EUROPEAN CHEMICALS AGENCY

Helsinki, 8 July 2019

Addressee: [redacted]

Decision number: TPE-D-2114475149-40-01/F
Substance name: Tetraammineplatinum (II) diacetate
EC number: 457-310-8
CAS number: NS
Registration number: [redacted]
Submission number: [redacted]
Submission date: 12/12/2017
Registered tonnage band: 10-100

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation ((EC) No 1907/2006) (the REACH Regulation), ECHA examined your testing proposal(s) and decided as follows.

Your testing proposal is modified and you are requested to carry out:

1. **In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum using the analogue substance tetraammineplatinum (II) dichloride (EC 237-706-5). It is at your discretion to perform in combination with the requested comet assay the in vivo micronucleus test and the toxicokinetic study.**

You have to submit the requested information in an updated registration dossier by ***15 July 2020***. You also have to update the chemical safety report, where relevant.

The reasons for this decision are set out in Appendix 1. The procedural history is described in Appendix 2 and advice and further observations are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

Authorised[1] by Claudio Carlon, Head of Unit, Hazard Assessment

[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

The decision of ECHA is based on the examination of the testing proposals submitted by you.

1. **In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2)**

Pursuant to Article 40(3)(b) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test under modified conditions.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex VIII, Section 8.4. provides that "Appropriate in vivo mutagenicity studies shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII."

The technical dossier contains an *in vitro* study in bacterial cells (2004) performed according to *OECD TG 471* with the registered substance that show positive results. The study is positive in in the bacterial strains S. typhimurium TA 1537 (with and without metabolic activation; MA), TA 98 (with MA) and in E. coli WP2 uvrA (with and without MA). ECHA observes that a positive result for the fifth strain (E. coli WP2 uvrA) has been obtained in the bacterial test indicative of potential cross-linking properties for the registered substance. The dossier also contains an *in vitro* gene mutation mouse lymphoma assay (2017) according to OECD TG 490 and GLP with the analogue substance Tetraammineplatinum dichloride (EC 237-706-5). The study shows that tetraammineplatinum dichloride induced mutations at the tk locus of L5178Y mouse lymphoma cells, when tested up for 3 hours in the absence and presence of S9 and for 24 hours in the absence of S9. Some *in vitro* studies in mammalian cells performed according to OECD TG 476 with the analogue substance Tetrammine platinum (II) hydrogen carbonate (CAS 123439-82-7) are also provided in the dossier. These studies also have a positive result with and without metabolic MA. Overall, the positive results either in bacteria or in mammalian cells indicate that the substance is inducing gene mutations under the conditions of the tests. In addition, the positive result in the strain E. coli WP2 is indicative of potential cross-linking properties of the registered substance.

The dossier also contains two *in vitro* cytogenicity tests performed according to OECD TG 473 (2007, 2008) with the registered substance, both with negative test results. ECHA, however notes that all the provided *in vitro* cytogenicity studies have deficiencies (e.g. insufficient number of metaphases analysed).

Some *in vivo* studies (UDS, 1999; *in vivo* SLRL, 1980; *in vivo* micronucleus, 1980; *in vivo* chromosomal aberration, 1981) performed with analogue substances (Tetrammine platinum hydrogen carbonate (no EC or CAS indicated), Tetraammineplatinum (II) dichloride EC 237-706-5; CAS 13933-32-9) are also provided. The studies are pre-guideline studies and are labelled with the reliability 3 (not reliable) and they all have a negative test result. Additionally, ECHA considers that none of them is adequate to address the *in vitro* concern for gene mutations.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the registered substance but shall be considered. Consequently, there is an information gap and you considered it necessary to generate information for this endpoint.

Hence, you have submitted a testing proposal for an *in vivo* Mammalian Alkaline Comet Assay to be performed with the analogue substance Tetraammineplatinum dichloride (CAS 13933-32-9, EC No 237-706-5). In addition, you propose to perform a concomitant micronucleus assay and combined/parallel toxicokinetic study.

ECHA notes that the proposed test is an appropriate test to further investigate effects on gene mutations *in vivo* as described in the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017), Chapter R.7a, section R.7.7.1. and figure R.7.7-1.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity in vivo. ECHA notes that 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.

ECHA has evaluated your proposal to perform the test with the analogue substance Tetraammineplatinum dichloride (EC 237-706-5; CAS 13933-32-9).

Grouping and read-across approach for toxicological and ecotoxicological information

Your registration dossier contains for the in vivo genotoxicity endpoint an adaptation argument in the form of a grouping and read-across approach under Annex XI, Section 1.5. of the REACH Regulation. ECHA has assessed first the scientific and regulatory validity of your read-across approach in general before assessing the individual endpoint.

You have sought to adapt information requirements for *in vivo* mutagenicity by applying a read-across approach in accordance with Annex XI, Section 1.5. According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (read-across approach). ECHA considers that the generation of information by such alternative means should offer equivalence to prescribed tests or test methods.

Based on the above, a read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for a toxicological or ecotoxicological property is reliable and should be based on recognition of the structural similarities and differences between the source and target substances[2]. This hypothesis explains why the differences in the chemical structures should not influence the toxicological/ ecotoxicological properties or should do so in a regular pattern. The read-across approach must be justified scientifically and documented thoroughly, also taking into account the differences in the chemical structures. There may be several lines of supporting evidence used to justify the read-across hypothesis, with the aim of strengthening the case.

[2] Please see for further information ECHA *Guidance on information requirements and chemical safety assessment* (version 1, May 2008), Chapter R.6: QSARs and grouping of chemicals.

Due to the different nature of each endpoint and consequent difference in scientific considerations (e.g. key parameters, biological targets), a read-across must be specific to the endpoint or property under consideration. Key physicochemical properties may determine the fate of a compound, its partitioning into a specific phase or compartment and largely influence the availability of compounds to organisms, e.g. in bioaccumulation and toxicity tests. Similarly, biotic and abiotic degradation may alter the fate and bioavailability of compounds as well as be themselves hazardous, bioaccumulative and/or persistent. Thus, physicochemical and degradation properties influence the human health and environmental properties of a substance and should be considered in read-across assessments. However, the information on physicochemical and degradation properties is only a part of the read-across hypothesis, and it is necessary to provide additional justification which is specific to the endpoint or property under consideration.

The ECHA Read-across assessment framework foresees that there are two options which may form the basis of the read-across hypothesis[3]- (1) (Bio)transformation to common compound(s)- the read-across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed and (2) Different compounds have the same type of effect(s)- the read-across hypothesis is that the organism is exposed to different compounds which have similar (eco)toxicological and fate properties as a result of structural similarity (and not as a result of exposure to common compounds).

Finally, Annex XI, Section 1.5. lists several additional requirements, which deal with the quality of the studies which are to be read-across.

i. Description of the grouping and read-across approach proposed by you

You consider to achieve compliance with the REACH information requirements for the registered (target) substance Tetraammineplatinum (II) diacetate (EC 457-310-8) using data from a structurally similar substance Tetraammineplatinum (II) dichloride (EC 237-706-5; CAS 13933-32-9) (hereafter the 'source substance').

You have provided a document to justify the read-across approach for human health as a separate attachment in the registration (section 13 of IUCLID, submission [redacted]). This report contains a category justification and a data matrix (human health). In your read-across documentation you propose read-across between the substances:

- *"Tetraammineplatinum (II) dichloride [CAS 13933-32-9; EC 237-706-5; registered (1-10 tpa) by* [redacted]
- *Tetraammineplatinum (II) dinitrate [CAS 20634-12-2; EC 243-929-9; registered (1-10 tpa) by* [redacted]
- *Tetraammineplatinum (II) diacetate [CAS 127733-97-5; EC 690-714-4; registered (10-100 tpa) by* [redacted]
- *Tetraammineplatinum (II) hydrogen carbonate [CAS 123439-82-7; EC 426-730-3; registered (10-100 tpa) by* [redacted]

ECHA notes that the identifiers you have provided for Tetraammineplatinum (II) diacetate in that document (see quote above) are not the same as for your registered substance (EC 457-310-8). However, ECHA understands that you intend to refer to your registered substance and there is a typographical error in the read-across justification document.

[3] Please see ECHA's Read-Across Assessment Framework (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across).

You use the following arguments to support the prediction of properties for the registered (target) substance from data for the source substance(s) within the group:

- *"In accordance with the RAAF (ECHA, 20171) [...] this read-across justification follows Scenario 5".*
- *"It is hypothesised that the target and source substances will behave in a similar way, undergoing (bio)transformation to common products, with no expected difference in the relative strength of effects within the category".*
- *"For this category of four tetraammineplatinum (II) salts, it is proposed that, in aqueous solution and in biological media (e.g. gastric fluid), there will be dissociation of the anions (acetate, chloride, hydrogen carbonate or nitrate), leaving the core tetraammineplatinum(II) complex as the common product and toxicologically active species".*
- *"In solution, the anions of these salts will dissociate from the core tetraammineplatinum(II) cation complex; this can be regarded as the common dissociation product of all four compounds in this category".*
- *"The proposed category is composed of simple salts of the tetraammine-platinum(II) cation complex. The typical anions of these salts are ubiquitous in mammalian physiological systems, and are not expected to contribute to the overall toxicity of the substance".*
- *Within this category, all four tetraammineplatinum(II) read-across group member substances are acting as both source and target compounds for several endpoints. In all of these species, the platinum is in the 2+ oxidation state, co-ordinated to four neutral ammonia molecules (giving an overall 2+ charge on the complex). Thus, the difference in anion (acetate, chloride, hydrogen carbonate or nitrate) represents the only structural difference between the compounds in this category. As such, all the human health toxicity data included in the dossiers, and in the Data Matrix, should be considered equally applicable to each of the four substances."*
- *"Further experimental justification for rapid dissolution and stability of the various tetraammineplatinum(II) complexes was obtained via 195Pt NMR. Tetraammineplatinum(II) diacetate, dinitrate, dichloride and hydrogen carbonate were added to artificial gastric body fluid (HCl, pH 1.5, 2h shaking at 37°C) at* [redacted] *mol/L. The obtained NMR spectra were compared to those of Tetraammine-platinum(II) dichloride in water (saturated solution and at* [redacted] *mol/L). All spectra showed a single and clear peak at δ =-2560 ppm, and confirmed that the same Pt complex was formed upon dissolution under acid, chlorine rich media (mimicking gastric conditions)."*
- *"Bacterial mutagenicity assays, all conducted to protocols in accordance with or similar to OECD test guideline (TG) 471, tested all four salts at levels of up to 1 mg/plate. At least two of the salts have been tested for mutagenicity in mammalian cells, as well. None of the four salts appeared to give significantly different results, with a mixture of (weak) positives and negatives depending on bacterial strains/mammalian cell types and presence or absence of metabolic activation. Crucially, several studies reported cytotoxicity and/or precipitation at the highest tested concentrations, indicating that the toxic effects were comparable between the salts."*
- *"Tetraammineplatinum(II) dichloride is selected as the representative substance for further genotoxicity testing because:*
    - *It has the most extensive genotoxicity dataset amongst the tetraammineplatinum(II) read-across group member substances.*

- *The 195Pt NMR spectra demonstrate that, in the stomach, all four salts would dissociate to form the same tetraammineplatinum(II) complex. The concentrations of the anions released would be negligible in comparison with the surrounding high-chloride environment.*
- *On a molecular weight basis, tetraammineplatinum(II) dichloride will provide the highest dose of the toxicologically-active tetraammineplatinum(II) complex."*

As an integral part of this prediction, you propose that the source substance and the target substance have similar properties for the above-mentioned information requirements. ECHA considers that this information is your read-across hypothesis.

ii. ECHA analysis of the grouping and read-across approach in light of the requirements of Annex XI, 1.5.

With regard to the proposed predictions ECHA has the following observations:

In order to meet the provisions in Annex XI, Section 1.5. to predict human health effects from data for a reference substance within the group by interpolation to other substances in the group, ECHA considers that structural similarity alone is not sufficient. It has to be justified why such prediction is possible in view of the identified structural differences and the provided evidence has to support such explanation. In particular, the structural similarities must be linked to a scientific explanation of how and why a prediction is possible.

ECHA agrees with the following aspects of your arguments:

1. The target and source substances will behave in a similar way, undergoing (bio)transformation to common products, with no expected difference in the relative strength of effects within the category.
2. In aqueous solution and in biological media (e.g. gastric fluid), there will be dissociation of the anions (acetate, chloride, hydrogen carbonate or nitrate), leaving the core *tetraammineplatinum(II) complex* as the common product and toxicologically active species.
3. The typical anions of these salts are ubiquitous in mammalian physiological systems, and are not expected to contribute to the overall toxicity of the substance.
4. The NMR spectra support the fact that the four selected salts fully dissociate after 2h at pH 1.5 and 37°C. ECHA however notes that you did not provide information on the kinetics of the dissociation process which adds uncertainty to your prediction. Accordingly, you did not fully demonstrate that the dissociation is fast enough and that test animals are not exposed to the parent compound. Based on the information you provided it may however be considered that the selected *tetraammineplatinum(II) complexes* will eventually be transformed to a common compound.
5. Choice of representative substance for further genotoxicity testing.
   The provided data set for *tetraammineplatinum(II) dichloride* and *Tetraammineplatinum (II)diacetate* seem equally extensive considering that the *in vivo* studies for *tetraammineplatinum (II) dichloride* are not reliable.
   On a molecular weight basis, *tetraammineplatinum(II) dichloride* will provide the highest dose of the toxicologically-active *tetraammineplatinum(II) complex:* ECHA agrees that, among the selected salts, *tetraammineplatinum(II) dichloride* is the

source substance for which the relative weight of *platinum (II) tetraammine* is the highest.

ECHA considers that despite some deficiencies, your hypothesis that the four selected tetraammineplatinum (II) salts (i.e., tetraammineplatinum (II) dichloride, tetraammineplatinum (II) dinitrate, tetraammineplatinum (II) diacetate and tetraammineplatinum (II) hydrogen carbonate) will dissociate to form a stable platinum (II) tetraammines under physiological conditions is plausible.

ECHA considers that your read-across approach may provide a reliable basis whereby the human health effects of the target substance may be predicted from data on the source substance. Hence, this approach is considered plausible in order to fulfill the general rules of adaptation as set out in Annex XI, Section 1.5. of the REACH Regulation.

iii. Conclusion on the read-across approach

For the reasons as set out above, ECHA considers that this grouping and read-across approach may provide a reliable basis whereby the human health effects of the target substance may be predicted using data from the source substance. Therefore the read across from the tetraammineplatinum (II) dichloride (EC 237-706-5; CAS 13933-32-9) (source substance) to Tetraammineplatinum (II) diacetate (EC 457-310-8) is considered plausible.

This approach is considered plausible for the purpose of the testing proposal evaluation. ECHA emphasises that any final determination on the validity of the read-across, including the grouping approach proposed by you, would be premature at this point in time. The eventual validity of the read-across hypothesis and grouping approach will be reassessed once the requested information is submitted.

You also propose that a concomitant micronucleus assay and combined toxicokinetic assessment are performed and that *"in the Comet assay, it is proposed that somatic cells are sampled from three tissues: the liver (systemically exposed tissue) and the glandular stomach and duodenum (site-of contact tissues). The duodenum tissue will be stored/frozen, and only analysed (Comet measurements taken) if both the liver and glandular stomach provide a negative response. Germ cells will also be collected at the same time, stored/frozen, and Comet measurements taken if either the liver or glandular stomach provide a positive response. It is proposed to conduct this study in rats following oral gavage dosing. Bone marrow is selected as the target tissue for micronuclei assessment. Inclusion of a parallel toxicokinetic study is proposed for the purpose of demonstrating that adequate target tissue exposure to the test substance has been achieved".*

ECHA notes that an *in vivo* MN study is not appropriate to follow up of an *in vitro* gene mutation concern (i.e. positive test results in the *in vitro* bacterial gene mutation assay and *in vitro* gene mutation assay in mammalian cells).

ECHA considers that an *in vivo* micronucleus test is an appropriate test to investigate effects on chromosomal aberrations (micronuclei) *in vivo* as described in the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.7.1. and figure R.7.7-1 (version 6.0, July 2017). However, as already explained above, the provided *in vitro* and *in vivo* cytogenicity studies were concluded to have deficiencies and

are therefore inconclusive regarding effects on chromosomal aberrations. In the absence of positive *in vitro* findings ECHA could therefore not request such an in vivo follow up study by itself.

However, as long as the investigations supplementing the Comet Assay do not lead to additonal animal testing and suffering, ECHA considers that it is at your discretion to perform the *in vivo* micronucleus test in combination with the requested comet assay and the toxicokinetic study.

You proposed testing in rats and by the oral route of administration.

According to the test method OECD TG 489, the test shall be performed in rats. 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.

In line with the test method OECD TG 489, the test shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism as well as 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.

When assessing your testing proposal, ECHA thus considers that the duodenum should not be stored/frozen as proposed, but should be collected and analysed at the same time as the other tissues. ECHA therefore decided to modify the conditions under which you are to perform the test. Regarding the proposal to store tissues by freezing them, ECHA further reminds you that freezing tissues is not recommended for the comet assay: the OECD TG 489 mentions in paragraph 5 that "laboratory should demonstrate competency in freezing methodologies [...] the freezing of tissues has been described using different methods. However, currently there is no agreement on how to best freeze and thaw tissues, and how to assess whether a potentially altered response may affect the sensitivity of the test".

Concerning your proposal regarding germ cells (i.e. "*germ cells will also be collected at the same time, stored/frozen, and Comet measurements taken if either the liver or glandular stomach provide a positive response*"), ECHA would like to:

- remind you that according to Annex IX/X, Section 8.4., column 2 of the REACH Regulation, if positive results from an in vivo somatic cell study are available, "the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered".
- encourage you to consider examining gonadal cells, as it would optimise the use of animals. ECHA notes that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive result would indicate that the substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. 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.

- remind you that freezing tissues is not recommended by OECD TG 489.

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, you are requested to carry out the proposed study with the analogue substance tetraammineplatinum (II) dichloride (EC 237-706-5; CAS 13933-32-9):

In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum. It is at your discretion to perform the *in vivo* micronucleus test in combination with the requested comet assay and the toxicokinetic study.

Notes for your consideration

ECHA reminds you that you may decide to take into account the potential cross-linking properties of the registered substance in the experimental setup of the comet assay and perform a modified comet assay in order to detect cross links. Hence, you may consider preparing and analysing two sets of slides: one set of slides submitted to the standard experimental conditions (as described in OECD TG 489); the other set of slides submitted to modified experimental conditions that enable the detection of DNA. The modified experimental conditions may utilise one of the following options: (1) increase of electrophoresis time, e.g. as described in reference 23[4] in the OECD TG 489; (2) treatment of isolated cells (either in suspension or embedded in the slides) with a chemical (e.g. MMS) or (3) treatment of isolated cells (either in suspension or embedded in the slides) with ionising radiation (options 2 and 3 are described e.g. in references 36-39[5] in the OECD TG 489 or Pant[6] et al. 2015). In order to ensure the robustness of the test result a specific positive control group of animals would be needed.

In the draft decision communicated to you the time indicated to provide the requested information was 12 months from the date of adoption of the decision. In your comments on the draft decision, you requested an extension of the timeline to 30 months. You proposed a tiered testing strategy of the different platinum sub-groups arguing that "*the aim is a strategy whereby the testing of the next tier group for in vivo genotoxicity will be reconsidered and refined based on the outcome of the previous tier testing to avoid unnecessary test animal suffering and vertebrate testing*". Furthermore, you stated that "*12 months might not be sufficient to test all groups (in the worst-case situation), as the next tier testing cannot be initiated before the results of the previous tier are available*".
ECHA notes that the testing proposals from the various platinum sub-groups that you refer to will be processed in batches and be referred to the Member States Competent Authorities as sub-groups at different time points. The registered substance (Tetraammineplatinum (II) diacetate) belongs to a sub-group with four members and is the first sub-group to be referred to MSCAs. Hence, you will receive the adopted decisions for the four sub-groups at

[4] Reference 23 of OECD TG 489 (2016): (23) Nesslany, F, Zennouche N, Simar-Meintieres S, Talahari I, NKili-Mboui E-N, Marzin D (2007), In vivo Comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds, Mutation Research/Genetic Toxicology and Environmental Mutagenesis, Vol. 630/1, pp. 28-41.

[5] References 36 to 39 of OECD TG 489 (2016): (36) Merk, O., G. Speit (1999), Detection of crosslinks with the Comet assay in relationship to genotoxicity and cytotoxicity, Environmental and Molecular Mutagenesis, Vol. 33/2, pp. 167-72; (37) Pfuhler, S., H.U. Wolf (1996), Detection of DNA-crosslinking agents with the alkaline Comet assay, Environmental and Molecular Mutagenesis, Vol. 27/3, pp. 196-201; (38) Wu, J.H., N.J. Jones (2012), Assessment of DNA interstrand crosslinks using the modified alkaline Comet assay, Methods in Molecular Biology, Vol. 817, pp. 165-81; (39) Spanswick, V.J., J.M. Hartley, J.A. Hartley (2010), Measurement of DNA interstrand crosslinking in individual cells using the Single Cell Gel Electrophoresis (Comet) assay, Methods in Molecular Biology, Vol. 613, pp. 267-282.

[6] Pant K, Roden N, Zhang C, Bruce C, Wood C, and Pendino K (2015) Modified In Vivo Comet Assay Detects the Genotoxic Potential of14-Hydroxycodeinone, an a,b-Unsaturated Ketone in Oxycodone. Environmental and Molecular Mutagenesis 56, 777-787.

different time points. This should allow you to reconsider and refine your testing, if relevant, for the different sub-groups. Consequently, ECHA considers that no extension of deadline is required to perform the testing on this first sub-group. Therefore, ECHA has not modified the deadline of the decision.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposals for examination in accordance with Article 40(1) on 4 April 2017.

ECHA held a third party consultation for the testing proposals from 22 June 2017 until 7 August 2017. ECHA did not receive information from third parties.

ECHA notified you of the draft decision on 28 September 2017 (submission number [redacted]) and invited you to provide comments.

You updated your registration on 12 December 2017 (submission number [redacted]). ECHA took the information in the updated registration into account, and amended the draft decision. The updated information is reflected in the Reasons (Appendix 1).

Hence, ECHA re-started the decision-making process by sending a new draft decision for your comments.

This decision does not take into account any updates after **7 January 2019**, 30 calendar days after the end of the commenting period.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

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

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

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

ECHA received proposals for amendment and did not modify the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

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

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-64 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided in your registration dossier is in compliance with the REACH requirements. The decision does not prevent ECHA from initiating a compliance check on the registration at a later stage.

2. Failure to comply with the requests in this decision will result in a notification to the enforcement authorities of the Member States.

3. In carrying out the tests required by the present decision, it is important to ensure that the particular sample of substance tested is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported. If the registration of the substance covers different grades, the sample used for the new tests must be suitable to assess these.

   Furthermore, there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.

4. If the required tests are conducted with an analogue substance in the context of a read-across approach, the identity of the test material used to perform the test should be specified in line with the ECHA's Practical Guide on "How to use alternatives to animal testing to fulfil your information requirements" (chapter 4.4). This is required to show that the test material is representative of the analogue substance identified in the read-across approach and used to predict the properties of the registered substance.