Petitioner AMVAC Exhibit 16

EPA OALJ Docket No. FIFRA-HQ-2022-0002

ENVIGO

Plan for completion of DCPA developmental thyroid studies:

1) **DCPA (Chlorthal Dimethyl): Dose Range Finding Pre and Post Natal Developmental Thyroid Study in Sprague Dawley Rats by Oral Administration (Envigo Study: BDG0204).**

We need to rerun Phase I of this study because the rat Luminex immunofluorescence assay which was used at the time the study was performed was not able to detect quantifiable levels of T4 and T3 in plasma from rat fetuses: and these are critical endpoints on the study. The assays were validated to the lowest possible limits of detection taking into account the limited available scientific literature on this subject.

As Phases II and III of the study were scheduled to start after Phase I, we acted quickly and those phases were not performed and will be performed once the rerun of Phase I has been completed. Detection of T4 and T3 and TSH in the dams and TSH in the fetuses was successful using the Luminex assay.

In order to overcome this challenge Envigo has now developed an LC-MS MS assay with much lower detection limits (pg/ml) which has been proven to detect T4 and T3 in Control fetuses. The assay, which detects T4 and T3 simultaneously, has been satisfactorily validated for accuracy and precision and we also have 1 month frozen stability data and are currently investigating stability for longer periods of storage. The Luminex assay is also currently being validated for detection of TSH in serum so that all 3 hormones are assayed in the same medium, serum.

The rerun of Phase I was authorised by UK Home Office and the in life phase and thyroid hormone assays have been successfully completed as follows:

Animal arrival 22 February 2017.

Start of dosing on Day 6 after mating: 6 March 2017

Completion of in life phase: 26 March 2017

Thyroid hormone assay results issued: 19 May 2017

The results from Phase I have been reviewed by US EPA.

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As per the results/discussion/conclusions of the US EPA HED Review dated November 16, 2017 of the comparative thyroid assay range-finding study and thyroid hormone methods data, the plan moving forwards is as follows:

Submission to EPA of the outline of the proposed design of the new range-finding pre- and post-natal developmental thyroid study (Envigo Study No. JV36WK) which will incorporate all aspects (data originally planned to be provided on Phases II and II of BDG0204) as requested by the HED review – May 2018

Submission to EPA of a summary table of validated thyroid hormone analysis methods and measured levels using these methods at different developmental stages – July 2018.

Submission to EPA of the QA audited final report for validation of method of analysis and stability of TSH in rat serum – July 2018.

Submission to EPA of the QA audited final report for validation of methods of analysis and stability of T4 and T3 in rat serum –July 2018.

Development and validation of bioassays for detection of DCPA in rat milk and plasma – started mid-March 2018. All work is completed and reports are being prepared.
Summary:

An LC-MS/MS method for the determination of DCPA in rat plasma has been successfully validated.

Acceptable accuracy (recovery), precision (repeatability), linearity (calibration) and specificity (interference) were observed over the concentration range 0.00600 to 1.50 mg/mL using a sample volume of 32 µL.

The method was validated at LOQ (0.0200 mg/L) and 50x LOQ (1.00 mg/L) in rat plasma.

The effect of the variability of the matrix (plasma effect) from different rats on the reliability of the method was shown to be negligible. Therefore, calibration with standards in solvent may be used for subsequent work or studies using this analytical method.

The reference standard was shown to be stable as follows:

Short-term storage in plasma:	2 hours (22°C)
Long-term storage in plasma:	34 days (-70°C)
Freeze-thaw cycles in Plasma:	3 (-70/22°C)
Whole blood:	1 hour (on ice)
Standard stock solution:	6 hours (22°C)
Standard working solution:	6 hours (22°C)
Standard stock solution:	44 days (4°C)

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Standard working solution: 44 days (4°C)

Extracted samples were found to be suitable for reinjection after storage for 20.5 hours at 4°C.

An LC MS/MS method for the determination of DCPA in rat milk has been successfully completed.

DCPA	Rat Milk
Linearity	Acceptable over the range, 0.00600 to 1.50 mg/L
limit of detection (LOD)	0.00600 mg/L
The limit of quantification (LOQ)	0.0200 mg/L
Recovery at LOQ level	Within acceptance criteria of 70 to 110%
Second parent to daughter ion transition	acceptable for confirmatory purposes
Matrix Effects	Mean results between the assessed levels of LOQ (0.0200 mg/L) and 50x LOQ (1.00 mg/L) Acceptable (not less than 0.80%)
Processed Sample Viability	8 days at 2-10°C
Freeze/thaw	1 cycle (-70°C/RT)
Short-term stability	2 hours at RT
long-term stability	34 days at -70°C

Submission of the full draft study plan for the range-finding pre- and post-natal developmental thyroid study (Envigo Study No. JV36WK) to EPA – November 2018

Submission of validation report for the DCPA assay in rat plasma (Envigo Study No. DC87NT) to EPA – March 2019

Submission of validation data for the DCPA assay in rat milk (Envigo Study No.CH09GN) to EPA – April 2019

Testing on the new range-finding pre- and post-natal developmental thyroid study (Envigo Study No. JV36WK) study will proceed as soon as possible following authorisation to proceed is received from US EPA.

The results will determine the doses for the definitive main pre and post natal developmental thyroid study (BDG0202) and the precise design and size of the study (i.e. if direct dosing of pups required) and if it is how many groups of pups will need to be dosed.

2) **DCPA (Chlorthal Dimethyl): Definitive Main Pre and Post Natal Developmental Thyroid Study in CD Rats by Oral Administration (Envigo Study:BDG0202)**

Testing will proceed as soon as possible once the range-finding pre- and post-natal developmental thyroid study (Envigo Study No. JV36WK) has been completed and all of the results from that study have been reviewed by US EPA and authorisation to proceed is received.

D. P. Myers 20 February 2019

Signed: David P Myers, BSc, PhD, IDT, Senior Toxicologist, Department of Toxicology

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