European Precious Metals Federation

WELCOME TO EUROPEAN PRECIOUS METALS FEDERATION

Logistics

Time:	09:00 – 14:00
Date:	Wednesday 28th August
Attendees:	**European Precious Metals Federation (EPMF)** Katrien Arijs (ARCHE) – Project Facilitator for EPMF (KA) Lindsay Aveyard (GPC Consulting) – Study Monitor Reprotox testing (LA) Mark Raffray (Consultant) – Study Monitor TK testing (MR) Nissanka Rajapakse (Johnson Matthey) – EPMF Ag Tox Expert (NR) Steven Verberckmoes (Umicore) – EPMF Ag Tox Expert (SV) **Covance** Hannah Izod – Business Development Director, CP&C (Benelux & Scan) (HI) David Myers – Senior Reproductive Toxicologist (DM) – 30 years experience in reprotox; started at Huntingdon Jason Shearer– Senior Study Director, Toxicology Operations (JS) – 10 months at Covance, previously at Charles River for 16 years; SD for palatability study Lynne Taylor – Scientific Manager, Metabolism (LT) (Huntingdon) – responsible for TK testing Derek Angus – Scientific Manager TK, Metabolism (DA) (Huntingdon) Simon Cadden – Senior Study Manager, Metabolism (SC) (Huntingdon); SD for TK study Ravneet Lidher – Customer Success Manager (RL) **Covance Via WebEx** Darran White – Laboratory Manager (Shardlow) (DW) Glenn Williams – Group Leader, Residue Analytical Service (Shardlow) (GW) James Munday – Science Lead, Immunology & Immunotoxicology (JM) Lorraine Edwards – Operations Manager, BBC (LE)
Meeting Location	Eye Research Centre *Barric Lane, Occold, Eye IP23 7PX*
Meeting purpose	To discuss the following project – including analytical support; • OECD 417 – Comparative In Vivo TK study in the Rat • Palatability study in the Rats • OECD 443 Preliminary Reproductive Performance Study in the Rat by Dietary Administration • OECD 443 Reproductive Performance Study in the Rat by Dietary Administration (Cohorts 1,2 & 3)

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Agenda – Wednesday 28th August

Item	Time	Agenda Item	Covance Representative
1	09:00 – 09:30	Arrival, welcome drinks & introductions Including brief introduction to EPMF and Ag reprotox and introduction Covance study directors and their previous experience with the EOGRTS (reprotox study director) and TK testing for metals (TK study director) Cf. slides 3-12 for introduction to EPMF and Ag reprotox. Jason Shearer will be the study director for the palatability study and Simon Cadden the study director for the TK testing. The study director for the prelim study and the EOGRTS is still to be assigned; Covance assured EPMF this will be somebody used to coordinating big reprotox studies and with experience with DNT & DIT. There are 12 reprotox study directors in Covance; 9 in Eye. Currently Covance has performed 6 in life EOGRTS spread over 4 study directors.	HI, DM, JS, LT, DA, SC & RL
2	09:30 – 11:00	Focused project discussions Darran White & Glenn Williams to join at **09:30** for the ICP-MS support Lorraine Edwards, James Munday and Caroline Cadwallader to join at **10:30** for the Ceruloplasmin (Cp) and Glutathione peroxidase (GPX) in serum	HI, DM, JS, LT, DA, SC & RL DW & GW via WebEx LE, JM & CC via WebEx
3	11:00 – 12:00	Tour of animal facility, necropsy & histology departments	HI & TBC
4	12:00 – 12:45	Lunch – table to be reserved in Covance restaurant	HI, DM, JS, LT, DA, SC & RL
4	12:45 – 14:00	Continued project discussions	HI, DM, JS, LT, DA, SC & RL
5	14:00	Meeting close	HI, DM, JS, LT, DA, SC & RL

To be completed during and post meeting

Open Actions

No	Action/Discussion	Status or comment
General		
1	Covance animal welfare policy and procedures in place to avoid prolonged and avoidable animal suffering Animal welfare policy overview provided by DM. Covance is licensed and governed by the UK home office with DM being the project licence holder at Eye. The site complies with all regulatory wishes. However there are some exceptions e.g. caging sizes for dams and their pups with agreement of a formal letter from the Home Office. There is an extensive Code of practice all employees have to comply with. E.g. there is a list of clinical signs to recognize severe effects and in case an animal shows severe effects during testing (e.g. max bw loss of 20% with no other effects, 15% if other effects of concern are identified), it will be euthanized immediately. No changes are anticipated because of Covance takeover. *Actions:* ***AP1****: Covance to provide further documentation/links on animal welfare policy, share Code of practice.* ***AP2****: Covance to check with QA when/by who the site was last inspected and inform EPMF.*	
2	Study schedule (p.5 quotation) and estimated final reporting (cf. regulatory timeline); study site locations The TK study will be located at the Huntingdon site since this is where the TK team/experience is. The ICP-MS method development and validation is currently scheduled to start early October. Other than that, study schedules are currently as described in the final quotation. However based on the discussions there are to be some changes to the study designs and we will need to reassess these schedules. Full details will be included in the revised quotation. The OECD 443 studies will be run at Eye and the TK study at Huntingdon. ***AP3****: EPMF to provide AgAc test sample ASAP for ICP-MS method development.*	
3	Study design considerations (p.6 quotation): adjusting dietary inclusion levels - Need to ensure that palatability study has covered the range of dietary inclusion levels that we could anticipate needing to reach in the DRF and OECD 443 (cf. drop of diet intake in the previously performed EPMF AgAc dietary study – cf. slide 14) - What is the evolution of the food consumption observed over the study course in previously performed Covance DRF / EOGRTS studies? (are	

	there timepoints during these studies where the animals eat more/less, e.g. lactation?) - Does Covance have experience with dietary admin for the EOGRTS? Cf. slides 24-26 for Covance's food consumption data of: • F0 rats before pairing • F0 rats during gestation: slight increase in food intake during d14-19 as the fetuses start to grow • F0 rats during lactation: higher consumption than during late gestation and steady rise as physiological demand increases • F1 rats: ○ food consumption increase over first weeks but then food consumption plateaus together with bw plateau (it should not go down as observed in the EPMF study) ○ absolute consumption is lower than in parents but there is higher intake per unit bw so much higher achieved intake of test substance at start of F1 generation compared with parents. Covance's standard is to work on a ppm / fixed concentration of test material in the diet throughout the study (so the mg/kg/day values are target dose levels, and the concentration in the diet is estimated by predicting bw and food intake). Whilst this might be appropriate for studies which have a short pre-mating period, since we are going for the full 10 weeks in the EOGRTS, it would mean that the F0 animals are likely to receive lower actual dosages towards the end of the 10 weeks as during this time their bw may double (especially on the breeder diet with high protein and fat content) but their food intake (and hence the amount of compound consumed) increases by a much smaller proportion. On the other hand, F0 animals during lactation and F1 animals when they begin to eat the diet (generally accepted at d16 post-partum but DM indicated that recent data suggest it may be at least 2d later) are likely to be overdosed. The way to counter this is to adjust the concentration in the diet. This is generally done weekly, based on predicted bw and food intake for the following week. The predictions are not always 100% accurate (especially if there are effects of the test material on either food intake or bw) but they maintain dose levels far closer to target levels than ppm concentrations achieve. Adjusting dietary inclusion levels during the study is possible but very difficult logistically (Covance has done it previously for a drinking water study) and will have serious cost implications. Some customers request to control food consumption during lactation and first weeks, in order to avoid overdosing. Because mating may take place over the 2 week period, it is difficult to predict female bw, and they are more variable (i.e. more than one concentration of diet might be required), and it becomes even more complex when females litter and their bw goes down but their food intake steadily increases over the lactation period. It is suggested to adjust dietary inclusion concentrations every 2 weeks only up until the last premating week, then fix the concentrations throughout	

	gestation and lactation (so the dose is based on the female's 'base' bw and food intake, not the fluctuations observed over pregnancy/littering and any food intake of the pups when they begin to eat the diet). Dietary concentration then are adjusted again at the beginning of the F1 generation, for the selected pups which will have different bw and intake – but there might be some overlap here with the diets for the adult animals. For the prelim, it is suggested to commence control animals 2 weeks before the treated animals so as to accurately predict the food intake but it is agreed that this will not be necessary as estimates of bw/food intake of the animals can be taken from HCD from previous 421/422 studies with very similar design. Also, if we decide to go to a 4 week pre-mating dosing period on the prelim (cf. also point 9), then there is a longer period and more data available for dose adjustment. For the palatability study, adjusting diet concentrations might interfere with assessing palatability, so it is agreed to have a single concentration for the entire 14 days (based on the d7 predicted bw / intake) for a truer picture of palatability / tolerability over the whole 14 days. During previous EPMF study, diet stability was not an issue but binding of Ag to proteins could be an issue. ***AP4**: Covance to provide cost implications for suggested adjustments of dietary inclusion levels (i.e. adjust every 2 weeks up until the last premating week, then fix the concentrations throughout gestation and lactation, adjust again at the beginning of the F1 generation).* ***AP5**: EPMF to decide about suggested adjustments of dietary inclusion levels.*	
	Impact of Brexit, e.g. on availability of supplies? There is a Brexit Task Force in Covance. Covance doesn't foresee any issues for the testing but advises to send test samples before end Oct in order to avoid possible problems with Customs.	
OECD 417 Comparative TK study (CC71MP) (p.10-11 quotation)		
4	p.10: GLP compliance: all raw data recorded in a GLP compliant way but no QA audit of the report/data Cf. discussion under point 21.	
5	p.10: Age of rats in TK study Ideally, this should be the same as immediately pre-mating on EOGRTS, i.e. 15 weeks. It is suggested to order 10-12 weeks animals and have 3 weeks acclimatization. However, as this is older than normal, this will have cost / timing implications.	

	***AP6**: Covance to provide cost / timing implication to use 15 week old rats for TK study.*	
6	p.11: Formulation analysis for gavage / i.v. dose preparations ***AP7**: Covance to provide cost implication for formulation analysis for gavage / i.v. dose preparations.*	
7	Possibility for pilot work to support the i.v. segment for bulk Ag i.v. admin of micronized powder should be feasible but pilot work is anticipated. Cf. also notes break-out discussion below.	
Palatability study (GH28GX) (p.12-13 quotation)		
8	If no prior experience with measurement of Cu / Se / ceruloplasmin and Se enzymes in serum, suggestion to use the palatability study samples to test procedure Should be possible to put these samples into method development confirmation.	
OECD 443 Prelim reprotox study (YC23RN) (p.14-17 quotation)		
9	p.14: 2 weeks or 4 weeks treatment before pairing? (cf. consideration 10 weeks treatment before pairing in the EOGRTS. 2 weeks of treatment before pairing is Covance's standard approach and has proven a very good design over the years to help choose suitable dose levels for the EOGRTS. However, it can only detect functional effects upon mating performance and fertility due mainly to effects in the epididymis in contrast to the 4 weeks of treatment which can also detect functional effects due to late stage effects in the testes, so the 4 weeks is a slightly more robust evaluation) The 4 weeks pre-pairing treatment option was suggested by DM following EPMF's question whether the 10 weeks pre-pairing treatment in the EOGRTS was taken into account for the prelim study. Since fertility is flagged as a possible issue, DM recommends 4 weeks as this is more robust and will give more information on how to adjust for the main EOGRTS. Covance to check what was costed; EPMF then to decide. ***AP8**: Covance to check if the 2 or 4 weeks treatment was costed.* ***AP9**: EPMF to decide about 2 or 4 weeks treatment.*	
10	Diet should be checked for adequate Cu content prior to use; is this on analytical datasheet provided by the supplier? What diet is used? The SDS VRF1 diet will be used, which is supplemented with several amino acids, vitamins and minerals, incl. Cu (cf. http://www.sdsdiets.com/pdfs/VRF1-P.pdf for analytical datasheet). This is a breeder diet which has a higher protein and fat content than a maintenance diet used on repeat dose studies. Its performance is superior to other breeding diets, incl. Teklad.	

11	p.16: Exposure assessment blood sampling: - F0 animals: 3 males and 3 females: should be same 3 animals at each timepoint - Covance's previous experience with bleeding F0 animals during this study: max. blood volume to be taken from females on d17 of gestation is 1 mL - Is day 17 as late as can be done (Guideline states 'late pregnancy')? The effect on Cu homeostasis is expected to occur during early gestation (Shavlovski et al. 1995). It is therefore suggested to sample half of the rats on d6 and half of the rats on d17. Statistical reliability of results will depend on variability of method. - Sampling schedule: cf. Excel file. Covance is requested to double check the amount of samples, especially for the F1 culled animals. - Strategy behind sampling of both F1 selected termination animals and also culled F1 animals (as distinct from only former)? There are roughly 4-5 culled pups per litter; strategy is related to balance number of samples. Since F1 culled offspring blood samples will be obtained (currently planned analytes: Ag/Cu/Cp), it might be sensible to also assess Se and a functional Se marker (GSH-Px). Whilst information exists for adult animals treated with Ag (including the EPMF biome work), essentially no data is available for offspring. It is noted that Cu and Cp measurements (in culled offspring and selected offspring) should be prioritized over Se and Se marker enzyme measurements. - *Note: need to await sample volume required for bioanalysis to decide whether or not we can achieve all samples currently required (other option: add more animals to study)* ***AP10**: Covance to update proposal with above suggestions.* ***AP11**: Covance to review sample schedule and confirm numbers are correct.*	
12	p.16: F1 animals observations: bodyweight needs to be measured at least twice weekly to coincide with the twice weekly food, and should be extended to cover the selected offspring to week 7 ***AP12**: Covance to update proposal for F1 animals bw observations.*	
13	p.17: Organ weights and tissue retention: suggestion to include reproductive organ weights ***AP13**: Covance to update proposal to include reproductive organ weights for F0 and F1 selected offspring.*	
14	p.17: Bioanalysis of organs/pups: since DNT cohort has to be included in EOGRTS design, suggestion to measure brain Ag for 1 culled pup/sex/litter and 1 F1 animal/sex/group at termination The current Covance proposal has TK on pups limited to whole animal homogenate. EPMF had previously discussed the option of examining Ag CNS distribution in pups if there were sufficient drivers to justify trying to get	

	that additional information. Now that the PfA proposal for DNT inclusion is accepted, i.e. a potential concern is acknowledged, it is suggested to measure Ag in brains in the prelim study. ***AP14**: Covance to provide costing to measure Ag in brain for 10+10 and 12+12. Brain and blood Ag to be measured in same pup. Covance to assess what is possible based on available pups.*	
15	p.17: Formulation analysis: suggestion to consider at least homogeneity check / achieved concentrations of diets ***AP15**: Covance to include formulation analysis once at start F0, once at start F1.*	
OECD 443 EOGRTS (YQ21BX) (p.18-26 quotation) *Note: design may need to be further refined according to outcome of the prelim study*		
16	Thyroid hormone analysis: - T3 would be useful to get a complete assessment of thyroid hormones if HCD are available for this age group? And TSH, based on ILSI_HESI recommendations - T4 analysis day 4 culls (optional): how much HCD are available for this age group? ***AP16**: Covance to review current available T3, TSH and T4 HCD for OECD 443 and advise if there is sufficient HCD to support.*	
17	Spleen cell immunophenotyping: what recent HCD is available? HCD is available; DM never saw effects so far. ***AP17**: Covance to review available spleen cell immunophenotyping HCD.*	
18	Seminology should be included for all P & F1 cohort 1A males. What equipment is used for this and who will be responsible? The person who pioneered this work at Eye more than 20 years ago, and had the most experience, retired last year but DM assured us that the 3 personnel who now do this were all trained by her and had good experience. The equipment is the Hamilton Thorne IVOS CASA (computer assisted sperm analysis), which is industry standard for motility and sperm counts. https://www.hamiltonthorne.com/index.php/sperm-analysis-systems/ivos Morphology is assessed by light microscopy – which is probably better than letting the machine do it as the algorithm for what is abnormal is complex and more prone to error.	
19	Formulation analysis: suggestion to add last pre-mating week ***AP18**: Covance to update proposal and add last pre-mating week for formulation analysis.*	

20	Who will conduct the neuropathology assessments and what is their experience with OECD 443? Covance's Associated Director of Pathology Ian Taylor will perform the neuropathology assessments. This will be peer reviewed by the Director of Pathology Vasanthi Mowat. *Post-meeting note: EPMF should carefully consider the technical approach (described in outline only at the meeting) relating to the morphometrics / neuropath procedures. This relates to previous TE discussions about the neurotox controversies around Ag, e.g. the conflicting information regarding hippocampal effects. We may need more information than is given in a standard Covance protocol. Externals will focus on this aspect in future. However, it may not be advisable to discuss Ag specifics with the Covance pathology team – they should be reading slides without the possibility of preconceptions.*	
Analytical support (p.2 quotation)		
21	ICP-MS analysis: for Ag, method for measurement in blood and tissues used in previously performed EPMF AgAc dietary study has been provided to Covance and method development cost (for Ag in the blood and Ag in the brain) has been reduced. For TK study, measurement of Ag in different tissues is needed; validation costs? Cf. slide 16 for updated costs for measurement Ag in blood and in brain. In the TK study, measurement of Ag in 7 tissues is requested (cf. 6 tissues on p.11 of quotation + uterus). If TK study is to be performed according to GLP, validation would still need to be performed in the different matrices, including accuracy, precision and non-analyte interference so will still involve a considerable amount of work to microwave digest each matrix to allow quantification. This would mean longer lead-in time for TK study + impact on availability of draft report. It is noted that in general, TK studies are rarely performed under GLP. The previous Covance TK study on Ag nitrate (performed at Covance Harrogate for ESTF) was performed under GLP. The current Ag TK dataset is fragmentary and its reliability is questionable. EPMF believes a definitive GLP compliant comparative TK study will facilitate its regulatory acceptance. In order to reduce impact on timing / costs, Covance suggests to do the blood analysis GLP compliant but the tissue analysis non-GLP compliant. ***AP19**: Covance to provide cost + timing implication for A) TK study with GLP compliant blood analysis but non-GLP compliant tissue analysis and B) fully GLP compliant TK study.* ***AP20**: EPMF to decide on GLP compliance TK study based on above.*	
22	For the analysis of Ceruloplasmin (Cp) and Glutathione peroxidase (GPX) in serum, commercial kits are available so method development costs are less applicable. Update of costs?	

	GPx enzyme(s) kit: objective is a functional assessment, i.e. effect of Ag treatment on activity. Hence an ELISA kit which just quantitates GPx protein presence will not be suitable. Abcam produce a kit which has been validated in rats for GSH-Px activity (colorimetric endpoint); other similar kits are available (www.abcam.com/glutathione-peroxidase-assay-kit-colorimetric-ab102530.html). As a final check, EPMF side may need to confirm that serum is the optimal blood compartment (as opposed to RBC fraction for instance). Timing: validation will run in parallel with the palatability study but not with the prelim study. It is difficult to give definitive costs on method development until the kits are tested. Currently costing is conservative. ***AP21**: Covance to provide further breakdown of method development costs.* Prelim study will be performed non-GLP. What is needed in terms of validation for biomarker assays to make them fit for purpose? This would reduce costs. Full validation will only be needed if parameters are included in the actual EOGRTS (which will be GLP compliant). However, DM notes that it is practically impossible to add on additional measurements to the EOGRTS as this would mean guideline deviations. Therefore, any add-ons should be done on the prelim study. ***AP22**: Covance to provide costing scenarios for phased validation for Cp, GPX, Cu and Se measurements.*	

Notes of breakout discussion – TK study segment (LT, DA, SC, MR)

Discussions were based on the outline plan detailed on p.10-11 of the Covance quotation (ver. 6). The high-level design for the repeat dose 28-day segment was considered to be appropriate as currently presented. Covance queried why group sizes of 5m+5f had been requested. It was proposed that reduction to 4m+4f would be sufficient, e.g. accounting for statistical power, potential loss of animals, inter-individual variability etc. This would still be in alignment with TG 417. The same amendment was also proposed for the single dose segment. Due to known gender differences in Ag TK, male and female sub-groups will be required.

***AP23**: EPMF to decide if 4m+4f design would be acceptable.*

For the single dose (p.o./i.v.) segment (Groups 14-39), MR suggested reduction of the proposed 3 treated group approach to 2 treated groups per test article per route. This should still meet the need of providing key comparative TK information, including bioavailability (F value) whilst accounting for the possibility of non-linear kinetics according to dose. It would obviously also be sparing of animals and cost/complexity. Oral dose levels could be aligned with the repeat dose segment/pre-443 study (avoiding dose levels likely to induce toxicity or be problematic for i.v. formulation). A detailed discussion took place regarding dose level setting

and selection of suitable vehicles (p.o. and i.v.) based on available Ag TK data which is considered to be robust. This included a Covance TK study on Ag nitrate. Covance suggested that it might be possible to reduce to 1 treated group per test article per route if there were no doubts about TK linearity. Covance confirmed that blood volumes required for the timecourse sampling were feasible. Timepoints suggested by Covance for the timecourse experiments broadly appear to be appropriate but may be revised slightly after further review of Ag TK data. It is possible that vehicle control group plans may need revision dependent on final selections of dosing vehicle. In view of the pending design changes, test article quantity requirements were not discussed at this stage, but these should be followed up as soon as possible (particularly for AgNP test article).

***AP24**: EPMF to provide key TK papers and Raffray Biosci TK survey report.*
***AP25**: Covance to assess data pack, including the Ag nitrate study. Consider existing data re main TK parameters, linearity of kinetics etc. Revise study outline proposal accordingly.*
***AP26**: MR to provide Covance with suggestions regarding sighting dose levels and potential dosing vehicles.*
***AP27**: EPMF to consider if the pared down design for TK program is acceptable.*

As anticipated, i.v. formulations of bulk Ag (micron-size) and AgNP represent challenges, e.g. Covance confirmed that micron-size suspensions were rarely used in TK work, and production of stable suspensions will require feasibility work. Covance referenced fallback alternatives if conventional i.v. administration was infeasible, such as infusion or even i.p. route. Preliminary work is dependent on confirmation of test articles, their availability, and required test article data (CoA, characterisation, SDS etc.). The timeline for test article availability will determine TK study start timing, also taking into account the necessary initial preliminary development work, e.g. on formulation.

***AP28**: EPMF to finalise test article logistics and delivery timeplan.*
***AP29**: Covance to confirm to KA what test article information inventory is required.*