EUROPEAN CHEMICALS AGENCY

Helsinki, 12 December 2019

Addressee: [redacted]

Decision number: TPE-D-2114492260-53-01/F
Substance name: Hexachloroplatinic acid
EC number: 241-010-7
CAS number: 16941-12-1
Registration number: [redacted]
Submission number: [redacted]
Submission date: 04/01/2018
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:

> **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 Diammonium hexachloroplatinate (EC 240-973-0; CAS 16919-58-7). 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 **19 March 2021**. 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 proposal submitted by you and scientific information submitted by third parties.

In vivo mammalian alkaline comet assay (Annex VIII, 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 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 two *in vitro* GLP studies in bacterial cells from years 2002 and 2004 performed according to OECD TG 471 with the registered substance that show positive and ambiguous results. The study from 2004 is performed in strains S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2 uvr A. The test substance induced reverse mutations in S. typhimurium strains TA98 and TA100 and in Escherichia coli WP2 uvrA under the experimental conditions. ECHA observes that a positive result for the fifth strain (E. coli WP2 uvr A) has been obtained indicative of potential cross-linking properties for the registered substance. The study from 2002 performed with strains S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2 uvr A had an ambiguos result in S. typhimurium strain TA100.

In the dossier there is another supporting Ames study (1993, publication) (non test guideline and non GLP) performed with the strains S. typhimurium TA 98 and TA 100 with a positive result in strain TA 98 (with metabolic actvation). Furthermore, in the dossier there is also a positive result, in an *in vitro* gene mutation in mammalian cells study (2003) (OECD TG 476; GLP compliant), when tested in the presence and absence of metabolic activation.

The positive results indicate that the substance is inducing 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.

ECHA notes that the dossier does not contain an *in vitro* cytogenicity study with the registered substance. However, in the (sub-)category for hexachloroplatinates to which the registered substance belongs, there are members that show positive but also negative results for *in vitro* cytogenicity indicative of inducing chromosomal aberrations under the conditions of the tests. Therefore, ECHA cannot exclude the possibility for the substance to induce chromosomal aberrations.

Hence, you have submitted a testing proposal for an *in vivo* comet assay with the analogue substance Diammonium hexachloroplatinate (EC 240-973-0; CAS 16919-58-7)
with a concomitant micronucleus assay and combined toxicokinetic assessment.

ECHA notes that the proposed test is an appropriate test to further investigate effects on gene mutations and/or potential 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

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 diammonium hexachloroplatinate(IV) (EC No 240-973-0; CAS No 16919-58-7).

Grouping of substances and read-across approach

The decision of ECHA is based on the examination of the testing proposal submitted by you for the registered substance Hexachloroplatinic acid, (EC No 241-010-7; CAS No 16941-12-1); hereafter referred to as "target substance"), proposed to be performed with a source substance Diammonium hexachloroplatinate(IV) (EC No 240-973-0; CAS No 16919-58-7) on the submitted read-across justification. ECHA has considered first the scientific validity of the read-across hypothesis (preliminary considerations below), before assessing the testing proposed.

Article 13(1) of the REACH Regulation requires information on intrinsic properties of substances on human toxicity to be generated whenever possible by means other than vertebrate animal tests, including from information from structurally related substances (grouping or read-across), "*provided that the conditions set out in Annex XI are met*".

According to Annex XI, 1.5 there needs to be structural similarity among the substances within a group or a category and furthermore, it is required that the relevant properties of a substance within the group can be predicted from the data for reference substance(s) by interpolation, and the data should be adequate for the purpose of classification and labelling and/or risk assessment.

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

You have proposed to cover the standard information requirement for in vivo genotoxicity (Annex VIII, Section 8.4., column 2) by performing the test with a source substance Diammonium hexachloroplatinate(IV) (EC No 240-973-0; CAS No 16919-58-7).

You have provided the following hypothesis/justification for the category approach: [...] "*this read-across justification follows Scenario 5. In this scenario, the proposed human health read-across is considered appropriate because 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 hexachloroplatinate(IV) substances, it is proposed that, in aqueous solution and in biological media (e.g. gastric fluid), the cations (ammonium, hydrogen, potassium or sodium) would dissociate, leaving the core hexachloroplatinate(IV) complex as the common product and toxicologically-active species. It should be noted that this represents a chemical transformation and not a "biotransformation"; no metabolism of the hexachloroplatinate(IV) complex is anticipated to occur in vivo".

Information/documentation submitted to support the grouping and read-across hypothesis

You have provided a read-across justification as a separate attachment in the endpoint summary 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:

- Diammonium hexachloroplatinate(IV) [CAS 16919-58-7; EC 240-973-0; 10-100 tpa]
- Dihydrogen hexachloroplatinate(IV) [a.k.a. hexachloroplatinic acid; CAS 16941-12-1; EC 241-010-7; 10-100 tpa]
- Dipotassium hexachloroplatinate(IV) [CAS 16921-30-5; EC 240-979-3; 10-100 tpa]

You further state that "additional data are utilised from the following substance, not currently subject to REACH registration:
- Disodium hexachloroplatinate(IV) [CAS 16923-58-3; EC 240-983-5]".

In your justification document you state that *"In all of these species, the platinum is in the 4+ oxidation state, co-ordinated to six chloride ions (giving an overall 2- charge on the complex). Thus, the difference in cation (ammonium, hydrogen, potassium or sodium) 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 (Table 2) below, should be considered equally applicable to each of the four substances".*

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

Based on the information provided, ECHA understands that the proposed read-across hypothesis is based on similar behaviour and (bio)transformation and toxicological properties of the target and source substances.

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

- 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.
- In aqueous solution and in biological media (e.g. gastric fluid), there will be dissociation of the cations (ammonium, hydrogen, potassium or sodium), leaving the core *hexachloroplatinate(IV) complex* as the common product and toxicologically active species.
- The typical cations of these salts are ubiquitous in mammalian physiological systems, and are not expected to contribute to the overall toxicity of the substance.

You have proposed that the source substance Diammonium hexachloroplatinate(IV) has similar toxicity regarding sub-chronic toxicity and therefore the properties of the target substance can be predicted from data obtained from the source substance.

ECHA concludes that the data provided supports the hypothesis that the source and target substances dissociate to a common hexachloroplatinate ion in solution complex is mainly dependent on pH and Cl concentration and that the effect of the cations is considered negligible.

ECHA therefore considers that there is an adequate basis for predicting the properties of the registered substance from the data obtained with the source substance.

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 registered substance may be predicted from data generated in a test with the source substance.

Hence, 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.

Considerations on the study design

Species, route of administration and the specifications regarding the vehicle control group

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.

Modification needed regarding the proposed sampling/freezing of the tissues

You also propose 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."*

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, ECHA considers that 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.

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. 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"*.

The concomitant micronucleus assay and combined toxiconetic assessment

You also propose that a concomitant micronucleus assay and a combined toxicokinetic assessment are performed and that *"[g]erm 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 indicated above there is no cytogenicity study available with the registered substance hence the potential concern for chromosomal aberrations cannot be excluded.

Thus, 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.

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 notes that you may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien et *al.*[2]) in addition to the other aforementioned tissues, 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, you should consider analysing the slides prepared with gonadal cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation. ECHA reminds 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 with the comet assay.

[2] O'Brien, J.M., Beal, M.A., Gingerich, J.D., Soper, L., Douglas, G.R., Yauk, C.L., Marchetti, F. (2014) Transgenic Rodent Assay for Quantifying Male Germ Cell Mutant Frequency. J. Vis. Exp. (90), e51576, doi:10.3791/51576

c) Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, you are requested to carry out the modified study with the analogue substance Diammonium hexachloroplatinate (EC 240-973-0; CAS 16919-58-7):

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 in combination with the requested comet assay the in vivo micronucleus test 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[3] 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[4] in the OECD TG 489 or Pant[5] 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 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 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"*.

[3] 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.

[4] 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.

[5] 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.

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.

Appendix 2: Procedural history

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 18 January 2018.

ECHA held a third party consultation for the testing proposals from 26 March 2018 until 11 May 2018. ECHA received information from third parties (see Appendix 1).

This decision does not take into account any updates after **27 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.

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments and referred the draft decision to the Member State Committee.

You did not provide any comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-66 written procedure 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 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.

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.