



European Precious Metals
Federation

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Silver Tox Experts Call

18 June 2019

Confidentiality reminder

DO	DON'T
<u>Application of competition law</u>	
Art. 101 and 102 TFEU may be applicable to the conclusion of any preliminary agreement and activities of any preliminary phase.	Don't assume that conflicts with competition law are excluded simply by the fact that the Agreement complies with the provisions of the REACH Regulation.
<u>Consultation in Matters of Competition Law</u>	
Consult an in-house legal expert or the compliance officer of your company or an external lawyer whenever there are uncertainties respecting compliance with competition law. Stop all meetings/discussions which are not in compliance with these Compliance Guidelines until a legal expert has been involved.	Don't assume that these Compliance Guidelines deal with all competition law issues exhaustively. Basically, compliance with Art. 101 and 102 TFEU can be determined only on the basis of market impact in each individual case. These Compliance Guidelines may therefore be regarded only as a means of providing general conduct recommendations.
<u>Activities in any preliminary phase and at any other stage of operation of the Consortium</u>	
Restrict cooperation within the scope of the preliminary phase to the initially defined goals and purposes of the cooperation.	Pursuant to Art. 101 and 102 TFEU, activities which have the object of the effect of preventing, restricting and/or distorting competition are prohibited within the scope of this Agreement, including: <ul style="list-style-type: none"> - Coming to agreement, including arrangements or collusions, about prices, markets and customers (see Art. 101 paragraph 1 a)-e) TFEU); - Joint boycotting of other companies; - The unjustified unequal treatment of trade partners; - The abusive exploitation of a dominating market position.
<u>Exchange of Confidential Information</u>	
Involve a Trustee for the exchange of Confidential Information.	The exchange of information concerning market behaviour and having the object or the effect of preventing, restricting and/or distorting competition is inadmissible; in particular, this relates to: <ul style="list-style-type: none"> - Production capacities; - Productions or sales volumes; - Import volumes; - Market shares; - Price policy; - Distribution and marketing terms; - Marketing strategies; - Information regarding the relationship with suppliers.
<u>Documentation on Cooperation</u>	
Keep minutes of all meetings which detail the subject of the meeting. In case of uncertainty, have the contents of the minutes reviewed by an external legal expert prior to sending them to all parties of the Agreement. Stop all meetings which are not in compliance with these Guidelines until a legal expert has been involved.	

List of participants

- Katrien ARIJS (EPMF)
- Arno BUTHE (Heraeus)
- Roland BRASCH (Heraeus)
- Eliot DEAG (JM)
- Olga LEMKE (BASF)
- Jelle MERTENS (EPMF)
- Mark RAFFRAY (Consultant)
- Nissanka RAJAPAKSE (JM)



Agenda

- Minutes and actions from 23 April meeting
- Process and timing EOGRTS TP
- Selection CRO for TK / DRF / EOGRTS testing
- Ag DRF / TK testing
 - DRF test design
 - TK test design
- AOB

FOR APPROVAL



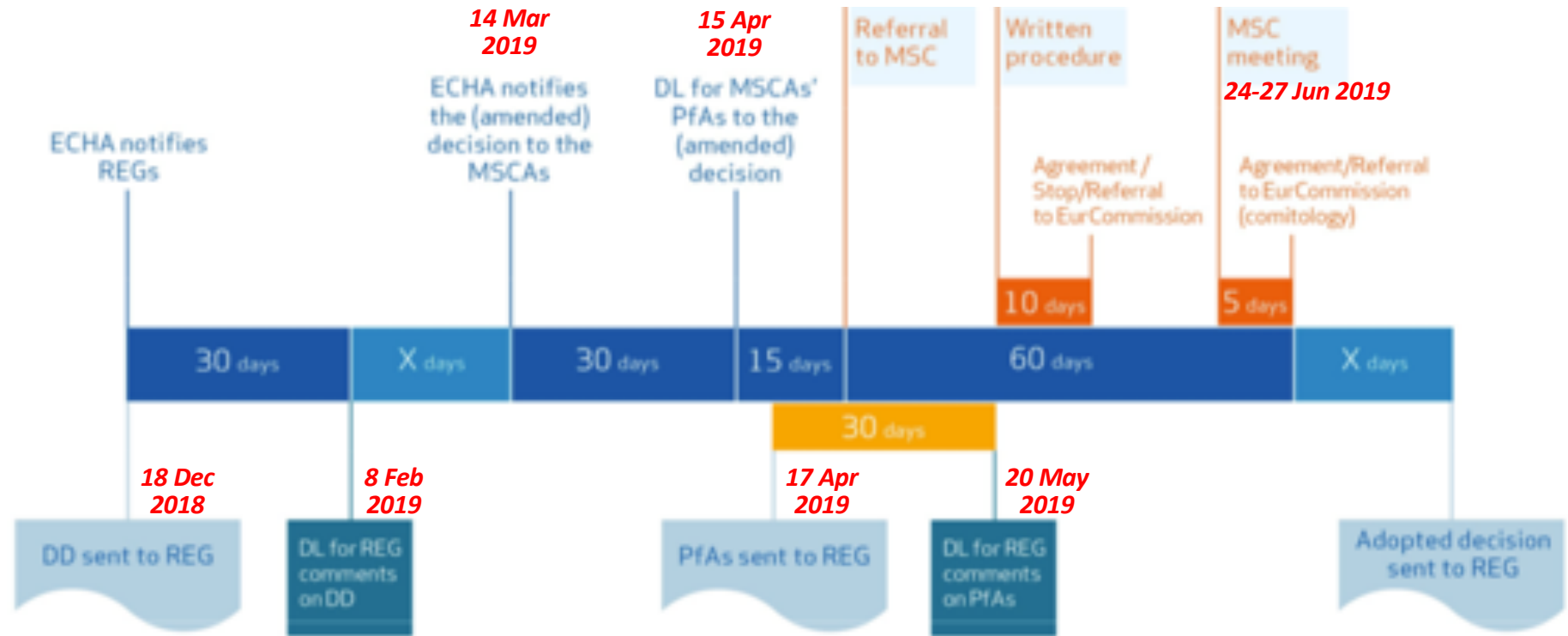
Approval of the minutes of the last meeting (23 Apr 2019) and status of action points

What?	Who?	Status
Ag EOGRTS TP - PfAs		
1	Further investigate available datasets Ag neurotox in response to PfAs from the Netherlands	M. Raffray DONE
2	Check with testing labs if we need to account for extra (testing/reporting) time if DNT cohort added to EOGRTS design	EPMF Sec DONE
AgAc gut biome study		
3	Follow-up with prof. Lison: <ul style="list-style-type: none"> • Ag localisation in brain, testis and kidney with AMG; • Histopath uterus and ovaries samples (via prof. Marbaix); • Offer for estrogen analysis. 	EPMF Sec ONGOING
Ag DRF/TK testing		
4	Request offers TK and DRF testing from CROs, taking into account suggestions from TE	EPMF Sec DONE
5	Provide offer for TK study monitoring	M. Raffray ONGOING
6	Check particle size, purity of elemental Ag forms in REACH dossier + ensure sample analysis before testing	EPMF Sec ONGOING
7	Agree on: <ul style="list-style-type: none"> • Time points TK sampling for TK and DRF testing; • Need for satellite TK group during TK testing; • Fasting of animals before sampling; • Dose levels for each substance for TK testing; • Nr of animals for TK testing; • GLP status of studies; • Selection test items; • Participation BASF metabolomics project. 	Ag TE ONGOING
8	Send some further background / publications on BASF metabolomics project + check if there are data in the database from nano metal forms	O. Lemke ONGOING

APPROVAL OF DRAFT MINUTES



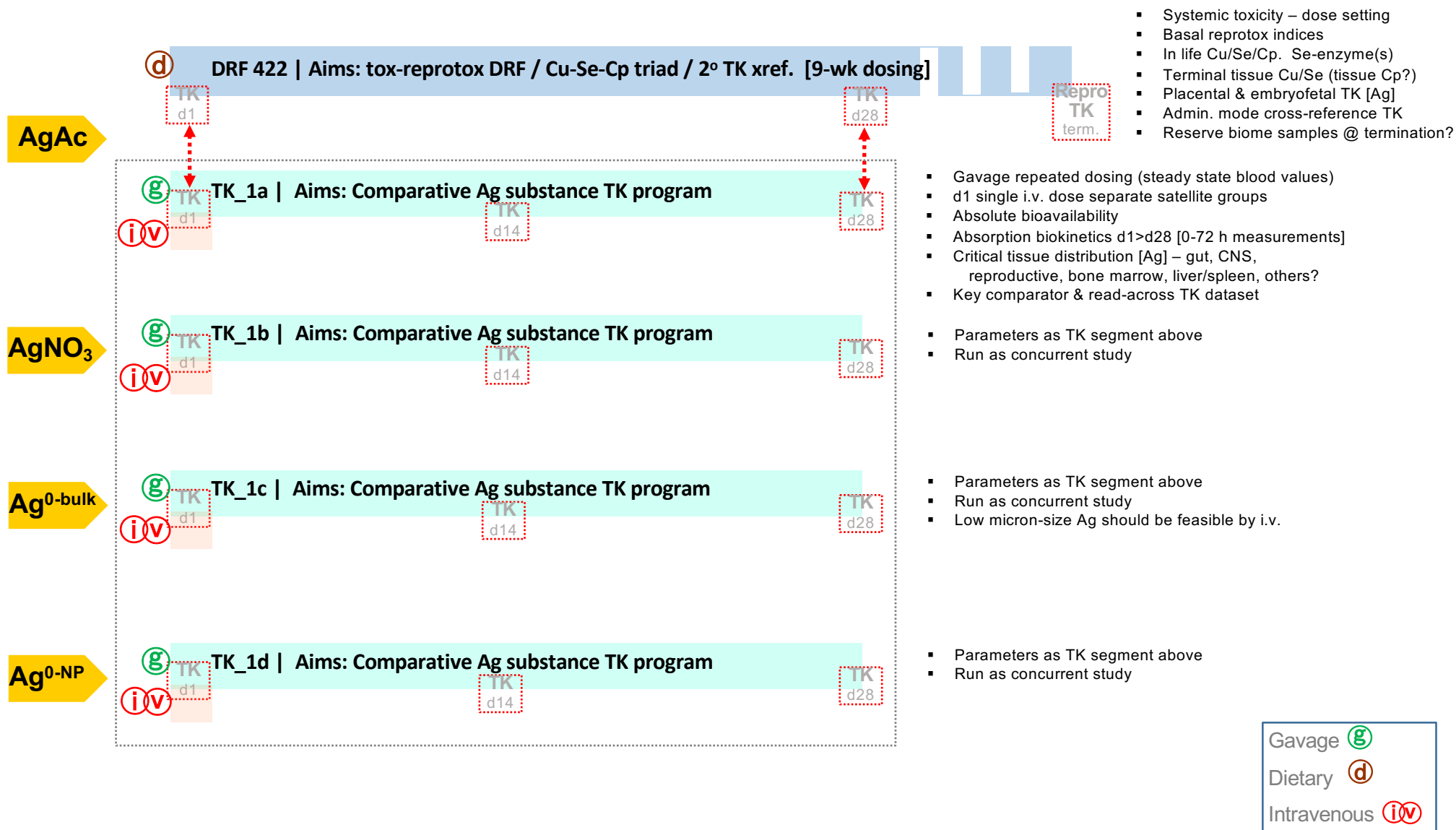
EOGRTS TP process and timing



NB: A decision can be adopted directly if no PfAs are received.

- DD Mar 2019: TP accepted so far by ECHA (30 months for testing)
- PfAs from NL (addition DNT cohort) ► EPMF comments submitted by 20 May 2019
- Agreement by MSC in **written procedure**, debrief at MSC meeting 24-27 June 2019 ► Final decision expected shortly after (EOGRTS with or without DNT)

Suggested study design DRF + TK



Selection CRO for TK / DRF / EOGRTS testing

- Reduce variability and for reasons of comparability ► perform TK, DRF and EOGRTS testing at **same test facility** (especially important for DRF and EOGRTS)
- Lab **experience** is key in choice of the CRO for EOGRTS (lab needs experience with EOGRTS + Cohorts 2 and 3) + **availability**
- Lab with most experience with EOGRTS incl. Cohorts 2 and 3: **Charles River (Den Bosch)** BUT first available time slot is late Q4 2020 (i.e. we would not make ECHA deadline and results will likely be too late to be taken into account during the AgNO3 CLH process (ATP inclusion))
- Site with second most experience: **Envigo (Eye)** and they have availability for the EOGRTS Q2 2020 (i.e. following the DRF testing)
- Envigo now merged with Covance ► we could benefit from Covance's experience on TK

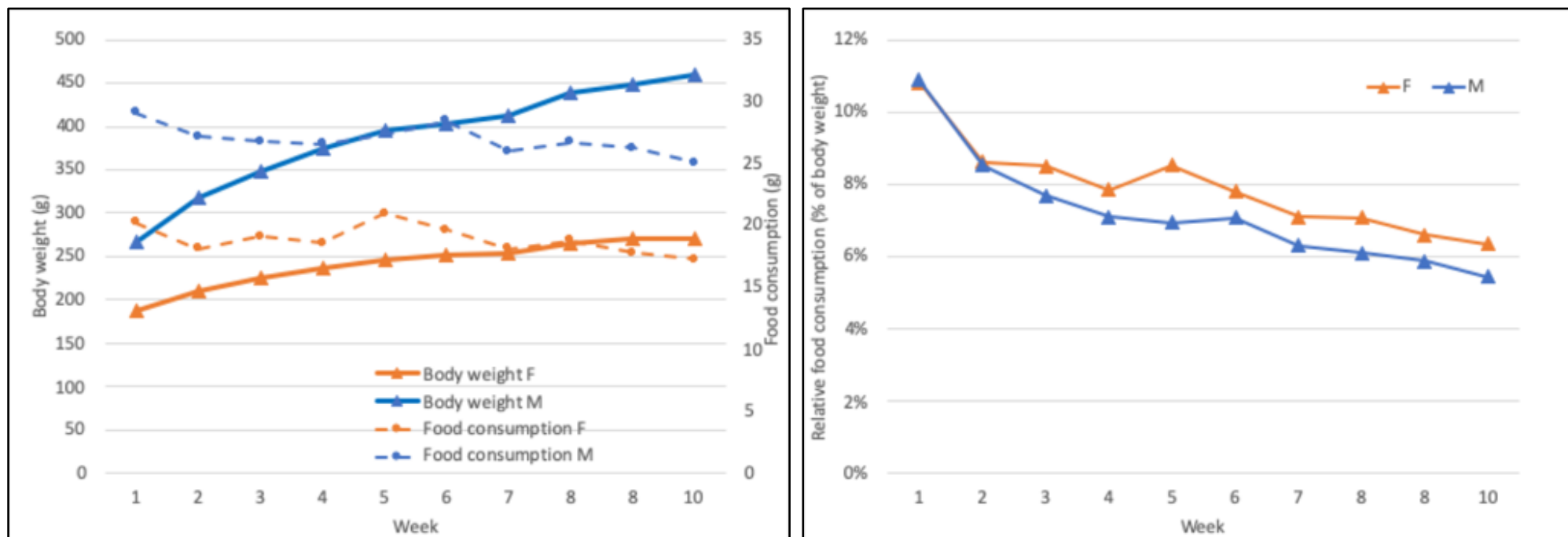
Timing Envigo

Study	Compliance/ Regulatory	Start	Draft report
Rodent Toxicity Studies			
Pre-study chemistry	GLP	July 2019	August 2019
14 day rat study (<i>palatability study</i>)	Non-GLP	September 2019	November 2019
Prelim to the OECD 443 (<i>DRF study</i>)	Non-GLP	November 2019	May 2020
OECD 443	GLP	May 2020	May 2021
TK study	Non-GLP	October 2019	February 2020



TK / DRF/ EOGRTS test design: dosing issue

Cf. AgAc gut biome study



Average dosing (% of nominal)						
	Females			Males		
Week	0,4 nominal	4 nominal	40 nominal	0,4 nominal	4 nominal	40 nominal
1	103,36%	104,83%	96,57%	101,20%	107,64%	97,96%
10	60,40%	63,42%	58,40%	49,90%	54,80%	49,39%
Average	74,97%	79,19%	70,57%	66,73%	71,05%	64,98%

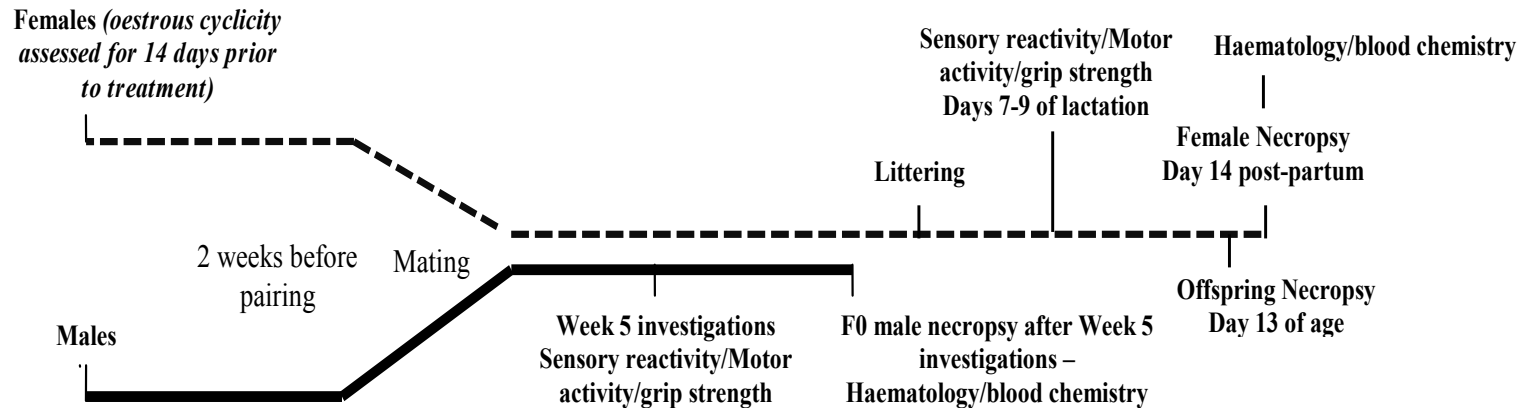
- Adjust dietary inclusion levels in DRF and EOGRTS as the growth rate of the rat changes throughout the study?

DRF test design

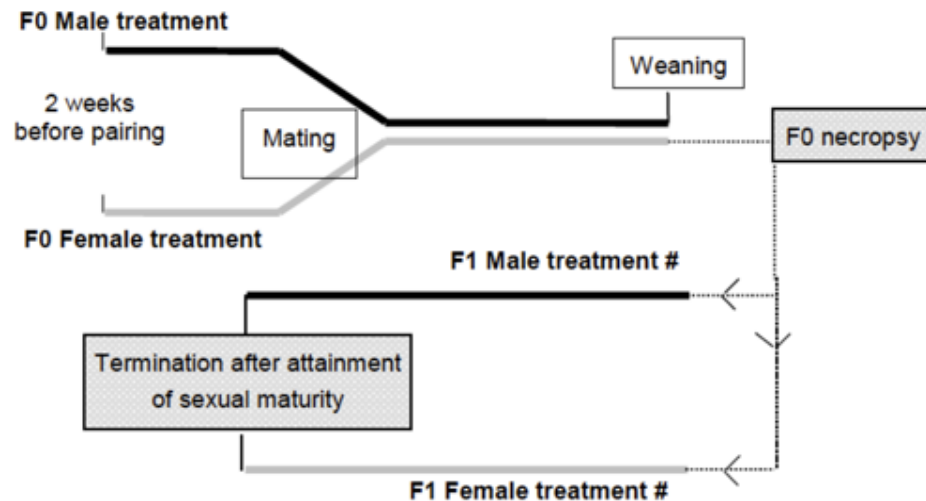
DRF study		Dietary					Satellite TK group?			
		Repeated dosing (diet) 63 d								
OECD 422	Dose levels	Control	1	2	3	4	1	2	3	4
	Test substance: AgAc	0 mg/kg bw/d	5 mg/kg bw/d	17 mg/kg bw/d	55 mg/kg bw/d	175 mg/kg bw/d				
	Nr of male animals	10	10	10	10	10	?	?	?	?
	Nr of female animals	10	10	10	10	10	?	?	?	?
- Treatment schedule and observations according to OECD 422										
- Palatability study needed										
- Additional measurements:										
* TK: Ag in whole blood at d1 (0-72 h) and at steady state (6 samplings/animal (5 time points 0-72 h: e.g. 3h, 6h, 12h, 24h, 72h + 1 time point at steady state))										
-> need for satellite TK group?										
(CRO to indicate that they have experience with / are comfortable taking blood at d28 (end of mating) without consequences to the dams)										
* Cu and Se in serum and in key tissues (especially reproductive tract) + assess the activity of one or more Se-enzymes (at necropsy)										
* Ceruloplasmin (Cp) in serum (and in key tissues if possible? CRO to confirm) (at necropsy)										
* Ag, Cu and Se in pups (sufficient nr of pups? Culled pups? Satellite animals? CRO to confirm)										
* Reserve gut biome samples at study termination										

DRF test design: duration

- Study design OECD 422: approx. 9 wk dosing



- Alternative study design suggested by Envigo: approx. 15 wk dosing



- F0: 8M+8F
- longer dosing so F1 offspring (10M+10F) will consume treated diet (from mid-lactation to termination) ► important for dietary study to avoid risk of doses being toxic for the pups in main EOGRTS

DRF test design: TK samplings

- **How many animals** to be sampled per group (sex / time point / dose level)? 3 or 4?
- **Time points** TK sampling (Ag in whole blood)?
 - Proposal F0 animals: at d1 (0-72 h) and at steady state; 6 time points total (5 time points 0-72 h: e.g. 3h, 6h, 12h, 24h, 72h + 1 time point at steady state: e.g. d28)
 - F1 animals?
- **Need for satellite TK animals?** Issues with using regular DRF animals:
 - sampling of female animals could affect outcome of the study
 - TK of non-pregnant and pregnant animals may be different: how reliable are the data for comparison with results TK study?
 - female animals will be at different stages of gestation on d28: issue with data reliability

DRF test design: other parameters

- **Cu and Se measurements** in serum and terminal tissues
 - F0 animals: serum measurements: at study termination?
 - F0 animals: which tissues? Reproductive organs only?
 - F1 animals?
- **Cp measurement** in serum: at study termination?
- **Se-enzyme(s)** in serum, (e.g. Glutathione Peroxidase (GPX)): at study termination?
- **Ag measurements in tissues?**
- Reserve **gut biome** samples at termination: F0 + F1 animals?



TK test design

TK study		Gavage						Intravenous										
		Repeated dosing 28 d			Single dose 0-72 h			Single dose 0-72 h										
OECD 417	Dose levels	1	2	3	1	2	3	1	2	3	Total test substances:	4 + control						
											Total doses/test subst.:	3 (1 for control)						
1	Vehicle control										Total admin and dosing modes:	3						
	Nr of male animals	3			3			3			Total groups:	39						
	Nr of female animals	3			3			3			Total rats (3 m/l / group):	234						
	Blood samplings/animal	6			6			7			Total rats (5 m/l / group):	390						
2	Test substance: AgAc										Total blood samples (3 m/l / group):	1482						
	Nr of male animals	3	3	3	3	3	3	3	3	3	Total blood samples (5 m/l / group):	2470						
	Nr of female animals	3	3	3	3	3	3	3	3	3	Total tissue samples (3 m/l / group):	1404	gut, brain, bone marrow, liver, spleen, rep					
	Blood samplings/animal	6	6	6	6	6	6	7	7	7	Total tissue samples (5 m/l / group):	2340						
3	Test substance: AgNO3																	
	Nr of male animals	3	3	3	3	3	3	3	3	3								
	Nr of female animals	3	3	3	3	3	3	3	3	3								
	Blood samplings/animal	6	6	6	6	6	6	7	7	7								
4	Test substance: bulk Ag																	
	Nr of male animals	3	3	3	3	3	3	3	3	3								
	Nr of female animals	3	3	3	3	3	3	3	3	3								
	Blood samplings/animal	6	6	6	6	6	6	7	7	7								
5	Test substance: Ag NP																	
	Nr of male animals	3	3	3	3	3	3	3	3	3								
	Nr of female animals	3	3	3	3	3	3	3	3	3								
	Blood samplings/animal	6	6	6	6	6	6	7	7	7								

- Pilot work on i.v. administration (especially the bulk Ag) will be required
- Ag measurement in whole blood (CRD to indicate cost per sample in case we want to add timepoints)
 - * Gavage, repeated dose: 6 samplings/animal (5 time points 0-72 h: e.g. 3h, 6h, 12h, 24h, 72h + 1 time point at steady state)
 - * Gavage, single dose: 6 samplings per animal (6 time points 0-72 h: e.g. 1h, 3h, 6h, 12h, 24h, 72h)
 - * I.v., single dose: 7 samplings/animal (7 time points 0-72 h: e.g. 0.25h, 1h, 3h, 6h, 12h, 24h, 72h)
- 3 male + 3 female (please include cost for Sm+Sf)
- 3 dosing levels per test substance

TK test design

- **Dose levels** for AgAc, AgNO₃, elemental bulk Ag, elemental nanoAg?
 - OECD417: high enough to allow for metabolite ID in excreta and plasma while not producing apparent tox
- **Time points** TK samplings (Ag in whole blood)? Proposal:
 - Gavage, repeated dose: 6 samplings/animal (5 time points 0-72 h: e.g. 3h, 6h, 12h, 24h, 72h + 1 time point at steady state)
 - Gavage, single dose: 6 samplings per animal (6 time points 0-72 h: e.g. 1h, 3h, 6h, 12h, 24h, 72h)
 - I.v., single dose: 7 samplings/animal (7 time points 0-72 h: e.g. 0.25h, 1h, 3h, 6h, 12h, 24h, 72h)
- **Nr of animals per group?**
 - 3 animals: £ 133.322 (tissue analysis not yet included; + £ 60k?)
 - 5 animals: £ 192.250 (tissue analysis not yet included; + £ 100k?)

Other discussion items

- **GLP status of studies?**

- DRF study + palatability study: offer Envigo non-GLP
- TK study: offer Envigo non-GLP
- EOGRTS: offer Envigo GLP

- **Selection test items?**

- AgAc, AgNO₃
- Elemental bulk Ag: granulometry, purity
- Elemental nanoAg: smallest form

- **Face to face meeting Envigo: second half of August?**





THANK YOU

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