Chloroplatinates sub-group & PGM Tox Experts: Discussion on occupational risk assessment in Pt REACH dossiers & Karstedt HLM assay

Chairman: Steven Verberckmoes (Umicore)

Participant list :

Steven Verberckmoes (Chairman, Umicore), Mark Raffray (consultant), Nissanka Rajapakse and Mark Hosford (Johnson Matthey), Olga Duerr and Michael Thiel (BASF), Rudolf Eller, Herbert Fuchs and Reimo Kinder (as from 10.45) (Heraeus), Mike Shepherd (Vale), David Kirkland (consultant; as from 10.45) and Jelle Mertens (PMC)

ACTION LIST

Action	Who?	When?	Status
Input / comments to proposed rewording document Mark Raffray via email to the group	Meeting participants	<5 August	
Check if / where / how Heederick study is referred to in DNEL reports	Jelle	<29 July	
Send participants DNEL info as included in IUCLID / CSR	Jelle	<29 July	
Check timing for next call with EBRC	Jelle	<29 July	
Check if slides are still available with Covance	Jelle via RSA	asap	
Contact LPT for including in vivo MN in OECD422	Jelle via RSA	asap	

1. Discussion on occupational risk assessment in Pt REACH dossiers (10-10.40)

Participants were welcomed.

Brief introduction to explain background for proposal Mark Raffray to re-introduce Benchmark Value ('BV'). The participants confirmed they read the documents.

It was repeated that PMC intends to finalise all Pt dossiers (except HHPA-2AE) at the same point in time (Q1 2017), but that the ClPt dossiers will be registered Q4 2017.

Proposal as drafted by Mark Raffray is not final:

-need to include explanation for including / derivation BV – level of detail needs to be well considered

-input from meeting participants welcomed (deadline 5 August 2016)

-for all uses/scenarios, exposure estimates <= BV (cfr table in Mark's proposal) except 'packaging and filling' scenario that was discussed and agreed as being not supported by PMC. Additional RMM required for safe use (cfr meeting 7 July)

-some exposure data = BV. There is a risk this is challenged in the future, but important note is that BV ≠ D(N/M)EL, and that this is a qualitative assessment (no RCR derived). Care needs to be taken on the language / justification used (reference to ECHA guidance). Considering all other options (cfr slides distributed), BV might be preferred.

The participants support and approve the proposal to include the BV approach in the ClPt Qualitative Occupational assessment.

Next steps:

-collect comments on BV document – Maxime Eliat to forward all PMC input to EBRC

-organise call with ClPt sub-group / PMC / EBRC 24/8 – 2/9

2. Discussion David Kirkland on Karstedt Concentrate HLM test (10.40-11.50)

Ahead of discussion with Kirkland, it was explained that (10.40-10.45)

-a positive in vitro test is not sufficient for classification

-it triggers considering an in vivo testing, which need to be included in the REACH dossier as a testing proposal

-if included as TP in REACH dossier, a decision by ECHA is not expected before second half 2017 (considering registration date and time needed to revise the dossier/TP)

-it may trigger hazard communication by the companies, and affect the public perception

-we aim to limit the amount of TP in the Pt dossiers (cfr aim of Pt genotox review by Kirkland), but Karstedt Concentrate is so far only Pt(0) compound so might not read-across with other Pt compounds

Prof Kirkland joined the call (10.45).
Mark R gave short introduction on the specific uses and chemistry of this compound.

Prof Kirkland explained that

-for biomedical uses, following ICHM7, genotoxic impurities need to be considered based on a positive Ames test (mutagenicity). In the case of Karstedt, the AMES assay has been concluded negative. Therefore this issue does not exist for this substance.

-according to today's OECD guidance three criteria are being used to determine the final conclusion of in vitro genotox tests. The HLM assay (only the 24+0h –S9 treatment!) is a clearly positive test based on the facts that 1) the data are significantly different from the control treatment AND 2) are significantly different from the historical controls AND 3) there is a dose-response relationship

-the potential cytotoxic effect at 0.7 µg/ml does not alter this interpretation, as the previous dose is well below the cytotoxicity range (50-60%) and also positive. If this were 40-50% cytotoxicity and negative, then this would be a different picture

-the heterogeneity of the replicates at 0.5 µg/ml is not unusual, as you exceed the historical control values. Bell-shaped dose-response curves are normal in HLM tests (eg due to cell cycle delay), and might have been more apparent if intermediate doses were tested as well.

-a robust test system has been used (p53 competent human cells), minimizing chances for false positives (in case of steep dose-response as in current testing)

-it is possible to discriminated cytotoxicity, by looking at apoptosis in time course experiments (apoptosis before or after MN induction/genotoxic actions). However, this is difficult to test, expensive, and of long duration. Not sure if regulators look at this testing when reviewing.

-it is possible to derive a threshold if it can be shown that KC induced micronuclei are the result of aneuploidity (change in # chromosomes) instead of clastogenicity (DNA strand breaking) This can be the case if KC is not affecting DNA directly, but for instance proteins (non DNA targets).

There are a few options discussed:

OPTION 1: do nothing and live with the result

-according to prof Kirkland, regulators will consider the HLM assay most likely as positive.

OPTION2: is the experiment reproducible?

-score more cells and see how the results change

-there might be a chance the 0.7 µg/ml treatment becomes clean

-unlikely the 0.5 µg/ml treatment becomes clean due to high value for replicate A

-there is chance that 0.3 µg/ml treatment becomes positive

-if shown to be not reproducible, we move away from 'clear positive', but remain 'equivocal'

-still need for in vivo data to overrule (and they need to be clearly negative!)

-there are no TK data available for KC. In this case, if in vivo outcome is negative (eg in vivo bone marrow assay), there is need to proof KC was systemically available (and reached the bone marrow in this case)

OPTION3: include in vivo endpoint in RDT testing

-in OECD guidance, it is 'encouraged to integrate MN in RDT studies if possible'

-include in OECD422 in vivo MN in blood erythrocytes via flow cytometry (at various times during 28d exposure). Testing proposal is not anticipated, as not additional animals are required (<-> stand-alone in vivo MN study). Taking blood samples does not affect other endpoints, and has no effect on normal homeostasis of blood marrow.

-if CRO has no experience and transport of samples is required, care should be taken to do this appropriately to ensure sample quality! (good and upfront communication CROs)

-target cells = young reticulocytes (CD71 as marker to be used in flow cytometry)

-pre-dosing sample per testing animal needs to be included

-required to measure blood plasma Pt levels (can be considered to omit in case toxic effects are observed in tissues due to systemic KC exposure.

-required to include 1 or 2 positive control groups for clastogenic and aneugenic effects - check with lab if they have experience and ask for historical control data. Positive control group only needs to be exposed last 2-3d of the 28d assay.

OPTION 4: additional FISH analysis of HLM slides

-this will also allow to check for aneuploidity events as the basis of the observed MN

-check with CRO if cell pellets are still available, and check chromosome damage using FISH

-done before within PMC for TCA

-quick and cheap option

Participant support options 3 and 4, and the PMC secretariat will check with

-LPT if the in vivo MN in blood erythrocytes via flow cytometry can be performed (in house or subcontracted)

-Covance still has the slides available, and if FISH can be performed

The group will be kept informed if further information is received.