Pt Genetox – meeting prof Kirkland & PMC Tox Experts

Participant list :

Prof David Kirkland (consultant), Steven Verberckmoes (Umicore), Mark Raffray (consultant), Arno Buthe (Heraeus), Nissanka Rajapakse and Mark Hosford (*via phone* - Johnson Matthey), Maxime Eliat and Jelle Mertens (PMC)

A detailed agenda is included in Annex I.

Slides (Annex II) and a Response to comments (RCOM) document (Annex III) are attached.

ACTION LIST

Action	Who?	When?	Status
Create table with properties of cis-platin per genotox/mutagenic assay and compare to the identified PMC Pt groups	prof Kirkland		
Include Pt(0) nano studies in review	prof Kirkland		
Provide Pt(0) nano studies to prof Kirkland	Mark Raffray	w/c 12 September	
Tetraammine Pt compounds & Potassium tetrachloroplatinate in vivo studies: add KL rank and any associated caveat to KL scoring	prof Kirkland	ASAP	
Tetraammine Pt compounds & Potassium tetrachloroplatinate in vivo studies: add KL input from above into REACH dossiers	BIBRA	<30 Sept	
Tetraammine Pt dichloride data gap: Perform mouse lymphoma TK assay with TPC in aqueous vehicle (	PMC	ASAP	
Write (brief) justification for additional mouse lymphoma TK assay with tetraammine Pt dichloride	prof Kirkland	w/c 12 September	done
Ames assay comparator information: include plate concentrations where	prof Kirkland		

available from studies into the review document			
Pt(0) Grouping: (1) differentiate Pt(0)massive vs Pt(0)nano with an associated critique of the relevance of nanoPt publications; (2) Karstedt concentrate to be considered as a separate sub-category	prof Kirkland		
Pt(IV)-anionic grouping: differentiate hexachloroplatinates vs hexahydroxyplatinates	prof Kirkland		
Adjust nomenclature for CAS 68133-90-4 to reference 'Dihydrogen hexahydroxyplatinate, compound with 2-aminoethanol' abbreviated as 'HHPA-/2AE in the review	prof Kirkland		
Potassium hexachloroplatinate is selected test substance for further in vivo testing in Pt(IV) anionic group	Bibra	<30 Sept	
Discussion with PGM WG about Pt nitrate: include a TP for an in vivo test (intragastric administration) vs defer with including TP till in vivo testing program of other Pt groups is finalised and reconsider in vivo testing on this compound afterwards.	PMC members	4 October	
Include combined Micronucleus/Comet assays in TP	Bibra	<30 Sept	
Do not classify any Pt substance for genotoxicity/mutagenicity until the follow-up in vivo testing is performed	Bibra/PMC	<30 Sept	

Summary of the meeting:

!! PLEASE NOTE THE BELOW SUMMARY NEEDS TO BE CONSIDERED TOGETHER WITH THE RCOM DOCUMENT DRAFTED BY PROF KIRKLAND (cfr. Annex III) !!

All participants were welcomed, and reminded of the anti-trust obligations.

The Scope of this genetic toxicology review performed by Prof. Kirkland was confirmed to consist of the following key deliverables:

1. Definition of the genetox profiles for a logical grouping of Pt substances.
2. Identification of any data gaps and recommendations for gap filling (via tests not requiring test proposals).
3. Recommendations for optimal in vivo assays (via test proposals).
4. For benchmark purposes, a comparison of the genetox profiles of platins vs non-platin Pt substances.

In its current form, the resultant review document is only intended for internal use.

Platinum nitrate:

-Known to be a UVCB substance.

-Increasing pH changes the substance's speciation.

-No further in vitro testing for this compound is required.

-Use of Pt nitrate as the representative test substance for the Pt(IV) cationic group was discussed together with waiving possibilities.

-Exposure based waiving for Pt nitrate is unlikely to be defensible.

-Alternatives reference substances within the group are PtO2, Pt sulfate or Pt tetrachloride. Pt sulfate and tetrachloride are not in PMC scope. PtO2 is being registered, but has completely different sameness characteristics to Pt nitrate. PtO2 has no genotox data at all, and is registered as Annex III exempted substance.

-The low pH of Pt nitrate is not a definitive waiver against further in vivo testing (i.e. because of animal welfare reasons). In the event of an attempted wavier, ECHA could take a precautionary view and read-across from another test result for a Pt substance (irrespective of sameness rationale).

STRATEGIES FOR CONSIDERATION by PMC members:

OPTION 1: Submit a TP for an in vivo test with Pt nitrate as test substance, based on intragastric administration rather than standard gavage to address animal welfare considerations. Due to this atypical technique, there may be logistics requirements which would limit CRO options for the study.

OR

OPTION 2: Defer a TP till in vivo testing program of other Pt groups is known, and decide afterward on (need to perform) in vivo testing on this compound.

DMSO:

-Mutagenic quenching potential of DMSO as a vehicle is acknowledged for chloroplatinates ('ClPt'), but is not clear for other Pt compounds.

-One in vitro result is discordant with the hypothesis that DMSO always quenches activity. This is an in vitro MLA assay with a Pt tetraammine compound (see next section).

Pt tetraammines:

-A structure/chemistry postulate may explain why the pattern of activity, e.g. in the Ames assay, differs from that of Pt substances with potential to form DNA adducts. It is argued that as the molecule is cationic, planar and amines groups can form H-bonds with DNA components, this fits with a DNA intercalator profile. Support for this working hypothesis is provided by the clear and consistent TA 1537 strain frameshift response in Ames assay for several tetraammine Pt substances.

-Although negative in vivo studies do exist for certain tetraammine Pt substances, these were either not conducted to current guidelines; or had methodological deficiencies (target tissue exposure not demonstrated; or were non-relevant assays (UDS assay for TPtHC). Hence all the negative outcomes are now considered non-robust, and a new in vivo study (TP submission) was agreed as appropriate.

-To address uncertainties related to an existing study relevant to tetraammine Pt group where DMSO was used as a vehicle, it was proposed to perform an in vitro mouse lymphoma TK assay with Tetraammine Pt dichloride using water as vehicle.

Conclusions:

° in vivo studies: add KL rank and any associated caveat to KL scoring, and include this input into the REACH dossiers.

° Write (brief) justification for additional mouse lymphoma TK assay with tetraammine Pt dichloride.

°perform in vitro mouse lymphoma TK assay with Tetraammine Pt dichloride in aqueous vehicle.

Platins:

-Cisplatin is most potent platin substance of those examined in the review, and has the most complete Pt genetox dataset, as well as carcinogenicity bioassay data (positive in rodents).

-Carboplatin and oxaliplatin have qualitatively similar mutagenic profiles to cisplatin but are typically less active. Transplatin—due to structural reasons – is inactive.

-The chloroplatinate complex salts being registered by PMC are structurally similar compounds, and are mutagenic but have markedly lower potency compared to platins.

Graphical representations of Pt genetox results:

-A simple plot of results for the various Pt groups is difficult to create, as 'potency' can be defined in many ways (eg as LOED, slope of dose-response curve, D20, number of revertants/µg etc.).

-Proposed alternative: prof Kirkland will attempt to assemble a strawman concept using a tabulation of Pt group versus key endpoint datasets. Cisplatin will be included as a reference substance, and its profile will be compared (via a qualitative rather than quantitative approach).

Nano-Pt(0):

-Not in scope of PMC, and not in scope as communicated to prof Kirkland.

-However, it was acknowledged that ECHA continues to maintain a focus on available nano data even when dossier submissions essentially relate to massive forms (Ag and other examples). Furthermore, external publications on the genetox of nanoPt are readily identifiable.

-Data are lacking on the genotoxicity/mutagenicity of Pt(0) metal massive. However, based on inertness, TK assessment etc., no effects are predicted. In strict terms, a data gap exists in relation to this endpoint in the dossier for Pt(0) metal.

-Based on WoE and waiving considerations, the high level decision was that no further Ames or mammalian cell testing was considered to be appropriate (also bearing in mind the potential for artefactual false outcomes with some assays).

Conclusions on the strategy applicable for Pt(0) metal:

-For Pt(0) metal genotox: primarily rely on exposure based waiving in REACH dossier.

-prof Kirkland to critique the nanoPt genetox studies in respect of any relevance to Pt(0) metal massive and include commentary in the Pt genetox review.

-It is not anticipated that there will be any inclusion of Pt(0) nano studies in the study set for the Pt(0) massive REACH dossier, but remarks on non-relevance and differentiation of this dataset may be included to pre-emptively address any ECHA focus.

Chloroplatinates:

-It is difficult to compare the potency of tetrachloroplatinate ('TCP') vs hexachloroplatinate ('HCP'). Reliable comparator assessment is only robust when compounds are tested simultaneously within the same assay.

-There is no conclusive (negative) in vivo dataset available for chloroplatinates.

-According to ECHA guidance: '*Follow up by assay to detect gene mutations required'.*

-An extra Ames test on TCP might be helpful to determine most suitable test substance for in vivo testing.

-Possible test substances for further in vivo testing for HCP-anionic group were considered:

-Hexachloroplatinic acid may be problematic to test due to its low pH and corrosive properties.

-Ammonium hexachloroplatinate demonstrated positive in vitro outcomes, e.g. Bünger et al. study.

-Potassium tetrachloroplatinate:

-Preferred to ammonium salt because of industrial volumes.

-Toxicological profiles K and NH4 salt reasonable expected to be similar.

-Potassium salt has most data available.

Conclusions:

-Include plate concentrations from Ames assays in Pt genetox review (e.g. from Bünger study) wherever possible.

-On balance, no further in vitro testing was recommended.

-Ammonium hexachloroplatinate is the logical reference compound for the hexachloroplatinate-anionic group in vivo testing.

-Potassium tetrachloroplatinate is the appropriate reference substance for the tetrachloroplatinate-anionic group in vivo testing.

Other Pt(IV) anionic group:

-It was agreed that hexahydroxyplatinate compounds should be differentiated as a separate sub-group within the Pt(IV) anionic category: Dihydrogen hexahydroxyplatinate and Dihydrogen hexahydroxyplatinate, compound with 2-aminoethanol

-similar structure to ClPt, but leaving group behavior predicted as different.

-HHPA: at neutral pH has low solubility vs HHPA/2AE higher solubility.

-During the meeting, prof Kirkland verified that for HHPA/2AE, the reaction component of 2 aminoethanol is not itself considered genotoxic/mutagenic.

-In terms of test substance selection, a lower solubility is sometimes better for testing (as this provide an uncomplicated suspension. The pH of HHPA was confirmed as 2.9 and thus is not a limiting factor for animal welfare (the low end threshold for the oral route is normally considered to be pH 2), TK assessment needs to be considered as part of any in vivo study.

Conclusions:

-Differentiate HHPA and HHPA/2AE from Pt(IV) chloroplatinates in the genetox review, and create two different profiles / TP recommendations.

-Standardise the naming of 68133-90-4 as 'Dihydrogen hexahydroxyplatinate, compound with 2-aminoethanol' in the review.

-Prof. Kirkland to make an updated recommendation in respect of use of HHPA or HHPA/2AE as test substance for further in vivo testing.

Oxidative damage DNA damage as a mode of action:

-Difficult to prove this effect as a sole Mode of Action.

-Not advised to do additional efforts in this field, won't change hazard assessment anyway.

-Suggestion is instead to commit funding to appropriate in vivo testing.

Drosophila testing:

-It was recommended that sex-linked recessive lethal (SLRL) mutation test where available be kept in the supporting studies section of dossiers (also taking into consideration KL rank).

Cleve's triammine / Cleve's salt

Cleve's triammine (Johnson publication) vs Cleve's salt (Uno and Morita publication):

-Considered as being different regarding genotoxic/mutagenic profiling due to chemistry considerations.

-Can remain in Pt genetox review, but not relevant to in REACH dossiers.

Klimisch ('KL') scoring:

-Prof Kirkland gave the opinion that KL ranking can be restrictive and misused. It is only relevant when considering negative studies (if a positive study, it is preferable to use instead a narrative to address deficiencies and relevance rather than only KL ranking).

In vivo test strategy; Micronucleus/Comet combined assay vs Transgenic rodent (TGR) assay:

-Per ECHA guidelines, selection for Micronucleus/Comet is principally for chromosomal damage follow-up vs Trangenic mutation assay ('TGR') for gene mutation follow-up.

-ILSI review dataset on 89 chemical compounds comparing predictivity/sensitivity of the Micronucleus/Comet assay:

-For 1 substance, Comet assay was not positive whereas TGR was clearly positive, but this was related to sex-specific effects of the test substance

-Detection of 'false positives': 15 substances were positive in MN/Comet and negative in TGR (for some, negative outcome because of too short exposure period in TGR). From these, 13 were tested for carcinogenic effects and 12 were positive. MN/Comet is considered at least as sensitive as TGR.

- ILSI report expected 1st draft spring 2017, and publication in 2nd half 2017.

-Relative costs were discussed, and acknowledged to be highly dependent on protocol specifics. Ballpark costs were for Micronucleus/Comet assay with TK approx. 60 k£ vs TGR approx. 250 k£. The TGR and associated analysis can also take much longer to complete (up to 9 months effort).

-Suggested capable CROs: Covance, Envigo.

Conclusions:

-Proceed with Micronucleus/Comet selection in all TP.

Classifications for mutagenicity:

-It was confirmed that based solely on in vitro data, there is no requirement to classify Pt substances for genotox/mutagenicity now.

-Even in terms of a best guess call, it is not possible for any prediction at this time as to whether there will be a need to classify (i.e. this will need to await in vivo testing).

Annex I

Agenda

1. *Welcome, tour-de-table and anti-trust* — *9 – 9.05*
2. *PGM chemistry – some introductory remarks* — *9.05 – 9.20*
3. *Discussion on Pt genetox review* — *9.20 – 13.20*
4. *(Lunch)*
5. *Conclusions* — *13.20 – 13.30*

Annex II: meeting slides

Annex III: RCOM document prof Kirkland