ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 22 November 2019

Addressee: [redacted]

Decision number: TPE-D-2114489550-43-01/F
Substance name: Dipotassium tetrachloroplatinate
EC number: 233-050-9
CAS number: 10025-99-7
Registration number: [redacted]
Submission number: [redacted]
Submission date: 18/12/2017
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 ***30 November 2020***. You shall also 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 VII, 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 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 several non test guideline *in vitro* studies in bacterial cells with the registered substance. The first study from 1979 shows a positive result in the only strain tested, that is *S typhimurium* TA 100 without metabolic activation system. The second study, also from 1979, performed in *S typhimurium* strains TA 98, TA 100, TA 1535, TA 1538 in the absence of metabolic activation, shows positive results in strains TA 98 and TA 100. The third study, from 1993, performed on bacterial strains *S typhimurium* TA 98 and TA 100 with and without metabolic activation system, shows a weakly positive test result on both strains.

Additionally, ECHA notes that you did not include tests with strains S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). Since the available tests were conducted, significant changes have been made to OECD TG guideline 471 so that additionally testing with S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101) is now required. Considering that the Ames studies, performed on other platinum substances, show a positive result in the fifth strain(s) ECHA considers that the registered substance could also have a potential cross-linking effect.

The technical dossier also contains two *in vitro* gene mutation tests in mammalian cells from years 1979 and 1980 performed similarly to the OECD TG 476 with the registered substance with a negative and ambiguous result respectively.

You have also included an *in vitro* micronucleus test (1997) performed similarly to OECD TG 487 without metabolic activation system that shows a positive result. In addition, you have also provided negative *in vivo* studies (1982 and 1981) performed similarly to OECD TG 475 and TG 474 and labeled with reliability 3 (not reliable) with negative test results in the technical dossier. However, since the studies have been conducted, significant changes have been made to the test guidelines. Therefore, the provided studies do no meet the current guidelines, nor can they be considered as providing equivalent data according to the criteria in Annex XI, 1.1.2. of the REACH Regulation.

The positive results indicate that the substance is inducing gene mutations and chromosomal aberrations under the conditions of the tests.

In view of the above, an appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations and chromosomal aberrations 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 *"alkaline comet assay (OECD Test Guideline 489), with a concomitant micronucleus assay and combined toxicokinetic assessment with the registered substance in rats by the oral route".*

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. Also, ECHA notes that the proposed test is appropriate to investigate effects on gene mutation and chromosomal aberrations *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 proposed testing by the oral route in rats.

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, 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.

You also propose that a concomitant micronucleus assay and a 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 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). As already explained above, ECHA notes that the provided *in vitro* and *in vivo* cytogenicity studies have deficiencies and are therefore inconclusive regarding effects on chromosomal aberrations. Hence, ECHA considers that it is at your discretion to perform the *in vivo* micronucleus test in combination to the comet

assay and any additional toxicokinetic study, as long as this will not impair the validity of and the results from each individual study.

In addition, ECHA 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. Moreover, regarding the proposal to store tissues by freezing them, ECHA 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 modified 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. It is at your discretion to perform the *in vivo* micronucleus test in combination with the 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[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.

Deadline to submit the requested information in this decision

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 deadline 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 would 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 are being processed in batches Hence, you will receive the adopted decisions for the various sub-groups at different time points. This should allow you to reconsider and refine your testing, if relevant, for the different sub-groups.

Therefore, ECHA has not modified the deadline of the decision.

[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.

Appendix 2: Procedural history

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 29 December 2017.

ECHA held a third party consultation for the testing proposals from 18 June 2018 until 2 August 2018. ECHA did not receive information from third parties.

This decision does not take into account any updates after **18 February 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 deadline.

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: 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.