EUROPEAN PRECIOUS METALS FEDERATION & COVANCE

Silver Acetate project;

OECD 417 & OECD 443 testing support

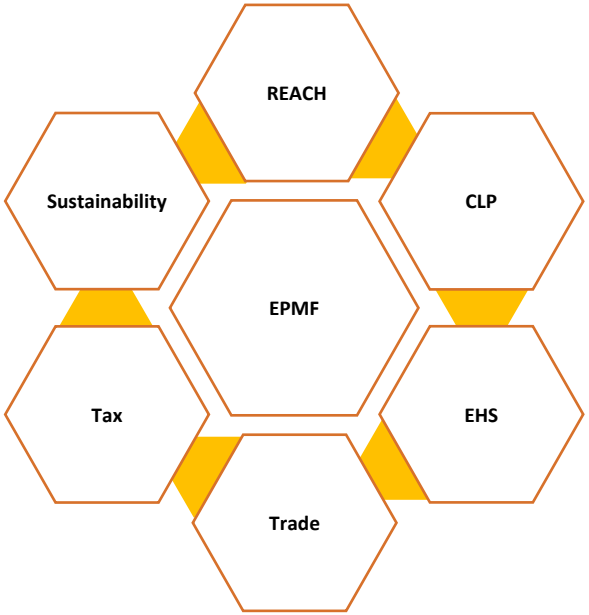
28th August 2019

COVANCE
SOLUTIONS MADE REAL®

Agenda

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)	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

Introduction to the European Precious Metals Federation



Website: www.epmf.be

Companies

National Federations

Precious Metals Industry

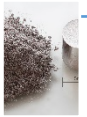
EPMF Ag project includes 8 substances/Dossiers:

1. Silver (incl. nano)
2. Disilver oxide
3. Silver nitrate
4. Disilver sulphate
5. Disilver carbonate
6. Silver chloride
7. Silver bromide
8. Silver iodide

Precious Metals Industry


Mining


Refining, recycling


Manufacturing, Importing


Banking, Trading


Electronics


Glass/Mirrors


Jewellery


Automotive


Alternative energy

Gold

Silver

Rhenium

Platinum


Catalysts/Chemical production


Healthcare

Ruthenium

Palladium

Rhodium

Iridium


Investment


Aerospace


Photography

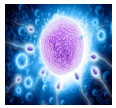
Silver reproductive toxicity: introduction

- Existing dataset for Ag ▶ multiple uncertainties & data gaps
  - Slide follows
- Industry has acknowledged need for definitive study
- REACH TP for EOGRTS submitted **2015** (rev. 2018)
  - Proposed test substance AgAc (outcome applicable to ionic Ag^+ irrespective of donor silver substance forming this ion)
  - Comprehensive ▶ design per latest OECD TG 443
  - Properly adapted ▶ e.g. ECHA design guidance integrated
  - Precautionary ▶ e.g. triggers (DIT cohort included; DNT cohorts initially not included but EPMF willing to include following PfA proposal for inclusion)
  - EOGRTS+ ▶ TK (adult, embryofetal), micronutrient homeostasis etc.
  - Discrimination ▶ assess whether indirect confounding effects occurring

Silver reproductive toxicity: uncertainties & data gaps

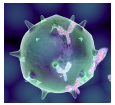
Ag^+ as toxicant: Relevance of SCAS 2-gen studies? Presence non-Ag constituents + TK data lacking.

New data - Sprando et al. (2017) OGRTS on AgAc: Devtox (pre- & post-natal). May be reliable study but several deficiencies (not GLP, no HCR data, omits key parameters like oestrus cycle, sperm parameters etc.). No linked TK or mechanistics (Cu?) work.

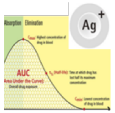


Fertility effects: Weight-of-evidence for Ag^+ equivocal. Indicators from general toxicity studies on Ag^+ & SCAS 2-gen studies unremarkable. AgAc OGRTS (Sprando) reported effect at HD only. Difficult to interpret (no stats/HCR data, key ♂, ♀ parameters omitted). If real, arguably not justified to categorise effect as 'severe'.

Potential for indirect effects: Now multiple studies in rodents demonstrating Ag^+/Ag effects on gut biome. Ag has potential to cause dysbiosis leading to 2º effects (incl. reproductive). Unclear as to magnitude biome effect vs degree of Ag^+ exposure ▶ cf. recent EPMF study



Developmental immunotox?: Evidence for adult animal immunotox due to Ag^+ lacking. Recent study (Babu et al., 2016) has suggested possibility of DIT in rat model. Limitations in protocol but industry consider it prudent to investigate further (EOGRTS DIT cohort)



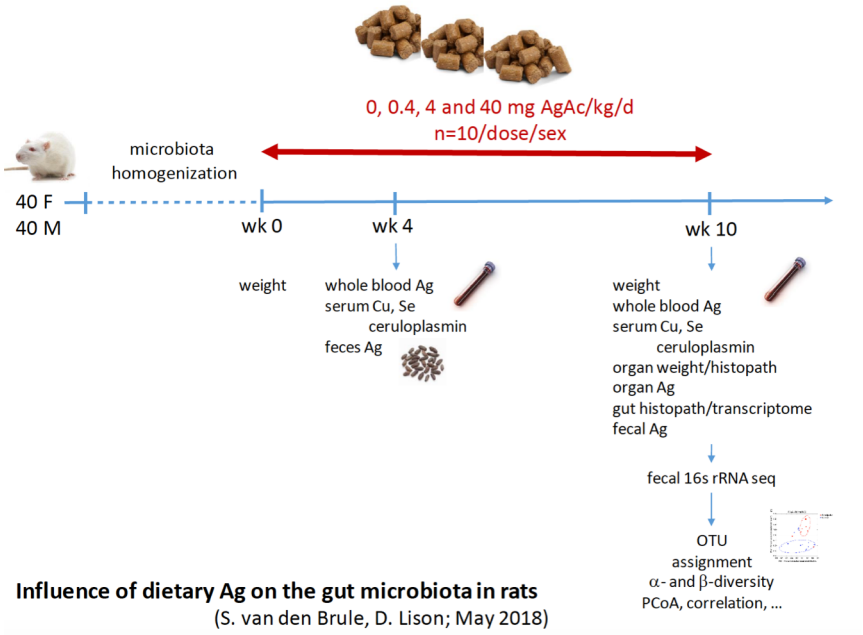
Ag toxicokinetics: Integration of all Ag substances. Fragmentary Ag TK dataset, often conflicting, incl. for reproductive tissues. Simple models (e.g. bioelution) don't represent reality. Robust TK needed for reprotox interpretation. Data for secure read-across lacking (e.g. AgCl vs other ionic forms; bulk Ag forms vs AgNP).

Silver reproductive toxicity: industry work programme

- EOGRTS key to definitive position
  - Appropriately extensive - full TG 443 conformance
  - Design integrates conservatism
  - Examining several potential effect axes of Ag^+
  - Ancillary investigations (TK, biome, DIT etc.) for added robustness
- Final decision from ECHA received 26 June 2019
  - EOGRTS in rats, oral route with the analogue substance AgAc
  - 10 weeks premating exposure duration for the parental (P0) generation
  - Dose level setting shall aim to induce systemic tox at the highest dose level
  - Cohort 1A (Repr tox)
  - Cohort 1B (Repr tox) without extension to the F2 generation
  - Cohorts 2A and 2B (DNT);
  - Cohort 3 (DIT)

Silver reproductive toxicity: AgAc effects on gut biome

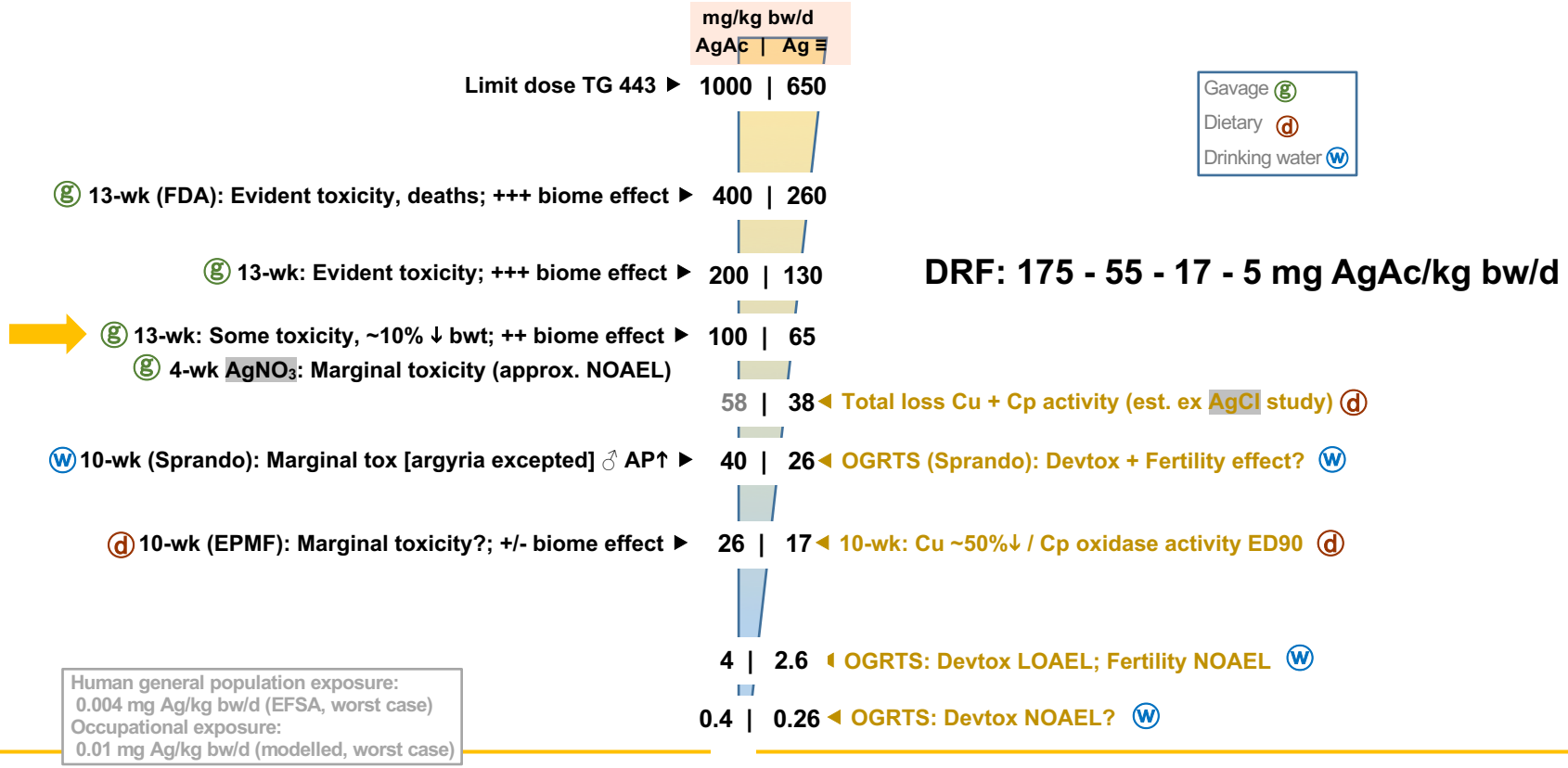
EPMF sponsored support study: Effects of Ag on gut microbial populations / influence re indirect toxicity



Influence of dietary Ag on the gut microbiota in rats
(S. van den Brule, D. Lison; May 2018)

- **Aim of study**: aid in interpretation of (adverse) reprotox studies; support dose-level setting for EOGRTS; further info on MoA (Cu, Se, Cp)
- **AgAc effects on rat biome**: statistically significant but not as remarkable as expected based on previous study results
- Study has also moved forward our knowledge on AgAc effects on Cp, Cu and Se (to be taken into account for further testing)

Ag dose level versus effect information

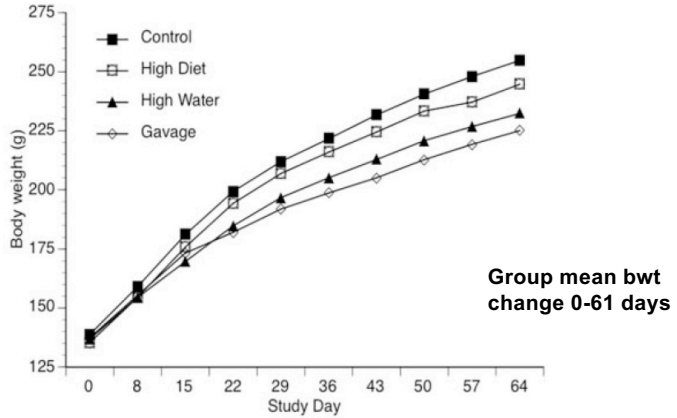


Mn case study: TK / toxicity equivalency of differing oral modes

TABLE 2. Mean (±SEM) Tissue Manganese Concentrations (μg Mn/g) Following 61 Exposure Days

Tissue	Control	Diet	Water	Gavage
Cerebellum	0.46 ± 0.01 (9)	**0.56 ± 0.03 (9)**	0.52 ± 0.02 (9)	**0.68 ± 0.02 (10)[a]**
Olfactory bulb	0.49 ± 0.03 (9)	0.49 ± 0.01 (9)	0.50 ± 0.05 (9)	**1.12 ± 0.13 (10)[a]**
Striatum	0.36 ± 0.01 (9)	0.43 ± 0.01 (9)	0.44 ± 0.01 (9)	**0.75 ± 0.07 (10)[a]**
Frontal cortex	0.25 ± 0.02 (8)	0.29 ± 0.01 (9)	0.27 ± 0.02 (9)	**0.73 ± 0.08 (9)[a]**
Bile	1.48 ± 0.20 (8)	**34.49 ± 5.68 (8)**	**38.63 ± 11.74 (9)**	**67.38 ± 7.91 (9)[a]**
Serum	0.66 ± 0.27 (9)	0.73 ± 0.16 (9)	0.42 ± 0.04 (9)	0.46 ± 0.03 (10)
Femur	0.32 ± 0.01 (9)	0.40 ± 0.02 (8)	0.40 ± 0.03 (9)	**0.66 ± 0.07 (10)[a]**
Spleen	0.13 ± 0.02 (9)	0.16 ± 0.01 (9)	0.13 ± 0.01 (9)	0.15 ± 0.02 (10)
Liver	2.62 ± 0.11 (8)	**3.30 ± 0.17 (9)**	2.94 ± 0.05 (9)	**3.36 ± 0.09 (10)**

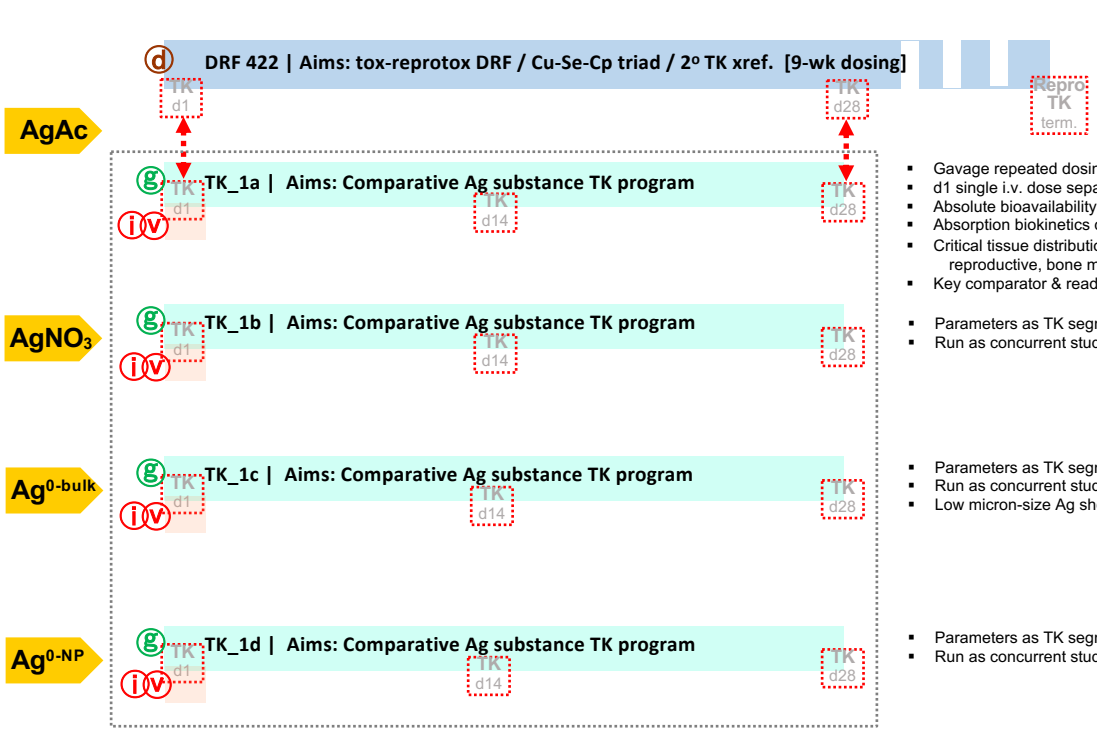
Number in parentheses = number of samples (*n*).
Bold text: $P < .05$; versus controls, significant ANOVA or Welch ANOVA followed by Dunnett's test.
[a]Increased when compared with all other groups ($P < .05$, Welch ANOVA and Tukey's HSD).



Group mean bwt change 0-61 days

- For Ag^+ no study which directly compares effect of different oral modes (gavage / drinking water / diet)
- Of all the metals, Mn TK profile most closely resembles Ag; work by Foster et al. (2015) may inform
- TK / toxicity equivalency of matched doses of $MnCl_2$ ~11 mg Mn/kg/day administered to rats (F344) via diet or drinking water or gavage
- Highest tissue exposures achieved via gavage (statistically significant)
- Some evidence that greater toxicity evident with gavage. Intuitively logical given (a) dose rate and general TK considerations; (b) diet matrix influences

Study design DRF + TK



- Systemic toxicity – dose setting
- Basal reprotox indices
- In life Cu/Se/Cp. Se-enzyme(s)
- Terminal tissue Cu/Se (tissue Cp?)
- Placental & embryofetal TK [Ag]
- Admin. mode cross-reference TK
- Reserve biome samples @ termination?

- Gavage repeated dosing (steady state blood values)
- d1 single i.v. dose separate satellite groups
- Absolute bioavailability
- Absorption biokinetics d1>d28 [0-72 h measurements]
- Critical tissue distribution [Ag] – gut, CNS, reproductive, bone marrow, liver/spleen, others?
- Key comparator & read-across TK dataset

- Parameters as TK segment above
- Run as concurrent study

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- Low micron-size Ag should be feasible by i.v.

- Parameters as TK segment above
- Run as concurrent study

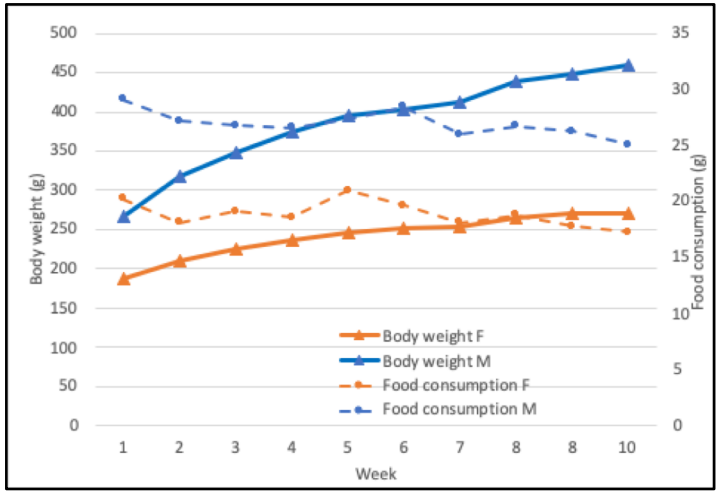
Gavage (g)
Dietary (d)
Intravenous (iv)

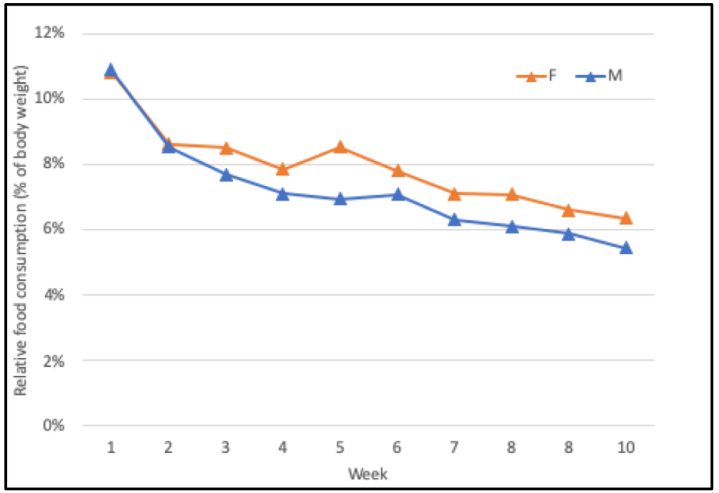
General Questions

1. Covance animal welfare policy and procedures in place to avoid prolonged and avoidable animal suffering
2. Study schedule (p.5 quotation) and estimated final reporting (cf. regulatory timeline); study site locations
3. Study design considerations (p.6 quotation): adjusting dietary inclusion levels
    I. 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)
    II. 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?)
    III. Does Covance have experience with dietary admin for the EOGRTS?

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?

OECD 417 Comparative TK study (CC71MP)

(P.10-11 QUOTATION)

4. GLP compliance: all raw data recorded in a GLP compliant way but no QA audit of the report/data
5. Age of rats in TK study
6. Formulation analysis for gavage / i.v. dose preparations
7. Possibility for pilot work to support the i.v. segment for bulk Ag
8. 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?

Bioanalysis Support Using ICP-MS

Covance Reference Number	Study details	Comments from Darran & Glenn	Cost (GBP) with 4% discount applied
NR98KN	Ag in the Blood - Method Development & Validation (ICP-MS)	Revised price based on method	12,864
BD55LW	**Se and Cu** in the Serum: Method Development &Validation (ICP-MS)	2 studies can now be combined	32,160
SX40QD	**Se and Cu** in the Testes and Ovaries (male and female reproductive organs): Method Development & Validation (ICP-MS)	2 studies can now be combined	32,160
MB42JH	Ag in the Homogenized Pup: Method Development & Validation (ICP-MS)	Price remains the same	25,728
KM26KX	Ag in in the Brain: Method Development & Validation (ICP-MS)	Revised price based on method	12,864

Palatability study (GH28GX)

(P.12-13 QUOTATION)

9. 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

OECD 443 Prelim reprotox study (YC23RN)

(P.14-17 QUOTATION)

10. 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)
11. Diet should be checked for adequate Cu content prior to use; is this on analytical datasheet provided by the supplier? What diet is used?
12. Exposure assessment blood sampling:
    - I. F0 animals: 3 males and 3 females: should be same 3 animals at each timepoint
    - II. 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
    - III. Is day 17 as late as can be done (Guideline states 'late pregnancy')?
    - IV. Sampling schedule: cf. Excel file. Strategy behind sampling of both F1 selected termination animals and also culled F1 animals (as distinct from only former)?
    - *V. 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)*

	Price / sample	F0 samples	F1 culled samples	F1 selected samples	Total samples
Ag in blood	£ 45,00	105	40	30	**175**
Cu in serum	£ 45,00	60	40	30	**130**
Se in serum	£ 45,00	60		30	**90**
Cu in repr organs	£ 60,00	64			**64**
Se in repr organs	£ 60,00	64			**64**
Ag in homogenised pups			32		
Ag in brain					
Cp in serum	£ 91,43	60	40		**100**
GPX in serum	£ 99,38	60			**60**

OECD 443 Prelim reprotox study (YC23RN) continued

(P.14-17 QUOTATION)

13. 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
14. Organ weights and tissue retention: suggestion to include reproductive organ weights
15. 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
16. Formulation analysis: suggestion to consider at least homogeneity check / achieved concentrations of diets

OECD 443 EOGRTS (YQ21BX)

(P.18-26 QUOTATION)

17. Thyroid hormone analysis:
    I. T3 would be useful to get a complete assessment of thyroid hormones if HCD are available for this age group?
    II. T4 analysis day 4 culls (optional): how much HCD are available for this age group?
18. Spleen cell immunophenotyping: what recent HCD is available?
19. Seminology should be included for all P & F1 cohort 1A males. What equipment is used for this and who will be responsible?
20. Formulation analysis: suggestion to add last pre-mating week
21. Who will conduct the neuropathology assessments and what is their experience with OECD 443?

Analytical support

(P.2 QUOTATION)

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?

Analytical support

(P.2 QUOTATION)

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Food Consumption Data

DATA COLLATED BY DAVID MYERS

Food consumption before pairing (g/rat/day) – F0 parents

Group /Sex		Week 1	Week 2
1M	Mean	27	27
	SD	9.7	11.5
	N	7	7

Group /Sex		Week 1	Week 2
1F	Mean	20	19
	SD	6.2	6.3
	N	7	7

Food consumption during gestation (g/rat/day) – F0 females

Note: Slight increase in food intake during Days 14-19 as the fetuses start to grow

Group /Sex		Day 0-6	Day 7-13	Day 14-19
1F	Mean	23	24	27
	SD	2.1	2.1	2.1
	N	24	24	24

Food Consumption Data…continued

DATA COLLATED BY DAVID MYERS

Food consumption during lactation (g/rat/day) - F0 females

Note: Higher consumption than during late gestation and steady rise as physiological demand increases

Group /Sex		Day 1-3	Day 4-6	Day 7-13	Day 14-20†
1F	Mean	37	47	61	80
	SD	4.5	3.8	5.7	5.7
	N	24	24	24	24

† Includes diet consumed by offspring

Food Consumption Data…continued

DATA COLLATED BY DAVID MYERS

Food consumption of selected offspring (g/rat/day) - F1 generation

Note: Absolute consumption is lower than in parents but there is higher intake per unit bodyweight so much higher achieved intake of test substance at start of F1 generation compared with parents

Group /Sex		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	16	22	24	26	28	27	28	28	28	27
	SD	10.8	27.5	12.6	8.6	10.7	9.3	8.4	8.6	6.6	7.8
	N	5	5	5	5	5	5	5	5	5	5

Group /Sex		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
1F	Mean	14	16	17	18	21	20	18	20	19
	SD	5.5	5.3	7.7	6.4	22.0	6.8	10.1	5.8	13.5
	N	5	5	5	5	5	5	5	5	5

CROP PROTECTION AND CHEMICALS (CPC)

An Introduction

August 2019

COVANCE
SOLUTIONS MADE REAL®

June 2019

LABCORP AND ENVIGO COMPLETE INNOVATIVE TRANSACTIONS

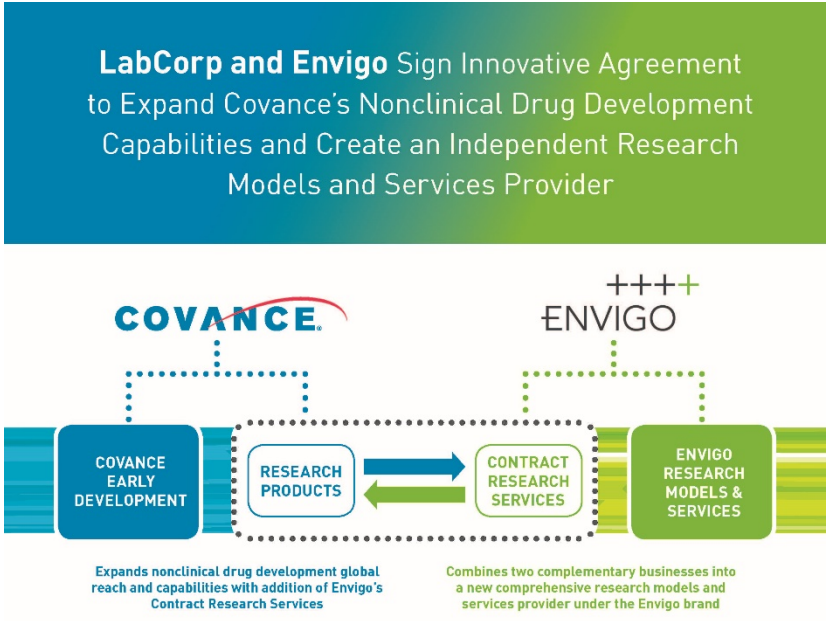
LabCorp Acquires Envigo's Global Nonclinical Contract Research Services Business, Envigo Acquires LabCorp's Covance Research Products Business

- LabCorp's Covance Drug Development segment has completed the acquisition of Envigo's nonclinical contract research services business, expanding Covance's global nonclinical drug development capabilities with additional locations and resources.
- Envigo's Research Models and Services business has completed the acquisition of the Covance Research Products business.
- The two companies will continue to collaborate through a multi-year, renewable supply agreement.

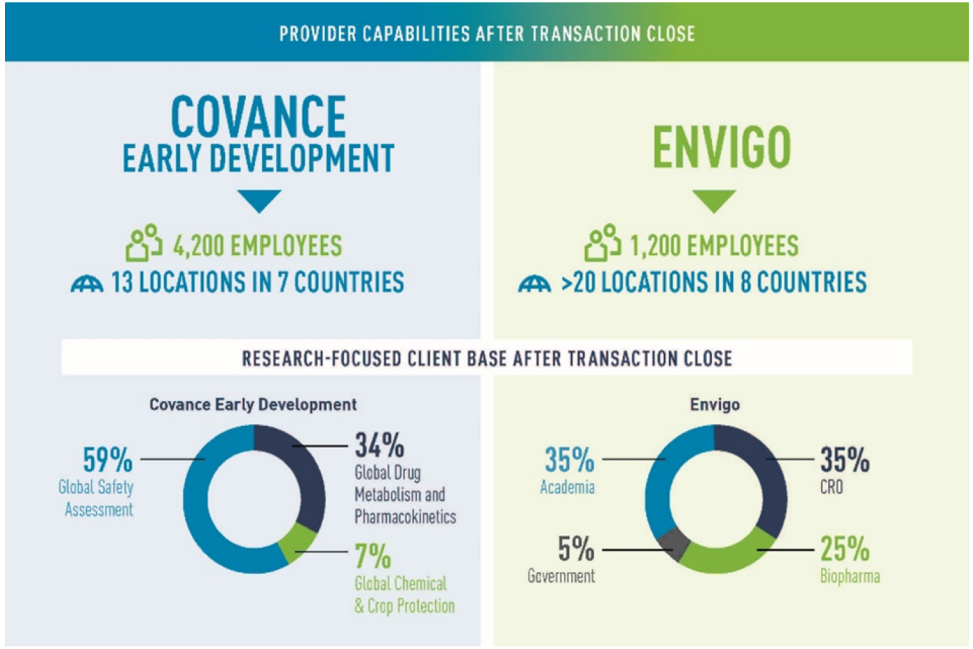
"*This unique transaction furthers a key element of our strategy to provide the biopharma industry with comprehensive drug development services that help bring innovative medicines*," said David P. King, chairman and CEO of LabCorp.

"*We are delighted to welcome our new colleagues from Envigo to Covance and look forward to driving growth and innovation with enhanced nonclinical research solutions that ultimately improve health and improve lives.*"

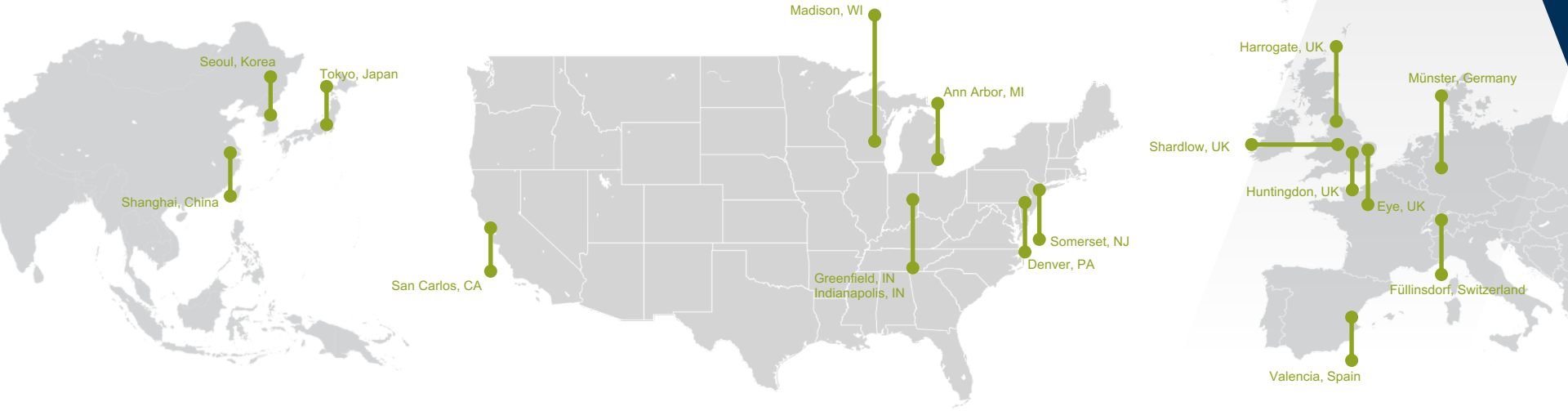
What's Happening…



What It Looks Like…



Covance Early Development Global Footprint



Laboratory sites as of June 2019

COVANCE
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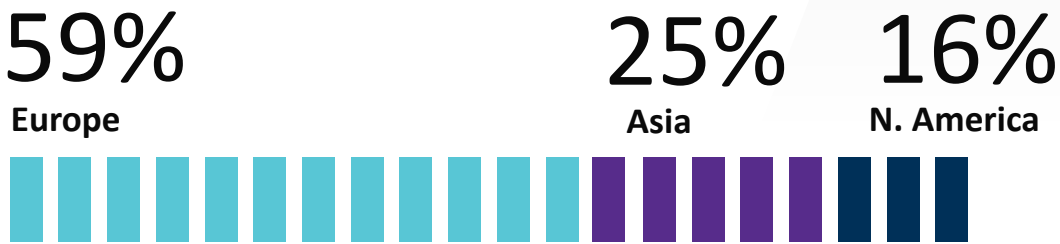
Crop Protection and Chemicals – Introduction

YOU'RE IN GOOD COMPANY

1,500
Customers globally

CPC supports more than 1,500 customers across industry, academia and government each year

- 15 of the top 20 crop protection and chemical companies

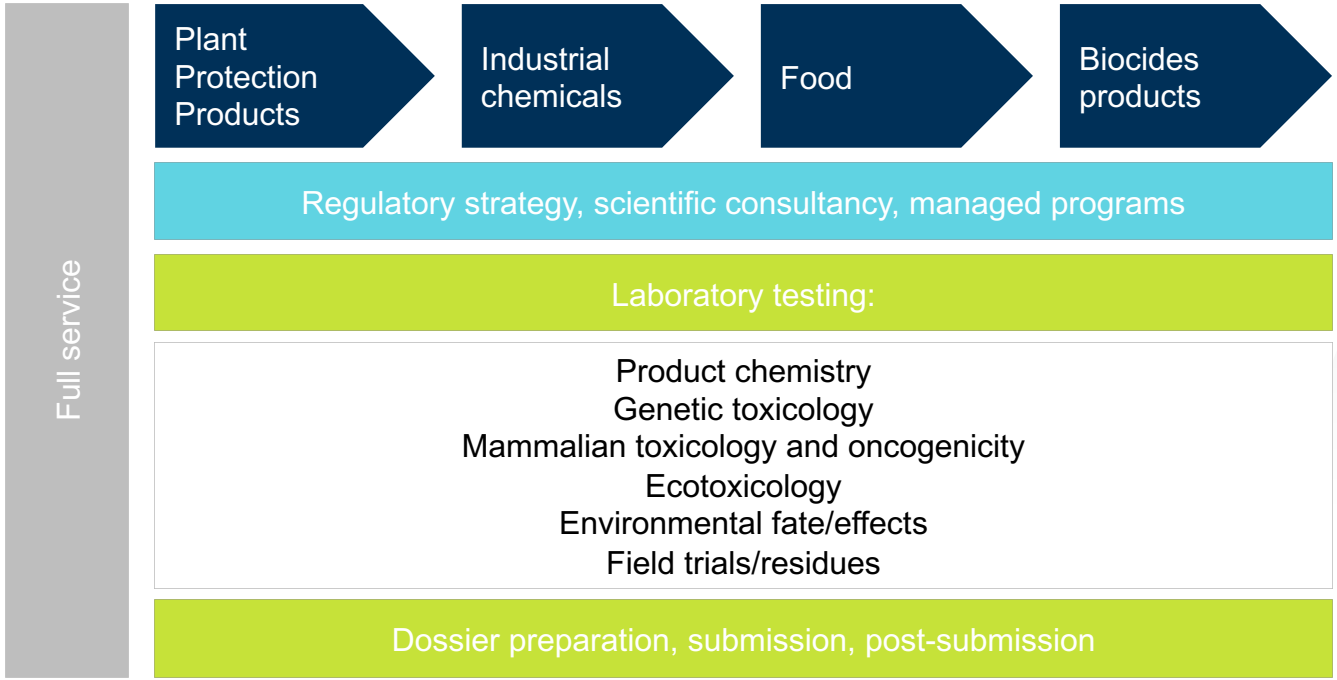


Core Strengths

We are in the business of selling solutions to meet regulatory needs that enable our customers to do business

Bringing product to market with our structured and integral consultancy

End to End Services for the CPC Industries



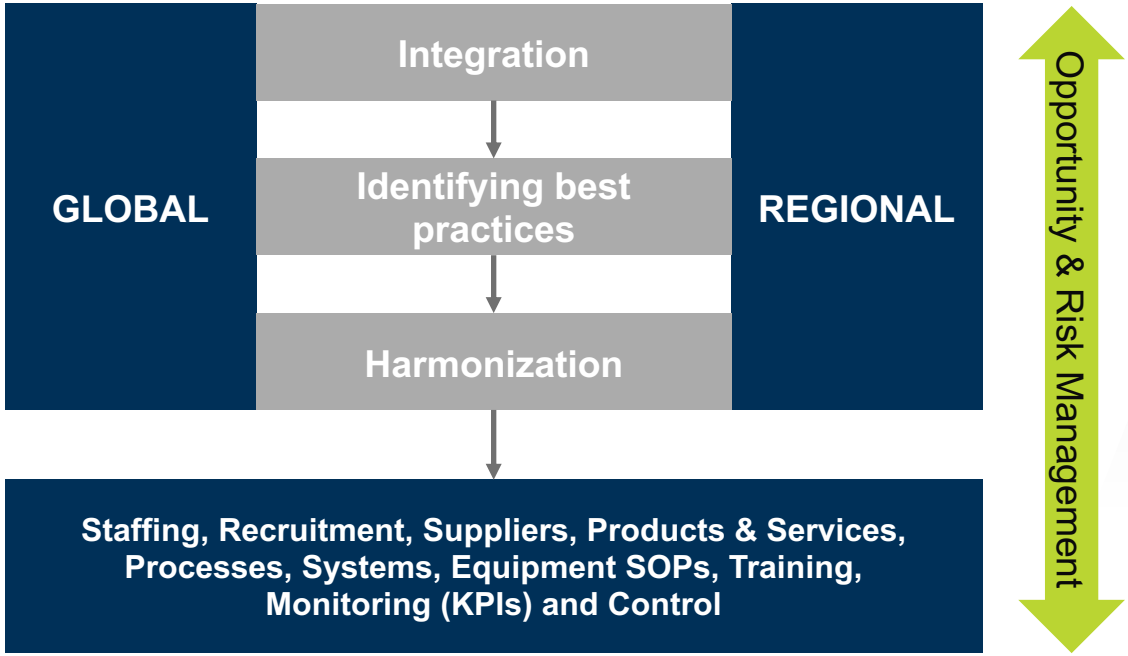
CRS - CPC Capabilities Grid

CPC Services	Eye	Huntingdon	Shardlow	Füllinsdorf	Valencia	Tokyo	Seoul
Physico Chemical Testing	X		X				
Environmental Fate	X	X					
Microbiology		X					
Terrestrial Ecotoxicology		X					
Aquatic Ecotoxicology & Biodegradation			X				
In Vitro Safety Assessment		X	X				
Animal Metabolism		X					
Regulatory Consultancy	X	X		X		X	X
Residue Analysis	X						
Environmental Risk Assessment	X	X	X				
Field Trials	X	X			X		
In Silico Testing		X	X	X			

Early Development, Analytical and CPC Capabilities Grid

Drug Development Services	Ann Arbor	Chantilly	Denver	Greenfield	Harrogate	Indy	Madison	Munster	Salt LC	San Carlos	Shanghai	York	Eye	Huntingdon	Shardlow	Somerset	Füllinsdorf	Valencia	Tokyo	Seoul
Discovery / Lead optimization	X			X	X		X	X			X		X	X						
In Vivo PK Screening				X	X		X							X						
Immunology & Vaccines			X											X						
Bioanalytical		X			X	X	X		X		X			X		X				
Drug Metabolism					X		X				X			X						
DART				X			X	X					X		X	X				
General Toxicology					X		X				X		X	X		X				
Genetic Toxicology					X									X	X					
Immunotoxicology				X	X		X	X						X						
Infusion Toxicology					X		X	X					X	X						
Inhalation Toxicology														X		X				
Ocular							X						X	X						
Safety Pharmacology					X		X	X			X			X		X				
Efficacy Pharmacology														X						
Pathology					X		X				X		X	X		X				
CMC Analytical Testing (biologics)				X	X							X		X						
Genomics														X		X				
Medical Device										X										
Physico Chemical Testing														X	X					
Environmental Fate													X	X						
Microbiology														X						
Terrestrial Ecotoxicology														X						
Aquatic Ecotoxicology & Biodegradation															X					
In Vitro Safety Assessment														X	X					
Animal Metabolism														X						
Regulatory Consultancy													X	X			X		X	X
Residue Analysis													X							
Environmental Risk Assessment													X	X	X					
Field Trials													X	X				X		
In Silico Testing														X	X		X			

Business Integration Model



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