Conference call on genotoxicity study programme of TCA
Observation of biologically and statistically significant induction in micronuclei

Participants:

Caroline Braibant (EPMF)
Mark Raffray (Johnson Matthey)
Steven Verberckmoes (Umicore)
Paul Whitehead (WCA)

Table 1. Actions agreed at the 12 Jul 2012 Au WG toxicology experts conference call (timing for all actions is ASAP or in due course)

	What?	**Who?**
1.	Cancel Aug 2012 planned HPRT/MLA study, share tentative MoGA and proposal to proceed with FISH/associated timescale study with Covance for discussion	PW
2.	Launch FISH study	PW+CB
3.	Contact David Kirkland to arrange timeslot for discussions when FISH timescale is known	PW
4.	Consider need for *ad hoc* literature search on Au I and Au III genotoxicity	PW
5.	Prepare summary of TCA dataset and read-across reference substance status and present to David Kirkland for assessment/advice	PW
6.	Decide on best *in vivo* assessment avenue/path	Au WG
7.	Re-evaluate read-across strategy from TCA to other Au compounds, from Au III to Au I more particularly	Au WG

Questions:

1. Comments on draft study results/report? Comments on the increased micronuclei induction? Comments pH, osmolality or protocol adherence? Comments on quoted control values being outside historical control range?
    - *In vitro* micronucleus assay in lymphocytes 3h and 24h exposure
    - Results (unlikely to change even following review from Study Director) show biologically and statistically significant induction in micronuclei at treatment levels up to precipitation (not corresponding to level of cytotoxicity 55%+) in 24h assay in the absence of S9
    - One control replicate of 3h –S9 treatment showed the presence of micronuclei just outside the historical control range , Study Director intends to analyse extra control replicates
    - Not influence of pH or pH regulation/neutralisation, osmolality, etc.
    - Even if not same potency as positive control material (vincristine), valid and acceptable result
    - Next step was mammalian cell HPRT gene mutation/MLA study starting end Aug 2012 if Ames and micronucleus were negative – now cancelled

2. TCA as *in vitro* clastogen/aneugen / Mechanism of genotoxic action (MoGA) of Au (III): complex Au compounds/salts inhibition of various key enzyme systems + thiol-reactive substances act via non-genotoxic mechanisms centred around interference with Topoisomerase II, which regulate the overwinding or underwinding of DNA (e.g. Se) (non-threshold effect)
    - Paul reviewed some of the articles recommended by Mark
    - Still need to find out actual effect, via e.g. FISH study (see below)
    - From a risk assessment viewpoint though, knowing whether effect is a clastogen or aneugen one will unlikely trigger any significant difference
    - Agreed to try and find out more about the potential MoGA of Au (III) with Covance and David Kirkland

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3. FISH study needed? FISH (fluorescence *in situ* hybridization) cytogenetic technique developed to detect and localize the presence or absence of specific DNA sequences on chromosomes
    - Aim is to discriminate between clastogen or aneugen effects
    - Supported, as it is expected/recommended by several authorities, and allows using existing momentum/dynamics available at Covance to progress science on TCA/Au III compounds
    - Cost = ~ 4000 £
    - Timescale: Paul to find out

4. *In vivo* micronucleus study in reticulocytes needed at all/to further elucidate MoGA, i.e. clastogenicity (chromosome damage) or aneuploidy (changes in chromosomal numbers)?
    - Likely to be needed, obtain feed-back from David Kirkland, as soon as available, based on WCA report summarising situation, and after the FISH analysis
    - Speculatively same result expected in *in vivo* study; give careful thought on tissue reactivity if TCA (extreme pH) has to be administrated to a test system + fact that it is a read-across reference substance
    - For information: Co case did not perform FISH and even if *in vitro* studies were positive, *in vivo* ones were negative
    - Consequence if study is negative: no classification; consequence if positive: classification as mutagenic category 2

5. Overall registration approach/timeline – further considerations
    - Timescale for resolution of genotoxicity issues is not critical since it is likely that an OECD 422 (repeat dose/reproscreen) study will be required, which is likely to influence the overall timescale for registration
    - Read-across from TCA → need to re-evaluate once full dataset becomes available
    - Read-across from Au III to Au I?

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