the sampling times for these additional haematological markers should be the same as the rest of the haematology and clinical biochemistry investigations.

223 In your comments on the draft decision, you agreed to the measure EPO.

3.5.8. Additional investigations of thyroid function

224 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).

225 In all repeated dose toxicity studies, investigations may need to be modified depending on the observed/expected effects from a given substance.

226 You propose to include additional measurements of thyroid function because this information is an information requirement based on the current version of the OECD TG 408.

227 The available OECD TG 408 ([redacted] 2015) was conducted prior to the inclusion of thyroid parameters into the OECD TG 408. The current version of the OECD TG 408 includes measurements of thyroxine (T4), triiodothyronine (T3), thyroid stimulating hormone (TSH) and thyroid gland weight which are sensitive to thyroid pathway perturbation. It also includes measurements of serum total cholesterol, low-density lipoproteins (LDL) and high-density lipoproteins (HDL) which are directly controlled by thyroid hormone action and contribute (with other thyroid endpoints) to evidence of thyroid function.

228 ECHA agrees that such investigations will provide important information regarding thyroid function.

229 However, this decision requires a conduct of the range finding study - a Sub-chronic toxicity study (according to OECD TG 408), in the same rat strain and with the same route of exposure as the proposed Combined Chronic toxicity/carcinogenicity study. The thyroid parameters listed above are now part of the OECD TG 408.

230 Therefore, the dose range finding study would provide the missing thyroid parameters.

231 You may at your own discretion include further thyroid investigations in the chronic toxicity phase of the study. Should you choose to do so ECHA recommends the same sampling times as those specified for cobalt blood measurements, see Section 3.5.5; animals must not be fasted.

232 In your comments on the draft decision, you agreed to the additional investigations of thyroid function.

3.5.9. Additional determinations of cobalt levels in target tissues

233 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).

234 In all repeated dose toxicity studies, investigations may need to be modified depending on the observed/expected effects from a given substance.

235 You propose to include additional measurements of cobalt levels in target tissues. You have identified the following target tissues: adrenal medulla, kidney, pancreas, bone marrow, liver and brain.

236 ECHA agrees with your proposal because examination of cobalt levels in target tissues will provide important information regarding distribution of the Substance.

237 However, the testis and the heart are target organs of cobalt substances based on existing toxicological studies ([redacted], 2014; ASTDR, 2004).

238 The mechanism of action of cobalt is not yet fully determined and you have not demonstrated that the target tissues organed that you have selected are representative.

239 Therefore, you must at the termination in the chronic toxicity phase and carcinogenicity phase of the study measure cobalt levels in the following tissues: adrenal medulla, bone marrow, brain, heart, kidney, liver, pancreas and testis. Measurements must be conducted in all animals in all dose groups.

240 In your comments on the draft decision, you agreed to the determinations of cobalt levels in target tissues.

3.6. Outcome

241 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 cobalt dichloride, EC number 231-589-4., as specified above.

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 13 December 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 60 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:

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

The initial draft decision contained two deadlines one for the combined chronic/carcinogenicity study (60 months) and one for the inhalation In vivo TGR and COMET studies (36 months). 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. The intermediate deadline has been extended to 48 months.

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

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

Appendix 3: Addressee(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]

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