~~Confidential~~

Helsinki, 19 April 2024

Addressees
Registrants of JS_Cesiumhydroxide as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
09 October 2017

Registered substance subject to this decision ("the Substance")
Substance name: caesium hydroxide
EC/List number: 244-344-1

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***26 July 2027***.

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

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

1. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test, also requested below (triggered by Annex VII, Section 8.4., Column 2)

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

2. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test, also requested below (triggered by Annex VIII, Section 8.4., Column 2)

3. Justification for an adaptation of the screening study for reproductive/developmental toxicity (Annex VIII, Section 8.7.1., Column 2) based on the request 5 below.

   If the extended one-generation reproductive toxicity study is not requested:

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

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

4. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4.4; test method: OECD TG 489) combined with in vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, or if justified, in mice, oral route. For the comet assay the following tissues must be analysed: liver, glandular stomach and duodenum. For the micronucleus test:

   - the aneugenic potential of the Substance must be assessed by using a centromere staining technique if the substance induces an increase in the

frequency of micronuclei in the OECD TG 474;
- target tissue exposure must be demonstrated if the result of the OECD TG 474 is negative.

5. Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method: OECD TG 443) in rats, oral route, specified as follows:
    - Ten weeks premating exposure duration for the parental (P0) generation;
    - 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 in request 5.3.3., or follow the limit dose concept. The reporting of the study must provide the justification for the setting of the dose levels;
    - Cohort 1A and 1B (Reproductive toxicity); and
    - Cohort 3 (Developmental immunotoxicity).

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

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

The reasons for the requests are explained in Appendix 1.

Information required depends on your tonnage band

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

In the requests above, the same study has been requested under different Annexes. This is because some information requirements may be triggered at lower tonnage band(s). In such cases, only the reasons why the information requirement is triggered are provided for the lower tonnage band(s). For the highest tonnage band, the reasons why the standard information requirement is not met and the specification of the study design are provided. Only one study is to be conducted; all registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the others under Article 53 of REACH.

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 request(s)
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 request(s)

Reasons related to the information under Annex VII of REACH

1. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test

1 Under Annex VII, Section 8.4., Column 2, an appropriate *in vivo* mammalian somatic cell genotoxicity study as referred to in Annex IX, point 8.4.4, must be performed in case of a positive result in any of the *in vitro* studies referred to in Annex VII, Section 8.4. The *in vivo* study must address the concerns raised by the *in vitro* study results, i.e. the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

1.1. Triggering of the information requirement

2 Your dossier contains positive results for the *in vitro* cytogenicity test ([redacted], 1994) which raises the concern for chromosomal aberrations.

3 Therefore, the information requirement is triggered.

4 In your comments to the draft decision, you provide information regarding the triggering of the study. ECHA has addressed your comment under Request 4.

1.2. Information requirement not fulfilled

5 The information provided, its assessment and the specifications of the study design are addressed under request 4.

Reasons related to the information under Annex VIII of REACH

2. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test

6 Under Annex VIII, Section 8.4., Column 2, an appropriate *in vivo* mammalian somatic cell genotoxicity study as referred to in Annex IX, point 8.4., must be performed in case of a positive result in any of the *in vitro* studies referred to in Annex VII or VIII, Section 8.4. The *in vivo* study must address the concerns raised by the *in vitro* study results, i.e. the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

2.1. Triggering of the information requirement

7 Your dossier contains positive results for the *in vitro* cytogenicity test ([redacted], 1994) which raise the concern for chromosomal aberrations.

8 Therefore, the information requirement is triggered.

9 In your comments to the draft decision, you provide information regarding the triggering of the study. ECHA has addressed your comment under Request 4.

2.2. Information requirement not fulfilled

10 The information provided, its assessment and the specifications of the study design are addressed under request 4.

3. Screening study for reproductive/developmental toxicity

11 A screening study for reproductive/developmental toxicity study (OECD 421 or OECD 422) is an information requirement under Annex VIII, Section 8.7.1.

12 You have not submitted any information for this requirement.

13 Therefore, the information requirement is not fulfilled.

14 In the comments to the draft decision, you indicate your intention to use a read-across adaptation based on the information available from a structural analogue, Cesium Nitrate and that you will provide this information in a future update of your registration dossier. As details on you read-across approach and supporting information are not yet available, no conclusion on its validity can currently be made. You remain responsible for complying with this decision by the set deadline.

3.1. Study design

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

16 As the Substance is a solid, the study must be conducted with oral administration of the Substance (Annex VIII, Section 8.7.1., Column 1).

17 Therefore, the study must be conducted in rats with oral administration of the Substance.

3.1.1. Justification for an adaptation of the screening study for reproductive/developmental toxicity (Annex VIII, Section 8.7.1., Column 2)

18 The present decision requests the registrants concerned to generate and submit a reliable EOGRT study (see request 5). According to Annex VIII, Section 8.7.1., Column 2, and to prevent unnecessary animal testing, a screening study for reproductive/developmental toxicity does not need to be conducted.

19 Therefore, to comply with the information requirement under Annex VIII, Section 8.7.1., you are requested to provide a justification for adaptation, as provided in Annex VIII, Section 8.7.1., Column 2.

20 In case the adopted decision no longer contains a request for a EOGRT study, you are required to provide a screening study for reproductive/developmental toxicity.

21 Therefore, you are requested to either submit:

- a justification for adaptation according to Annex VIII, Section 8.7.1., Column 2, based on request 5; or
- a screening study for reproductive/developmental toxicity as per the study design described in 3.1. in case the EOGRT study is not requested in the adopted decision.

Reasons related to the information under Annex IX of REACH

4. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test

22 An appropriate *in vivo* mammalian somatic cell genotoxicity study is an information requirement under Annex IX, Section 8.4.4., if there is a positive result in any of the *in vitro* studies referred to in Annex VII or VIII, Section 8.4.

4.1. Triggering of the information requirement

23 Your dossier contains positive results for the *in vitro* cytogenicity test ([redacted], 1994) which raise the concern for chromosomal aberrations.

24 Therefore, the information requirement is triggered.

4.2. Information provided

25 You have adapted this information requirement by using Annex IX, Section 8.4.4., Column 2. To support the adaptation, you have provided the following information:

(i) Mammalian Bone Marrow Chromosome Aberration Test, GLP, OECD TG 475 (2012) conducted with the Substance

4.1. Assessment of the information provided

4.1.1. The provided study does not meet the specifications of the test guideline

26 To be considered adequate, the study has to meet the requirements of the OECD TG 475. Therefore, the following specifications must be met:

a) the mitotic index and the mean number of cells with aberrations per group are reported for each group of animals;

b) a clear negative outcome is concluded when the data available shows that bone marrow exposure to the Substance, or its metabolite(s), occurred;

c) the negative control data is ideally within the 95% control limits of the distribution of the laboratory's historical negative control database.

27 In study (i):

a) the mitotic index and the mean number of cells with aberrations per group were not reported for each group of animals;

b) you did not demonstrate that bone marrow exposure to the Substance, or its metabolite(s), occurred;

c) you did not report if the negative control showed a response within the historical control range of the laboratory.

28 The information provided does not cover the specification(s) required by the OECD TG 475.

29 In your comments to the draft decision, you indicate that you will provide the missing information under points a) and c) above in a future update of your registration dossier. You however claim that this information "*not a mandatory field in IUCLID*" and that "*ECHAs own guideline was not listing these specific details*".

30 ECHA takes note of your intention to provide additional information on the existing study from your dossier. However, this information is not yet available in your dossier, no

assessment can currently be made. Furthermore, ECHA emphasizes that OECD TG 475 specifies the information that needs to be reported in order to adequately describe the methodology applied and the results obtained in a specific study. These reporting requirements includes points a) to c) as listed above.

31 Regarding point b) above, you mention that "*systemic bioavailability of Cs administered by gavage after acute and repeated dose exposure has clearly been shown*" and you will include data "*from other Cs salts, Cs carbonate (acute exposure) and CsCl (repeated dose exposure)*".

32 ECHA understand from your comments that you consider that providing information on point b) above is not necessary. However, ECHA emphasizes that the examination of the bone marrow exposure to the tested substance is one the mandatory conditions listed in the paragraph 44 of the OECD TG 475. This condition must be fulfilled to confirm the reliability of the conclusion drawn from such study when negative results are obtained. Therefore, ECHA maintains that this information must be provided.

33 On this basis, your comments on the draft decision do not change the assessment's outcome.

34 Based on the above, your adaptation is rejected and the information requirement is not fulfilled.

35 In your comments to the draft decision, you state that you will "*carefully assess, if a new animal study (being the last resort under REACH) is really the only option taken into account all the information available already*". You also state that you have "*received the same data request for several soluble Cs salts based on more or less the same rational* […]" and that you "*will certainly consider the read across option for soluble Cs salts*".

36 As this strategy relies on a read-across approach that has not yet been fully described and justified, as well as on data which is yet to be provided for the source substance(s) (including bridging studies and supporting information), no conclusion on the compliance of the proposed adaptation can be made. You remain responsible for complying with this decision by the set deadline.

4.2. Test selection

37 Under Annex IX, Section 8.4.4., Column 1, the *in vivo* mammalian somatic cell genotoxicity study must address the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

38 The positive *in vitro* results available in the dossier indicate a concern for chromosomal aberration.

39 The *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) can be combined in a single study (OECD TG 474, paragraph 37c; OECD TG 489, paragraph 33; Guidance on IRs & CSA, Section R.7.7.6.3). While the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations.

40 The combined study, together with the results of the *in vitro* mutagenicity studies, can be used to make definitive conclusions about the mechanism(s) inducing *in vivo* mutagenicity and lack thereof. Furthermore, the combined study can detect effects in both distant organs, such as the bone marrow or the liver, and at site(s) of contact, such as the glandular stomach, the duodenum or the lung. Investigating several genotoxic endpoints and different tissues in a combined study is necessary to reduce the uncertainties associated with not testing all organs and to generate complementary information that provides a

comprehensive overview of the genotoxic potential of the Substance. Moreover, the combined study can help limit the number of tests performed and the number of animals used.

41 Therefore, the comet assay combined with the MN test is the most appropriate study for the Substance.

42 In your comments to the draft decision, you claim that there is no specific OECD TG for combining the *in vivo* comet assay and the *in vivo* micronucleus test into a single study and that this situation, according to you, causes "*legal uncertainty*".

43 However, ECHA notes that both the OECD TG 474 (paragraphs 36 and 37) and the OECD TG 489 (paragraphs 7 and 33) contain detailed recommendations on how to combine the *in vivo* comet assay and the *in vivo* micronucleus test into a single study as well as references to scientific publications demonstrating the successful combination of both tests.

4.3. Study design

44 According to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified. According to the test method OECD TG 474, the test may be performed in mice or rats. Therefore, the combined study must be performed in rats, or if justified, in mice.

45 Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

46 In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

47 In your comments to the draft decision, you argue that the Substance does not raise any concern for gene mutation from the *in vitro* test results in bacteria and mammalian cells and that the *in vivo* comet assay is not appropriate to address the chromosomal aberration concern coming from the positive *in vitro* chromosomal aberration study (1994). You repeat your argument that performing a new *in vivo* study is not necessary as the provided OECD TG 475 (2012) with the Substance and toxicokinetic information available in your dossier on other cesium substances are sufficient to address the chromosomal aberration concern identified *in vitro* for the Substance. You further disagree with testing both the glandular stomach and the duodenum as sites of contact in the comet assay and consider analysis of the glandular stomach as sufficient since concentrations of the Substance are expected to be higher than in the duodenum after oral administration. You base your argument on the OECD TG 489 and a publication from Kirkland *et al.* (2015), which both indicate that only one site of contact needs to be investigated to detect genotoxic substances.

48 ECHA disagrees with your statement that an *In vivo* mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test is not an adeqaute follow-up syudy to investiguate further concern for chromosomal aberration when no concern for gene mutation is indicated. ECHA considers the i*n vivo* comet assay as a suitable test to investigate chromosomal aberrations *in vivo*, in particular when combined with the *in vivo* micronucleus test. The *in vivo* comet assay can detect genotoxic effects that can potentially lead to gene mutations and/or chromosomal aberrations and can be used to investigate different tissues, including sites of contact. As the Substance has been shown to induce

chromosomal aberrations and not gene mutations *in vitro*, positive *in vivo* comet assay results can be assumed to reflect the potential of the Substance to induce chromosomal aberrations *in vivo*, in the liver and at site-of-contact tissues. Additionally, combination of the *in vivo* comet assay with the *in vivo* micronucleus test will provide information on the mutagenic effects of the Substance in the bone marrow, a distant target organ, and allow a clarification of its chromosomal aberration mechanism(s), *i.e.* clastogenicity and/or aneugenicity.

49 Regarding ECHA's request to investigate, in addition to the liver, both the glandular stomach and the duodenum as site-of-contact tissues in the *in vivo* comet assay, is based on physiological differences between the various tissues and uncertainty regarding differences in exposure following oral administration. As you indicate in your comments to the draft decision, the glandular stomach may be more exposed to the Substance than the duodenum. However, ECHA notes that the Substance is corrosive and that you self-classified it as Skin Corr. 1A. In your comments to the draft decision, you also refer to the corrosive properties of the Substance that can cause massive local tissue damage in the gastro-intestinal tract. On that basis, a higher toxicity is expected in the glandular stomach than in the duodenum. According to paragraph 54 of OECD TG 489, positive findings in the comet assay can be due genotoxicity but may also result from target tissue toxicity and subsequent increases in DNA migration […] and increases in DNA migration in the presence of clear evidence of cytotoxicity should be interpreted with caution. For these reasons, ECHA disagrees that an analysis of the glandular stomach is sufficient and considers that investigation of two sites of contact, with potentially different levels of confounding cytotoxicity, is necessary and will help in the interpretation of the findings in the *in vivo* comet assay.

50 According to the test method OECD TG 474, in order to demonstrate the ability of the study to identify clastogens and aneugens, you must include two concurrent positive controls, one known clastogen and one known aneugen (OECD TG 474, paragraph 25, Table 1).

51 The combination of the OECD TGs 489 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen et al. 2011 [1]).

[1] Bowen DE et al. (2011) Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. *Muta Res.*;722:7–19.

4.3.1. Assessment of aneugenicity potential

52 If the result of the in vivo MN test is positive, i.e. your Substance induces an increase in the frequency of micronuclei, you must assess the aneugenic potential of the Substance. In line with the OECD TG 474 (paragraph 42), you should use one of the centromere labelling or hybridisation procedures to determine whether the increase in the number of micronuclei is the result of clastogenic events (i.e. micronuclei contain chromosome fragment(s)) and/or aneugenic events (i.e. micronuclei contain whole chromosome(s)).

53 In the comments to the draft decision, you argue that aneugenicity assessment in case of positive results in the requested combined *in vivo* comet assay and *in vivo* micronucleus test is not necessary as (i) clastogenic effects were shown in the *in vitro* chromosomal aberration test with the Substance, (ii), there is no difference in the hazard assessment, risk assessment or classification and labelling between substances inducing aneugenic effects or clastogenic effects *in vivo*, and (iii) there are technical challenges to chromosome centromere staining in the *in vivo* micronucleus test.

54 Regarding point (i), you indicate in your dossier that the *in vitro* chromosomal aberration test with the Substance (1994) shows the induction of structural chromosomal aberrations as well numerical chromosomal aberrations (polyploid cells) at the maximum concentration of 1500 µg/mL in the 48h-treatment without metabolic activation. ECHA agrees with your conclusions that the study results raise a concern for chromosomal aberration. However, while the OECD TG 473 stresses the fact that the test is not designed to measure aneuploidy and that polyploidy alone does not necessarily indicate an aneugenic potential and can simply reflect cell cycle perturbation or cytotoxicity, ECHA considers that potential aneugenic properties of the Substance cannot be excluded based on the study results.

55 Regarding point (ii), ECHA disagrees that there is no difference in the hazard and risk assessment of aneugenic substances and clastogenic substances since aneugenic effects are considered as having a non-linear dose (concentration)-response curve and a threshold could in principle be identified for aneugenic substances and trigger specific risk management measures, contrary to clastogenic effects. However, ECHA acknowledges the fact that, if the Substance is confirmed to have both clastogenic and aneugenic properties, risk assessment would normally be based on its clastogenic effects as worst case. As the *in vitro* and *in vivo* data in your dossier are not sufficient to conclude on the mutagenic properties of the Substance and the *in vivo* micronucleus test can detect both clastogenic and aneugenic substances, ECHA considers it as the most appropriate *in vivo* follow up test to clarify the chromosomal aberration mechanisms of the Substance, in particular when combined with the *in vivo* comet assay, which will allow a broader coverage of target organs compared to the *in vivo* micronucleus test alone.

56 Regarding (iii), and as highlighted in the OECD TG 473, an *in vitro* micronucleus test would be recommended for the detection of aneuploidy *in vitro*. As an *in vitro* micronucleus test with the Substance was not conducted and the exact mechanism of chromosomal aberration is not fully clarified, aneugenicity assessment in case of positive results *in vivo* is necessary. According to OECD TG 474 (paragraph 42), 'anti-kinetochore antibodies, FISH with pancentromeric DNA probes, or primed *in situ* labelling with pancentromere-specific primers, together with appropriate DNA counterstaining, can be used to identify the nature of the micronuclei (chromosome/chromosomal fragment) in order to determine whether the mechanism of micronucleus induction is due to clastogenic and/or aneugenic activity. Other methods for differentiation between clastogens and aneugens may be used if they have been shown to be effective.'

4.3.2. Investigation of target tissue exposure

57 The applicable test method OECD TG 474 states that "*If there is evidence that the test substance(s), or its metabolite(s), will not reach the target tissue, it may not be appropriate to use this test*". Additionally, a negative test result can be considered reliable only if "*Bone marrow exposure to the test substance(s) occurred*".

58 Therefore, to ensure that the data generated are adequate for hazard identification, you must take blood samples at appropriate times and measure plasma levels of the Substance and/or its metabolites (OECD TG 474, paragraph 40), unless exposure of the bone marrow can be demonstrated through other means, e.g. by showing a depression of immature to mature erythrocyte ratio (OECD TG 474, paragraph 48).

59 If the Substance is negative in this test, but it is not possible to demonstrate that bone marrow exposure to the Substance occurred, then ECHA will consider any remaining uncertainty concerning the mutagenic potential of the Substance and whether to request any further information.

60 In your comment to the draft decision, you indicate that "*There are enough data at hand about the bioavailability of the Cs salts after single and repeated dosing in rats. Therefore, additional data are not required to address this issue.*" ECHA reminds you that a "*blood*

sample should be taken at appropriate time(s) in order to permit investigation of the plasma levels of the test substances for the purposes of demonstrating that exposure of the bone marrow occurred, where warranted and where other exposure data do not exist" (OECD TG 474, paragraph 40). The examination of the bone marrow exposure to the susbtance is one the conditions listed in the paragraph 48 of the OECD TG 474 that must be fulfilled in order to assess if the results are clearly negative.

4.3.1. Germ cells

61 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other aforementioned 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.

62 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.

5. Extended one-generation reproductive toxicity study

63 An extended one-generation reproductive toxicity (EOGRT) study (OECD TG 443) is an information requirement under Annex IX, Section 8.7.3., if the available repeated dose toxicity studies indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity. Furthermore Column 2 defines the conditions under which the study design needs to be expanded.

5.1. Triggering of the information requirement

64 You claim that "Based on the current data set and the observed test item effect on the male reproductive system …a two-generation reproduction toxicity study (OECD 416) or an extended one-generation reproductive toxicity study (OECD 443) appear scientifically unjustified and not to be in line with animal welfare regulation as no considerable knowledge gain is expected from this study."

65 However, your statement is contradicted by the following studies in your dossier, which indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity, *e.g.*:

- a sub-chronic study (i) conducted with the Substance (2012) indicates effects in the testes and the epididymides weight at the Mid-and High doses, with related histopathological findings observed at the High Dose; decreased intensity of spermatogenesis (9/10), and lack of mature spermatozoa in the seminiferous tubuli of testes (9/10) and in the ductuli of epididymides (9/10). Sperm was also affected at the Mid-and High Doses.
- a sub-chronic study (ii) conducted with the analogue substance Cesium Chloride EC 231-600-2 (2016, report [redacted]) indicates severe effects starting at the Mid-dose: tubular degeneration/atrophy in the testes, effects on the sperm maturation (statistically significant changes in sperm morphology, motility (increase of beat cross frequency only) as well as significant reductions in cauda epididymal sperm numbers.

66 To summarize, the available repeated dose toxicity studies conducted with the Substance itself and the analogue Substances indicate adverse effects on reproductive organs.

67 Therefore, the information requirement is triggered.

68 In your comments to the draft decision, you consider that the reported fertility effects are secondary to systemic toxicity and that there is no scientific reason to perform an EOGRT study to further investigate fertility effects. More specifically, you claim that "*the reproductive toxicity needs to be seen also in the context of systemic toxicity and influence on electrolyte levels, particularly potassium that can explain the sequence of the observed effects*".

69 ECHA considers that the potential link between the severe effects on male fertility and the decrease of potassium and uremia observed is speculative. ECHA notes that you have provided no experimental evidence to support of your claim. In addition, the study (i) concludes that *"Damage in spermatogenesis (smaller than normal testes, reduced weights of testes and epididymides, decreased intensity of spermatogenesis, accompanied with lack of mature spermatozoa in the seminiferous tubuli in the testes and in the ductuli of epididymides, and decreased number of spermatids in a proportion of seminiferous tubuli) after the 90-day oral (gavage) administration in Hsd.Brl.Han: Wistar rats* [are] *considered to be toxicologically relevant."*

70 Therefore, ECHA maintains that the reported male fertility effects in both sub-chronic studies are indicating adverse effects on reproductive organs and therefore trigger the need to conduct an EOGRT study under Annex IX, section 8.7.3, column 1.

5.2. Information provided

71 You have not provided any information to fulfil this information requirement.

72 Therefore, the information requirement is not fulfilled.

5.3. Study design

5.3.1. Species and route selection

73 As the Substance is a solid, the study must be conducted in rats with oral administration of the Substance (Annex IX, Section 8.7.3, Column 1).

5.3.2. Pre-mating exposure duration

74 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.

75 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.).

76 Therefore, the requested pre-mating exposure duration is ten weeks.

5.3.3. Dose-level setting

77 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, paragraph 22; OECD GD 151, paragraph 28; introductory part of Annex IX/X to REACH; Annex I, Section 1.0.1. to 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.

78 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; Annex I, Section 3.7.2.4.4. of the CLP Regulation) or severe suffering such as persistent pain and distress (OECD GD 19, paragraph 18) in the P0 animals.

79 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.

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

(2) 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

(3) 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

(4) 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

(5) the highest dose level in P0 animals must follow the limit dose concept.

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

82 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.

5.3.4. Cohorts 1A and 1B

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

5.3.4.1. Histopathological investigations in Cohorts 1A and 1B

84 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, paragraph 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.

5.3.4.2. Splenic lymphocyte subpopulation analysis

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

5.3.4.3. Investigations of sexual maturation

86 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, paragraph 12 in conjunction with OECD TG 443, paragraph 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.

5.3.5. Cohort 3

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

88 Existing information on the Substance itself and the source substance EC 222-492-8 derived from the available studies reported in your dossier show evidence of immunotoxicity including changes in haematological parameters and alterations in immune systems organ weight as spleen and thymus:

- concerning the sub-acute toxicity study with the Substance, a significant reduction of the thymus weights relative to brain weight is observed at the Mid- and High Dose (-28 % and -32 % and respectively).
- concerning the sub-chronic study with the Substance, the mean thymus weight (absolute and relative to brain weight) in the male animals) and the absolute mean thymus weight in the female animals were less than the control in animals administered at the High Dose. It was accompanied by involution of severe degree in one female and of mild degree in one male.
- concerning the sub-chronic study with the source substance Cesium Chloride EC 222-492-8, an increase of the spleen weight for females at high dose for 13 weeks was reported and following 16 weeks of recovery. An increase of the extramedullary haemopoiesis was observed in males at the High Dose for 13 weeks (with High Dose, ECHA refers to 127 mg/kg bw/d). A decrease of the thymus weight was also reported at the high dose and still observed following 8 weeks of recovery. Total leucocyte counts were increased in Week 13 for animals at the High dose, associated with increased neutrophil, lymphocyte, eosinophil and monocyte counts in both sexes.

89 In your comment to the draft decision, you consider that the effects described above are likely secondary to other inflammatory changes because they occurred at doses '*where 3 males and 3 females died or had to be killed for humane reasons.*' ECHA understands that you refer to the high dose (253 mg/kg bw/day) that has been stopped after 59 days due the reported mortality in males and females. ECHA emphasizes the dose we are referring to is 127 mg/kg bw/d where no mortality is reported after 13 weeks. Regarding your comments on inflammatory changes, ECHA did not identify any finding indicating inflammation that could explain the effects observed in the thymus at 127 mg/kg bw/day. In addition, the sub-acute and sub chronic studies on the Substance available in your dossier do not exclude an effect of the test material on the thymus.

90 Therefore, the concern for immunotoxicity remains and the cohort 3 is triggered.

91 To summarize, the available repeated dose toxicity studies indicate changes in haematological parameters and alterations in immune system organ weights such as spleen and thymus.

92 Therefore, the Substance itself and analogue substance show dysregulation of the immune system.

93 Because the immune system is under development in the post-natal period, the dysregulation of the immune system could have a more severe impact on developing organisms.

94 For the reasons stated above, the developmental immunotoxicity Cohort 3 must be conducted.

5.3.6. Further expansion of the study design

95 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 Annex IX, Section 8.7.3., Column 2. 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.

6. Long-term toxicity testing on aquatic invertebrates

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

6.1. Information provided

97 You have provided:

(i) a long-term toxicity study on *Daphnia magna* (2012) performed according to the OECD TG 211 (2012) with the Substance.

6.2. Assessment of the information provided

6.2.1. The provided study does not meet the specifications of the test guideline

98 To fulfil the information requirement, a study must comply with the OECD TG 211. Therefore, the following specifications must be met:

Reporting of the methodology and results

d) the nominal test concentrations and the results of all analyses to determine the concentration of the test substance in the test vessels are reported;

e) the full record of the daily production of living offspring during the test by each parent animals provided;

f) the number of deaths among the parent animals (if any) and the day on which they occurred is reported.

99 In study (i):

Reporting of the methodology and results

a) you have not provided the tested nominal concentrations nor the results of the analytical determination of exposure (only mean measured values are reported);

b) the full record of the daily production of living offspring during the test by each parent anima is not provided;

c) the number of deaths among the parent animals (if any) and the day on which they occurred is not reported.

100 Based on the above, the reporting of the study is not sufficient to conduct an independent assessment of its reliability. Without the information on b) to d), it is not possible to evaluate whether the validity criteria of the test method were met and the interpretation of the results is appropriate.

101 On this basis, the specifications of OECD TG 211 are not met.

102 Therefore, the information requirement is not fulfilled.

103 In your comments to the draft decision, you provided the missing information and addressed the study deficiencies identified above. However, as the information is currently not available in your registration dossier, the data gap remains. You should therefore submit this information in an updated registration dossier by the deadline set in the decision.

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

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

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

The compliance check was initiated on 7 October 2022.

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 did not amend the requests.

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

(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.

With that detailed information, ECHA can confirm 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 (https://echa.europa.eu/manuals).