Helsinki, 8 July 2019

Addressee: [redacted]

Decision number: TPE-D-2114475152-53-01/F
Substance name: Tetraammineplatinum dichloride
EC number: 237-706-5
CAS number: 13933-32-9
Registration number: [redacted]
Submission number: [redacted]
Submission date: 10/04/2019
Registered tonnage band: 1-10

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 VII, 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 registered substance. 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 and scientific information submitted by third parties.

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

a) Examination of the testing proposal

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 VII, Section 8.4. of the REACH Regulation. Column 2 of Annex VII, Section 8.4. provides that "Further mutagenicity studies shall be considered in case of a positive result".

The technical dossier contains a key *in vitro* study in bacterial cells (1980) performed similarly to OECD TG 471 with the registered substance that show positive results in the strains S. typhimurium TA 1537, TA 98 and TA 100 (with and without metabolic activation). The dossier also contains three supporting non guideline and non GLP *in vitro* studies in bacterial cells from years 1979 and 1993. One of the studies shows a weak positive result and the two others show a negative result. However, the supporting studies have deficiencies in study design and are therefore not adequate (e.g. the recommended five strains not used, no details on used controls given). ECHA furthermore observes that none of the provided studies contains the fifth strain required by the OECD TG 471. Moreover ECHA notes that a positive result for the fifth strain indicative of potential cross-linking properties has been obtained with the analogue substance tetraammineplatinum (II) diacetate (EC 457-310-8) a member of this Tetraammineplatinum(II) salts category. The dossier also contains an *in vitro* gene mutation mouse lymphoma assay (2017) according to OECD TG 490 and GLP with the registered substance. 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. 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 bacterial strain E. coli WP2 with the analogue tetraammineplatinum (II) diacetate is indicative of potential cross-linking properties.

The dossier also contains *in vitro* cytogenecity tests performed according to OECD TG 473 (2007, 2008) with the analogue substance tetraammineplatinum (II) diacetate (EC 457-310-8) and several OECD TG 473 studies (2007, 2008) performed with the registered substance, all with negative test results. All the provided *in vitro* cytogenicity studies have deficiencies (e.g. insufficient number of metaphases analysed).

Some *in vivo* studies (UDS, *in vivo* SLRL, *in vivo* micronucleus) performed both with the registered substance and an analogue substance (Tetraammineplatinum (II) hydrogen carbonate EC 426-730-3) are also provided in the dossier. All 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. 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 with the registered substance Tetraammineplatinum dichloride (EC 237-706-5; CAS 13933-32-9) with a concomitant micronucleus assay and combined toxicokinetic assessment.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity in vivo. ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

ECHA considers that you have adequately demonstrated the need to perform the proposed test. ECHA considers that the proposed test is appropriate to investigate effects on gene mutation *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.

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 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.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. For the reasons explained further below the information provided by third parties is not sufficient to fulfil this information requirement.

The third party has indicated a support of the study design proposed by the registrant *"[...] as it aims to obtain the maximum amount of information from a single study and [...] also use the results for read-across to other substances in the category"*.

ECHA acknowledges that in view of optimal animal use and useful additional information it is at your discretion to perform the studies in combination to the comet assay.

c) Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, you are requested to carry out the proposed study with the registered substance subject to the present decision:

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

d) *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[2] 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[3] in the OECD TG 489 or Pant[4] 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*

[2] 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.

[3] 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.

[4] 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.

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) dichloride) 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 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 12 December 2017.

ECHA held a third party consultation for the testing proposals from 28 February 2018 until 16 April 2018. ECHA received information from third parties (see Appendix 1).

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

ECHA notes that there was a change of the Lead Registrant from [redacted] to [redacted] on 10 April 2019 after the proposals for amendment were notified to the Registrant. ECHA informed the Registrants that the procedure for the examination of the testing proposal continued on the registration of the newly appointed Lead Registrant and that any further communication in relation to this testing proposal will be addressed to him.

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, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of the Member States.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests 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 by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally 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