



PGM Tox Experts and Working group

Minutes, Brussels, 9 October 2019 (9-12)

Chair: Michael Thiel (BASF, Germany)

Co-chair: Arno Buthe (Heraeus, Germany)

ACTION	WHO?	WHEN?
Inform EPMF Secretariat in case colleagues want to be added to PGM TE Mailing list	Companies	
have Rh13 dissolution with increasing DMSO concentrations analysed by CRO	EPMF Secretariat	<Spring 2020 BtB
Finalise Pd PNEC derivation and circulate to members	EPMF Secretariat	Q4 2019
Pd PNEC - Verify safe use and inform EPMF Secretariat if safe use can not be demonstrated	Companies	Q4 2019
Decide on appropriateness of solid PtN for full OECD407/421 assays	EPMF Secretariat and companies	Novemer2019
Share draft protocols in vivo skin sensitisation with TE to ensure proper vehicle selection	EPMF Secretariat and TE	When available
Ship test samples for DPRA assay to Uni Wisconsin	Sample providing companies	ASAP
Decide on appropriateness of test samples, and ship to Charles River	EPMF Secretariat and companies	ASAP after finalisation DPRA assays
Share draft protocols for bioanalytical development work with TE	EPMF Secretariat and TE	After finalisation DPRA assays
Follow IPA mutagenicity testing program with nanoPt	EPMF Secretariat and TE	
Continue inclusion PGM ES in CHESAR as proposed	EPMF Secretariat	Q4 2019
Proceed with datagap-filling and dossier update RhCl3 to Annex VIII dossier	EPMF Secretariat	2020



Run further in vitro muta assay(s) with tris(nitrato-O)nitrosylruthenium	EPMF Secretariat	Q4 2019
Forward nitrosyl muta publications to TE	EPMF Secretariat	Oct 2019
Develop workplan for master thesis at INRS (Canada) and monitor project	EPMF Secretariat	Q4 2019 – Q2 2022
Prepare TSCA notification after draft report AMES test with tris(nitrato-O)nitrosylruthenium	EPMF Secretariat	ASAP after draft report

The minutes summarise the discussions and decisions taken during the meeting, and need to be consulted in parallel with the slides as presented (and made available) to the participants.



1. Welcome and Introduction

- 1.1. Reminder on Confidentiality and Competition Law
- 1.2. Tour de table and apologies
- 1.3. Approval of the agenda
- 1.4. Approval of the minutes of the last meeting (9 October 2018) and status of action points

Michael Thiel welcomed the participants reminding the anti-trust and competition law guidelines.

The meeting started by a tour de table (see list of participants in annex).

The agenda of the meeting has been approved.

The status of the action points of the spring BtB meeting in April 2019 has been summarized:

- Some are ongoing and come back later in the meeting for further discussion/development
- members are requested to inform the EPMF secretariat in case they have colleagues that need addition to the PMG WG/TE mailing list
- it is agreed that the investigation of RhI3 dissolution with increasing DMSO concentration is outsourced to a CRO.

The draft minutes of the spring BtB meeting (1 April 2019) have been approved.

2. Palladium and compounds

- 2.1. Update ongoing testing:
 - RDT/Reprotox assays Pdacac, PdCl₂ and Na₂PdCl₄

The status of the ongoing RDT/Reprotox assays has been summarised:

- Pdacac: the in-life phase of the full OECD422 assay was finalised mid May 2019. Reporting is ongoing and a draft report is expected mid October 2019. In the current Pdacac dossier, Pd(OH)₂ is used as read-across substance (NOAEL 1000 mg/kg/d). The NOAEL in the Pdacac assay will be significantly lower (high dose = 30 mg/kg/d), and will thus trigger a review and lowering of the DNELs in the Pdacac dossier. Consequently, the risk assessment will be reviewed accordingly and trigger more stringent risk management measures (RMM) to ensure safe use.
- PdCl₂: an uncommon palatability effect was observed in the first dose range finding (DRF) study using dietary administration. Therefore, it was agreed to perform a second DRF using gavage administration and a lower dosing regime (high dose 300 mg/kg/d). This DRF will start 14 Oct 2019.
- Na₂PdCl₄: substance stability has been shown using far-IR spectroscopy. The DRF has been finalised and did not show major effect on males/females up to the high dose (10000 ppm test item in diet). The full OECD407/421 assays need inclusion in the LPT workplan.

- outcome review Pd ecotox

The Pd ecotox dataset (company study reports and identified scientific studies (<mid 2019)) has been reviewed by Arche. Fourteen (out of fifteen) company study reports were given a Klimisch 1-2 score, and 2 (out of thirteen) relevant scientific studies were given a Klimisch 1-2 score. The Klimisch 3-4 studies were



not considered in the further assessment. These Klimisch 1-2 reports/publications cover in total 6 different Pd compounds.

Based on currently available scientific and industrial knowledge regarding aquatic Pd speciation, it was concluded that:

- Pd(IV) species are rapidly reduced to Pd(II) when added to aquatic medium
- free Pd(2+) ionic concentration $\ll 10^{-15}$ M
- Pd is mainly available as inorganic Pd complexes in aquatic test media
- ‘simple’ inorganic Pd compounds, Cl-coordinated, NH₃-coordinated and mixed Cl-NH₃-coordinated Pd complexes will ultimately respeciate to [Pd(OH)₂]⁰ complexes. However, it is mentioned that kinetics of this respeciation (especially from NH₃ to OH) might be very slow, and experimental information on this topic is lacking (*cf. agenda point under AOB ‘INRS master thesis’*).
- Pdacac is considered different from all other compounds (organic ligand, low water solubility).

Acute and chronic test data are summarised in the slides. In general, algae are the most sensitive aquatic test species (both acute as chronic), and toxic thresholds for all test items (except Pdacac!) are similar. For Pdacac, the lowest EC10 values is observed with *Daphnia* reproduction.

Considering all evidence in a weight-of-evidence argumentation, the proposal is to create two groups for ERV/PNEC derivation:

- group 1: Palladium and all inorganic Pd substances
- group 2: Pd acac

An indication of PNEC values is given in the slides. For group1, it is possible to take geometric mean values for the algal responses rather than a worst-case value based on a single test (multiple test data available for a single test species + data generated under comparable conditions). The (preliminary) aquatic PNEC values are higher than those currently applied in the environmental risk assessment for Pd.

The participants agree to apply the default assessment factor (AF) of 50 for aquatic endpoints, and not the lower AF=10 because we don’t have chronic fish data to justify this decrease. It was considered not required to perform a chronic fish test to be able to use the AF=10 (this test would require inclusion of a testing proposal in the REACH dossier, and is expensive (long exposure, high amount of test item and analytics)). This position can be reconsidered once the final PNECs are known and the members have the option to check their site specific risk assessments. At this point in time, there are no alternative methods (like QICAR (Quantitative Ion Character-Activity Relationship)-models) available to predict the chronic toxicity to fish.

The testing with Pd acetate (update Annex III->VII) is ongoing. For in vitro skin sensitisation testing, it was concluded that testing was not possible because no stable solution/suspension could be generated in test medium and saline. The third validated solvent for these assays (DMSO) has not been used upon EPMF request. IPA has tried alternative solvents for the ‘modified DPRA’ assay, but these have not been validated in the current OECD protocol and are thus not used. The next step is to test these test items in an in vivo assay. It was mentioned that also for in vivo assays, generation of stable suspensions is critical. Therefore, the TE will be consulted as soon as draft protocols are available to validate vehicle selection.



3. Platinum and compounds

3.1. Update ongoing testing:

-speciation PtN in HCl (195Pt NMR testing)

The extended DRF for platinum dinitrate (PtN) has been finalised. The extension was required to increase the dosing regime up to 10000 ppm (in diet) because of absence of effects up to 3000 ppm (in diet) during the first 14 days of the DRF. The testing is currently on hold due to the substance ID (SID) investigation requested by ECHA. SID clarification has been finalised and accepted by ECHA, the dossier has been updated by the Lead Registrant (LR) and co-registrants have been requested to update their dossier as well.

The ¹⁹⁵Pt NMR speciation analysis of the solid PtN and PtN solutions when dissolved in HCl (pH1.5, 'gastic bioelution'-type of setup) is ongoing. This will help us to decide if the speciation of the solid PtN and PtN solutions upon gastric administration is comparable, and if solid PtN can be used as test item for the full OECD407/421 assays (which are currently on hold). Results of this analysis are expected later in October.

-status update Pt in vivo mutagenicity testing

A final decision has been received from ECHA for Tetraammineplatinum dichloride (deadline for dossier update mid July 2020), a final decision for diammonium hexachloroplatinate is expected <end 2019 and Dipotassium tetrachloroplatinate is expected soon after. The final decisions for HHPA-2AE and platinum dinitrate are expected mid-end 2020.

It was agreed by the TE to perform an in vitro DPRA assay at Uni Wisconsin (Dr Schäfer) to get an indication of sample purity, and more specifically absence of residual chloroplatinate impurities at detectable levels. Although not being completely solid to ensure sample quality for in vivo muta testing (ie, avoidance of false positives), the 'modified DPRA' assay is considered a best alternative. Samples are currently being shipped by the sample providing companies. Once the DPRA assay is finalised and the samples are considered OK for in vivo mutagenicity testing, the companies will be requested to ship all test samples to Charles River (contract lab for in vivo muta testing) for:

1. Simultaneous bioanalytical method development & development/validation for dosing formulations for all 5 test items
2. In-life testing of the test items for which we have received ECHA approval (at this point in time, only tetraammineplatinum dichloride)

The analytical work is challenging and critical, and should be performed with due care. Therefore, the draft protocols will be shared with the TE ahead of experimental initiation.

3.2. Pt metal: need for in vitro muta testing

It is specified in the Pt genotox review by prof Kirkland that:

- there are no genotox test data for Pt metal
- there are indications for genotoxic effects of nanoPt in eukaryotic cells
- there are indications that the effects are related to released Pt ions.

Therefore, it might be advisable to generate (in vitro) test data for Pt metal in order to confirm the (hypothesised) absence of any genotoxic/mutagenic potential i.e. via AMES (eventually read-across from



PtO₂?), in vitro gene mutation and in vitro cytogenicity/micronucleus assays. The cost for the latter two assays is estimated at €35K.

It was agreed by the participants that this additional testing does not need to be initiated now, and that we should:

- wait till we have a better view on the experimental results from the in vivo muta assays within EPMF
- follow the progress of the nanoPt muta testing within IPA. The nanoPt testing is considered a worst-case testing for Pd powders.

Afterwards, the need for in vitro testing with Pt metal can be reconsidered.

3.3. HHPA and HHPA-2AE: Status and update of Pt ES transfer to CHESAR

The PGM exposure scenarios (ES) are currently being included in CHESAR. When including the Pt ES in CHESAR, it was noticed that hazard conclusions in IUCLID didn't always match those of the ES, and that in some cases the DNELs were reported on test item basis in IUCLID and compared to elemental Pt exposure concentrations in the ES. Therefore, it is suggested to re-align IUCLID and the ES, which triggers an increase of the risk characterisation ratio (RCR), although the RCRs will remain below 1 (i.e. safe use will remain). For local dermal exposure/risk, it is proposed to tackle this purely qualitatively (i.e. remove local exposure concentrations expressed in µg/cm²). For systemic dermal exposure/risk, the required risk management measures will be implemented. The participants agree with the proposed way forward.

4. Rhodium and compounds

4.1. Update ongoing testing (annex III -> VII)

The testing for update of Annex III->VII dossiers is ongoing, except for rhodium triacetate where the test item is still pending.

4.2. Scope extension: update RhCl₃hydrate to Annex VIII dossier

Upon request of a registrant, the dossier of RhCl₃hydrate needs updating from Annex VII to VIII. There are some datagaps identified without read-across possibilities (tox to algae, tox to microorganisms, RDT/Repro screening and toxicokinetics assessment). The cost for datagap filling and updating of the dossier (ie, inclusion data in the dossier, DNEL derivation, ES and CSR generation) is estimated at €400K. The work is included in the 2020 workplan and 2020 draft budget. The participants agree with the proposed way-forward and the required budget. Regarding mutagenicity, reference will be made to the existing TP for in vivo mutagenicity included in the rhodium sulphate dossier. It was briefly discussed if the planned in vivo mutagenicity test could be combined with the RDT/Repro screening test that is now required for RhCl₃hydrate, but this was not the case.

5. Ruthenium and compounds

5.1. Update ongoing testing (annex III -> VII)

The testing for update of Annex III->VII for tris(nitrato-O)nitrosylruthenium is ongoing. In the AMES assay, there were clear and concentration related increases in number of revertants observed. The CRO is



currently preparing the draft report. If this information is confirmed, and following ECHA guidance, a positive AMES assay would trigger further mutagenicity testing. It is proposed to test this substance further with an in vivo MN assay, and eventually a 2nd in vitro assay (MLA/hprt assay). The need for an in vitro MLA/hprt assay will be decided when the results of the in vitro MN assay are available. The budget for these 2 additional assays is estimated at €35K, and included in the 2020 draft budget.

There is already some scientific evidence available of genotoxic/mutagenic action of other nitrosyl compounds. This suggests that the observations in the AMES assay might be related to the nitrosyl moiety rather than the ruthenium moiety (note that the in vitro assays with Ru(IV) oxide, RuCl₃ and TetradoRu are all solid negative). The references will be sent to the TE.

ToxTracker can be considered as a next step to investigate further the MoA.

The agreed way forward:

- draft TSCA notification once draft AMES report is available
- perform an in vitro MN assay, and decide afterwards on the need to perform an in vitro MLA/hprt assay and/or ToxTracker
- forward publications on nitrosyl mutagenic action to TE

6. Iridium and compounds

6.1. Update ongoing testing (annex III -> VII)

The testing for update of Annex III->VII dossiers is ongoing

7. Draft budget 2020

The 2020 draft budget has been presented. No remarks have been made by the participants.

Post-meeting note: following the discussion on further in vitro mutagenicity testing with Pt powder, €35K has been removed from the Pt metal budget.

8. AOB, next meeting(s) and closing remarks

Two points have been included under AOB:

-Project proposal Steven Verpaele (Nickel Institute) on occupational monitoring

The project has been presented during the IPA STF meeting earlier. The participants have strong reservations about the scope and realism of the project. Although considered relevant for other metals like Ni and Co, it is agreed to not join this project as EPMF.

-EPMF Secretariat proposal to sponsor master thesis student at INRS (Canada)

Considering the existing uncertainties related to PGM speciation in aquatic media, the kinetics hereof, and the unclarity for PGMs of the generic metals toxicity hypothesis that free metal ions (rather than metal complexes) are responsible for toxic effects, it is proposed to sponsor a Master



Thesis at INRS (Canada). This project would run (roughly) May 2020 – April 2022 under supervision of prof Fortin (INRS) and prof Le Faucheur (Uni Pau, France). Palladium and platinum (both as tetrachloro and tetraammine complex) would be used as test item, and algae would be the test organisms (as most sensitive test species). The cost of this project is approx. €60K for two years. Both supervisors have a good and long experience with PGMs and the analytical capacities of (mainly Uni Pau) are well-recognised. Part of the project will be the publication of the results in peer-reviewed literature to ensure recognition of this work. The participants support this proposal, and the budget has been included in the 2020 draft budget (as presented during the meeting).

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Annex 1: Participants

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