Petitioner AMVAC Exhibit 34

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: July 15, 2021

SUBJECT: **DCPA.** Review of a Protocol for an Oral (Gavage) Comparative Thyroid Assay (CTA), with a Lactational Exposure Assessment in Sprague Dawley Rats.

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I. CONCLUSIONS:

The Agency has reviewed the draft study protocol for a definitive Oral (gavage) Comparative Thyroid Assay (CTA) with a lactational exposure assessment in Sprague Dawley rats for DCPA. Recommendations on the submitted protocol are summarized below in the Discussion section.

II. ACTION REQUESTED:

The registrant would like for the Agency to comment on their definitive CTA protocol. To provide comments on dose selection, the registrant has submitted both of their completed dose range-finding studies (MRID 50663603 and 51591701) for the Agency to review and method validation studies for the detection of T3, T4, and TSH (MRID 50663602 and 50663601).

III. BACKGROUND:

Dimethyl tetrachlorophthalate (DCPA) is a chlorinated benzoic acid herbicide whose pesticidal mode of action involves the inhibition of cell division of root tips in target plants. DCPA is used to control many annual grasses and broadleaf weeds for a variety of agricultural crops and ornamental varieties (e.g., broccoli, onions, tomatoes, cabbage, cauliflower, dogwood, and azaleas). Following oral administration of DCPA to rats, the affected target organs include the liver, the thyroid, and the kidney. Chronic administration of DCPA led to the development of thyroid follicular cell adenomas/carcinomas (rats), hepatocellular adenomas/carcinomas (rats), hepatocholangiocarcinomas (rats), and hepatic adenomas (mice).

In 2002, the Agency recommended that a confirmatory study showing the comparative short-term thyroid toxicity of DCPA in adults and offspring be required. In addition, the Agency recommended that the study include an evaluation of thyroid hormone levels and liver induction (D281320, T.C. Dole, 08-JUL-2002). In 2011, the Agency identified the CTA as a data gap and affirmed its 2002 recommendation that the study be required (D386637, C. Olinger, 27-MAY-2011).

In 2013, following a 90-day Data Call-In (DCI) regarding the Health Effects Division (HED) toxicology data requirements included in the Human Health Risk Assessment Scoping Document on DCPA, the registrant submitted four protocols for the Agency to review. Following review of one protocol, the single and repeat exposure dose range-finding study protocol with DCPA in male and female juvenile Sprague Dawley rats, the Agency identified concerns and concluded that further review of the other three submitted protocols was not warranted. Before conducting any thyroid assays, the Agency recommended that the registrant submit for review a new range-finding protocol for juvenile (PND 11) rats and the range-finding study on young adult rats that the registrant referred to in the aforementioned protocol (TXR 0056835, D413170, L. Taylor, 19-NOV-2013).

In 2014, the Endocrine Disruptor Screening Program (EDSP) Tier 1 Assay Weight of Evidence Review Committee (T1WoERC) of the Office of Pesticide Programs (OPP) and the Office of Science Coordination and Policy (OSCP) conducted a weight-of evidence (WoE) analysis of the potential interaction of DCPA with the thyroid hormone pathway. The T1WoERC and OSCP concluded, based on a WoE approach, that DCPA demonstrated a potential for interaction with the thyroid hormone pathway in the absence of overt or systemic toxicity. In the male pubertal assay (MRID 48615905) (PND53), serum thyroxine (T4) levels were decreased in a dose-dependent manner at dose levels ≥500 mg/kg/day ($p<0.001$, ↓78-81%). In the female pubertal assay (MRID 48615905) (PND 42), serum T4 levels were decreased in a dose dependent manner at ≥500 mg/kg/day ($p<0.001$, ↓47-55%). In the amphibian metamorphosis assay (AMA) (MRID 48670304), an increased incidence of thyroid gland hypertrophy and increased follicular cell height were observed. In addition, there was concern that the point of departure (POD) may not be protective of potential thyroid toxicity in the young. Therefore, the EDSP T1WoERC and OSCP recommended that the registrant conduct a special thyroid assay in pregnant animals, fetuses, postnatal animals, and adult animals (TXR 0057165, G. Akerman, 29-JUN-2015).

In 2015, following a meeting with the Agency, the registrant submitted a revised CTA dose range-finding study protocol. First, the registrant planned to measure thyroid hormone levels following the administration of propylthiouracil (PTU) as a positive control while optimizing collection of blood from young animals (MRID 50357301). Then, the registrant proposed to conduct a Phase I range-finding study to optimize doses as well as sampling times and to determine whether DCPA was sufficiently present in maternal milk, eliminating the need to directly dose the pups. Also, the registrant proposed to modify their methods of kill and anesthesia. The Agency concluded that the protocol revisions were adequate. The Agency recommended that the registrant submit the positive control data and the results from the dose range-finding study before beginning a definitive CTA study (TXR 0054026, L. Taylor, 16-APR-2015).

In 2017, the Agency reviewed the preliminary Phase I-a results from the dose range-finding pre- and post-natal developmental thyroid study (MRID 50663603) to provide comments on the time-point for thyroid assessment and dose selection. Also, the Agency reviewed a positive control data report using PTU, a method validation report using LC-MS/MS to measure T3 and T4, and an immunoassay methodology that was used to measure all thyroid hormones (T3, T4, and TSH). The Agency concluded that the T3 and T4 validation data using LC-MS/MS was adequate, thyroid hormones levels (T3, T4, and TSH) be measured by LC-MS/MS, and the registrant provide adequate validation data for TSH using LC-MS/MS. Then, the Agency recommended that the registrant conduct a new range-finding study with the same dose levels (used in Phase I-a) and assess all thyroid hormone levels two hours post dose administration using LC-MS/MS. Given that thyroid hormone levels in the positive control data were assessed using the immunoassay method and the registrant planned to use LC-MS/MS instead of the immunoassay, the Agency concluded that the positive control data was not useful in demonstrating that the testing facility could successfully measure thyroid hormone levels. The Agency recommended that the new range-finding study provide thyroid hormone data, and incorporate all aspects such that the results would directly determine the dose levels, time points, and the potential for DCPA to be transferred in the milk avoiding the necessity of the direct dosing of pups in the definitive study (TXR 0057666, D444017, L. Taylor, 16-NOV-2017).

In 2019, the Agency reviewed a study plan for the dose range-finding and definitive CTA that was submitted by the registrant. The Agency recommended that the registrant submit a detailed study protocol that includes only the Phase I portion with the DCPA measurements in milk and thyroid hormone measurements in serum. While the Agency concluded that HPLC-MS/MS was adequate to measure T3, T4, and DCPA in both plasma and milk, the Agency recommended that the final study report contain internal standards and calibration curves. In reference to the immunoassay, the Agency recommended that the testing facility provide method validation data for the assessment of thyroid hormones to demonstrate similar results as those indicated by the manufacturer (TXR 0057935, D420813, O. Triplett, 17-SEP-2019). In 2020, the Agency concluded that the updated protocol submitted by the registrant for the range-finding study was adequate with recommendations (TXR 0057999, D456384, O. Triplett, 19-MAR-2020).

Currently, the registrant has submitted two method validations (MRID 50827701 and 50827702) in rat milk and rat plasma, respectively, to detect DCPA using Liquid Chromotography with Tandem Mass Spectrometric Detection (LC-MS/MS); two method validation studies (MRID

50663601 and 50663602) to detect thyroid hormone levels by immunoassay (TSH) and LC-MS/MS (T3 and T4), respectively; two dose range-finding studies in the rat (MRID 50663603 and 51591701); and a draft protocol for a definitive CTA dated July 2021 to be conducted by Covance Limited Laboratories Eye, UK that was submitted by AMVAC Corporation.

IV. DISCUSSION:

The definitive CTA study protocol, dose range-finding study results, and method validation studies were reviewed by the Agency. The recommendations from this review are provided below:

Specific Recommendations on the Submitted Study Design

Section 1: Introduction

1.2 Location of Study Procedures: "All in-life animal procedures will be conducted at Covance Eye."

Agency Comment: The Agency recently discovered that Covance purchased Envigo. While the previous studies, including method validation and dose range-finding studies, were conducted at Envigo, the definitive CTA will be conducted at Covance. The Agency recommends that the registrant clarify whether the definitive study will be performed at the same facility where the method validations and range-finding studies occurred as there are potential concerns regarding potential inconsistencies in standard operating protocols across two different laboratories.

1.6 Rationale for Dose Selection: "In this study, dose levels of 0.1, 1, 10, and 100 mg/kg/day DCPA will be investigated. Low and intermediate dose levels will be evaluated to understand any dose related trends. A dose level of 2 mg/kg/day will be used for the PTU positive control groups as this was tolerated in MRID 51591701 and caused the expected effects on the thyroid, including decreased T4 and T3, increased TSH, increased thyroid weight and thyroid follicular cell hyperplasia and hypertrophy."

Agency Comment: The most recent chronic dietary endpoint for DCPA is based on a decrease in serum T4 hormone levels and thyroid histopathology in the rat chronic/carcinogenicity study at the LOAEL of 10 mg/kg/day. The chronic dietary POD is 1 mg/kg/day. The Agency recommends 0.05, 0.1, 1, and 10 mg/kg/day as the dose levels for the definitive CTA to identify a clear NOAEL and LOAEL.

Abnormal changes at the 0.1 mg/kg/day dose level, which include an increase in triiodothyronine (T3) hormone levels in female fetuses (↑20%), and a decrease in plasma thyroid stimulating hormone (TSH) levels in male and female fetuses (↓25% and ↓21%, respectively) are observed. The Agency recommends that this dose level be repeated in the definitive CTA with an increased sample size, as a decrease in TSH is not typically observed in the suite of hormone changes. In female fetuses, a dose response with T4 tracks with the pubertal assay results at ≥1mg/kg/day. At 10 mg/kg/day, T4 levels were significantly decreased in male and female fetuses (↓74% and

↓77% ($p<0.01$), respectively), and the results from the range-finding studies suggest that the fetus is the most sensitive life-stage to DCPA compared to the offspring and adults.

Therefore, the Agency recommends 0.05 mg/kg/day as the low dose, 10 mg/kg/day as the high dose for the definitive CTA study, and a dose level of 2 mg/kg/day for the positive control, PTU.

Section 2: Proposed Schedule

2.3 Study Design-Phase II: "Treatment on Phase II is confined to pregnant/lactating females since evaluation of milk and plasma DCPA levels in MRID 51591701 demonstrated that DCPA is bioconcentrated in milk at much higher concentrations than in dam plasma."

Agency Comment: The Agency agrees that the method validation study for the detection of DCPA in milk is acceptable (MRID 50827701). In the dose range-finding study (MRID 51591701), detectable levels of DCPA are present in the milk above the limit of quantitation at dose levels ≥ 0.06 mg/kg/day and the levels of DCPA in the milk increase with dose. Thus, the Agency agrees that the direct dosing of pups is not necessary for the definitive CTA.

Section 6. Test System

6.1.5 Identification

Numbering: Unique for each adult animal. All F1 offspring numbered individually within each Litter on Day 1 of age.

Method: Micro-chip (adult animals) – post mating;
F1 (F1 generation offspring) – toe tattoo

Cage Labels: Uniquely identifying the occupant(s)

Agency Comment: The Agency recommends that the study is blinded.

Section 7. Serial Observations

7.1 General Observations/ Measurements

7.1.2 Mortality:

Premature sacrifice Animals found dead, or killed for reasons of animal welfare	Animals may be killed for reasons of animal welfare. A necropsy is performed as soon as possible.

Agency Comment: The Agency recommends that dams dying during the study should be examined macroscopically for any structural abnormalities or pathological changes that may have influenced pregnancy.

7.1.3 Bodyweight: "All adult animals Phase I: Days 2, 3, 6-20 after mating
Phase II: Days 0, 3, 6-20 after mating and PND 1, 4, 7, 11, 14,18, 20 and 21"

Agency Response: The Agency recommends that animals should be weighed on day 0, at termination, and at least at 3-day intervals during the dosing period.

7.2 Thyroid hormone analysis

- "The target time of blood sampling for measurement of thyroid hormone levels is 10:00-14:00 hours: The time of sampling at each time point will be balanced across the groups to minimize any potential confounding effect introduced by time variation."

Agency Comment: Due to the circadian rhythms of thyroid hormones, the Agency recommends that sample collection should occur at approximately the same time of day, randomized across dosage groups, preferably in the morning hours at which time the basal levels should be present.

- "Anesthetic Isoflurane (target maximum of 2 minutes of anesthesia) – the time taken to anesthetize each animal will be recorded in the raw data"

Agency Comment: The Agency recommends that decapitation be the preferred method of sacrifice and the registrant provide information to show that the anesthetic does not impact thyroid hormones.

7.2.1 Adults

Animals	**Time Point Relative to Dose Administration**
Phase I – Groups 1-6 females will be sampled on Day 20 after mating	2 hours (± 10 minutes) after last dose administration (132 samples)
Phase II – Groups 7-12 females will be sampled on PND 21	2 hours (± 10 minutes) after last dose administration (132 samples

Agency Comment: The Agency recommends that the thyroid hormone levels for all lifestages be measured three hours following the administration of DCPA instead of two hours as suggested by the registrant. The metabolism data (MRID 42262601) suggests that after three hours, the peak level of the radiolabeled compound is present in the blood at a low dose (1 mg/kg). In addition, newly submitted milk transfer data demonstrate that three hours post-dosing provides sufficient time for the chemical to pass through the milk, as the data show that residues in the milk are detected above the limit of quantitation at dose levels ≥ 0.06 mg/kg/day.

- **T3 and T4 analysis:** "Details such as preparation and handling of calibration standards and QC samples, as well as information about standard solutions will be documented in the analytical raw data. Working solutions, calibration standards and QC samples may be shared with related studies. Where this occurs, appropriate details will be documented in the report"

Agency Comment: The Agency recommends that a reference and/or an internal standard be used during thyroid hormone analysis, as appropriate for the assay.

- **Acceptance criteria:** "Based on the fact that thyroid hormones are endogenous compounds, the acceptance criteria are widened. The RE of at least 67% of the QC samples overall within the batch should be within ±20% of their nominal concentration, including at least 50% at each concentration level."

Agency Comment: The Agency recommends that the relative error (RE) of at least 67% of the QC samples overall within the batch should be within 20% of their nominal concentration including at least 75% at each concentration level.

- **TSH analysis:** "Serum samples will be analyzed using Luminex Milliplex MAP assay (method number IAI/0017 with a lower limit of quantification of 123 pg/mL, factoring in dilutions); this method has been validated under Covance Study Number SL13SG."

Agency Comment: The Agency acknowledges that the registrant plans to use LC-MS/MS to measure serum thyroid hormone levels (T3 and T4) and Luminex to measure plasma TSH levels. The Agency recommends that the registrant use LC-MS/MS rather than Luminex as the method to measure all thyroid hormone levels, including TSH. Previously, the registrant and the Agency concurred that LC-MS/MS would be the preferred method to analyze serum hormone levels of TSH, and the registrant agreed to submit TSH validation data using LC-MS/MS. In the dose range-finding study (MRID 51591701), wide variability was observed in collecting TSH measurements. In the method validation for the measurement of TSH via Immunoassay (MRID 50663602), the registrant notes that both the inter- and intra-assay precision variability is ≤ 30%; however, in the assay characteristics from the manufacturer, the variability for inter- and intra-assay precision is 10% and 15%, respectively. If the registrant decides to analyze TSH levels by LC-MS/MS, then the Agency recommends that the registrant submit TSH validation data via LC-MS/MS.

7.2.2 Phase I - Fetuses: Groups 1-6:
"Sample Collection: Pooled fetal blood from each litter (not by sex)"

Agency Comment: The fetal blood should be collected and pooled by sex within litters for biochemical analysis.

7.2.3 Juveniles: Groups 7-12: "PND 4 (two animals of one sex/litter; nominally males from litters of dams with an odd animal number and, females from litters of dams with an even animal number."

Agency Comment: The Agency recommends that on PND 4 and PND 21, pup blood should be collected from one randomly chosen male and female offspring per litter. If necessary, to increase sample volume, blood from a given number or all culled pups may be pooled by sex within litters.

Section 8. Terminal Investigations
"The methods of sacrifice will be appropriate for the life stage being evaluated and the data to be collected."

Agency Comment: The Agency recommends decapitation as the preferred method of sacrifice. In addition, the agency recommends that all animals are sacrificed by the same method regardless of life stage. If the registrant elects to use a different method of sacrifice, then the agency recommends that the registrant provide data to show that hormone levels are not impacted by this method.

8.1.10 Fixation: "Thyroid ((with parathyroid) including section of trachea) and liver from two offspring (1 male and 1 female, where possible except PND 4) from each litter at each stage of development (fetus/PND 4 and 21)."

Agency Comment: The Agency recommends that pup thyroids should be collected on PND 4 and PND 21 from one randomly selected male and female per litter for pathological analysis.