RuCl3: outcome of the OECD 407 and design of the OECD 421

Participants list:

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1. Discussion on OECD 407

RSA summarized the outcome of the OECD 407 (cf. RuCl3 report in annex 1). The main issues are:

- To define which of the recovery groups should progress into recovery phase - suggestion is to progress high dose only Control and High Dose recovery were agreed. It should be cost neutral for Mid-High Dose recovery group to also proceed regarding in-life stage. However, there may be incremental cost for further terminal procedures on that group (organ weights, tissue retention) – **RSA should clarify**.
- To use preliminary information from the OECD 407 to help select dose levels for subsequent OECD 421 study

Indications of target organ organ involvement and other information relevant to the above decisions can be obtained in the near-term from:

- Necropsy macroscopic observations outcome
- Organ weights
- Terminal bleeds

This information should be available by next week.

Questions raised:

- Whether the dietary analysis had shown conformance with expectations (as a possible explanation for bodyweight fluctuations). RSA responded that they had no anaylsis data suggesting discrepancies had occurred, and previous dietary analysis by the CRO had been acceptable.
- About the d21 bodyweight gain reduction seen in some treated female groups and the potential reasons. No specific reason has been identified, the change was not so big.

2. Discussion on OECD 421

RSA summarized the schedule and point of discussions (cf. Annex 1). RSA insisted on the fact that the longer treatment period will have to be taken into account for the dose level settting.

It was agreed that:

1. **OECD 421 new test guideline (2015) will be used.**

2. We need a revised protocol from CitoxLab to reflect this and ensuring that we check in detail endrocine parameters including the approach for morphometric endpoints. RSA will obtain and the protocol **will be reviewed by RSA, PMC and Mark Raffray** and with possible referral to Tox Experts WG if issues are identified.
3. Based on the timeline, a decision must be taken on the current tentative start date of the study by end of next week (19 August 2016 at the latest) but we agreed that this will only be feasible if the information on 407 (necropsy, organ weights and teminal bleeds) will be available by then. We need to ensure that a informed decision on the 421 design is taken and some flexibility can be allowed in the overall timeline if needed.
4. Because of capacity issues with hormone analysis at CitoxLab, the CRO has suggested the possibility that their sister laboratory in France perform this segment of the study. However, there are timeline risks associated with this option. An alternate option would be to sub-contract the work to a different CROs (e.g. LPT) —this approach would require that PMC insist on sub-contractor use. A third option would be to allow CiToxLab to perform analyses in-house but include a penalty clause in the contract if they default on timeline. Having a sub-contractor could slightly complicate the monitoring of the study but due to the importance of the deadline the group decided to push for a sub-contractor (ideally LPT who has the capacity and could respect the deadline).
5. Costs: as the offer is presented now and taking into account a sub-contractor (LPT) the overall budget needed will be of 120.000€. However, the current proposal is not detailed and clear enough to ensure a good review of the costs proposal. **RSA will ask for an updated quote based on the OECD 421 (new guideline) and with a sub-contractor for hormone analysis. RSA will also ask if a 10 % of the amount can be paid at the reception of the final report.**
6. Setting the dose level should be actioned when initial indicators from 407 are available (by 19 August latest). **RSA will send to PMC a proposal for dose levels (a conventional 3 treatment groups is envisaged), including all the justifications and rationales to ensure that the tox experts have a full picture and can make a quick and well informed decision.**
7. Timeline: we can give some flexibility to the lab for the final report but the draft report must be available by end of February at the latest to ensure the minimum impact on the overall Ru workplan which needs to be registered Q3 2017.

Annex 1:

Ruthenium Chloride

Study code: 15/266-100P: A 28-Day Dietary Toxicity Study in Wistar Rats With 14-Days Recovery

Gr. No.	Group Designation	Target Dose Level (mg/kg bw/day)	Diet Conc. (ppm)	Animal Numbers			
				Main*		Recovery**	
				Males	Females	Males	Females
1	Control	0	0	5	5	5	5
2	Low Dose	30	500	5	5	–	–
3	Mid Dose	100	1500	5	5	–	–
4	Mid High Dose	300	5000	5	5	5	5
5	High Dose	1000	15000	5	5	5	5

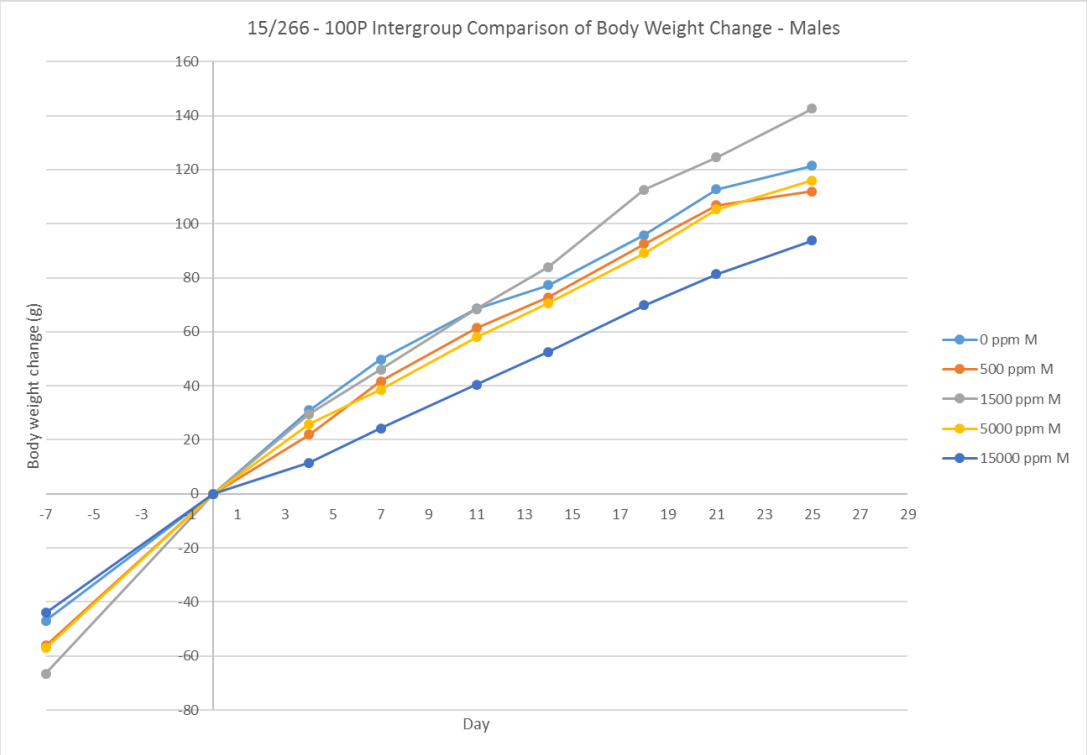
* Main animals will be euthanized after 28 days of daily treatment (Start of treatment, Day 0).
** Recovery animals (5/sex control, mid high dose and high dose groups) follow a 28-day daily treatment, with a 14-day recovery period.

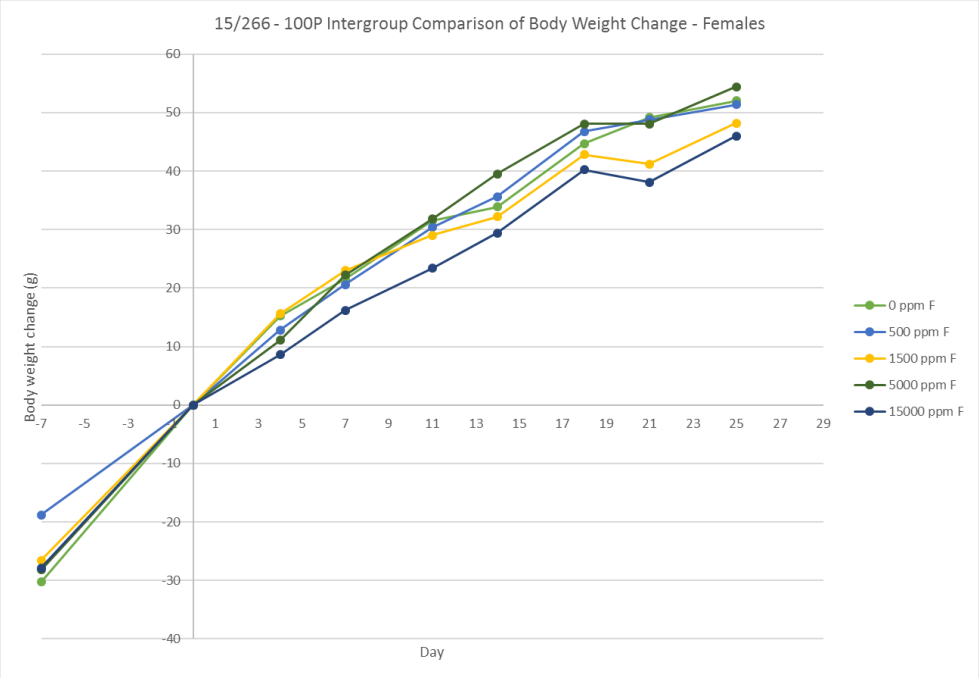
Day 27 today

Clinical observations:

Both the males and females given 5000 or 15000 ppm started to produce dark faeces from day 11 and continue to do so. No other observations.

Body weight gain



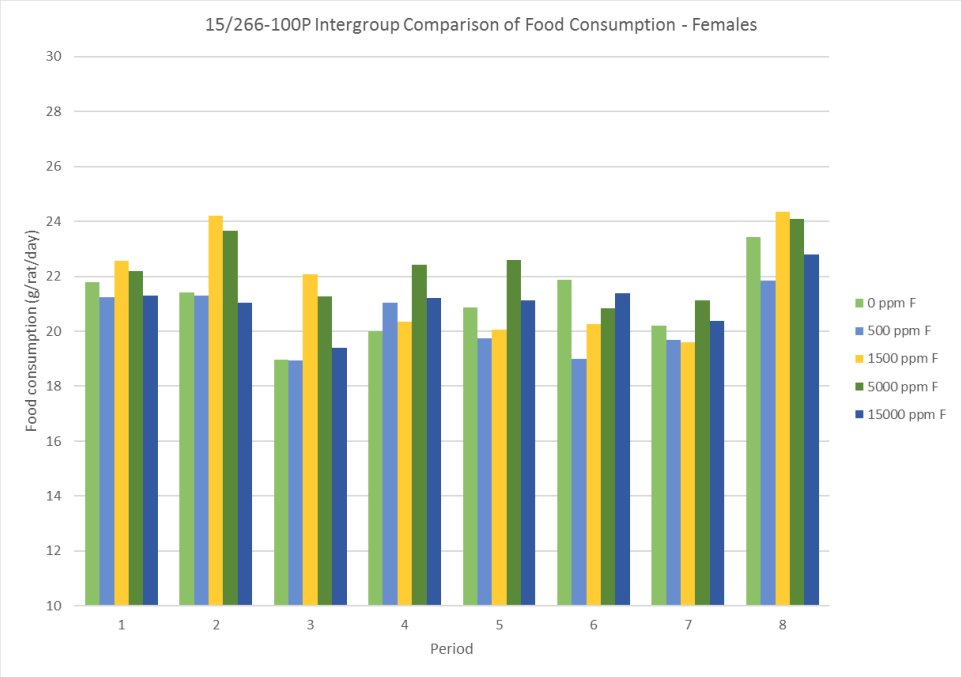


Body weight day 25:

	Day 25	Dietary concentration of RuCl3				
		0 ppm	500 ppm	1500 ppm	5000 ppm	15000 ppm
Males	Body weight (g)	437.1	439.4	477.8	441.1	417.0
	% difference	-	+2.3	+9.3	+0.9%	-20.1%
Females	Body weight (g)	268.3	256.4	259.4	268.4	259.2
	% difference	-	-4.4	-3.3	-0.1	-3.4%

Males more severely affected than females

Food consumption: unaffected by treatment



Last necropsy 25 August 2016.

Draft report no later than 25 November 2016.

ACTION:

To agree fate of recovery group - suggestion is to progress high dose only

To select dose levels for subsequent study

Study code: 15/266-xxxx: Reproduction/Developmental Toxicity Screening Test OECD 421 (2015)

- Scheduled to commence 6 September with diet preparation 12 September so treatment will commence ~ 20 September. Possibility of animals being available a week earlier but diet prep dates need to be confirmed for this option.
- New study design is longer than previous so males exposed for ~ 6 weeks and females for up to 9 weeks.
    - Need to consider dose levels in relation to expended period of dosing
    - Need to consider impact on report date. CiToxLAB agreed (under pressure) to deliver draft report for old study design before end January 2017 based on completion of in life phase ~ mid/end October. New study design is longer and will complete in life mid November. CiToxLAB have so far failed to confirm reporting time line – suspect unlikely to be January 2017.
- New study design includes additional end points essentially those to detect potential endocrine disruptors.

- Most significant measure is of T4 (and TSH). CiToxLAB Hungary does not have this capability but CiToxLAB France does. Option to subcontract to CiToxLAB France or to ANO. To be discussed further.

Study design shown overleaf.

ANNEX 2 DIAGRAM OF THE EXPERIMENTAL SCHEDULE INDICATING THE MAXIMUM STUDY DURATION, BASED ON A FULL 14-DAY MATING PERIOD