# Petitioner AMVAC Exhibit 43

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

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

**OFFICE OF CHEMICAL SAFETY**
**AND POLLUTION PREVENTION**

**MEMORANDUM**

**DATE:** **March 21, 2022**

**SUBJECT**: **DCPA.** A Review of the Existing Thyroid Toxicity Data and Residual Uncertainty Related to the Lack of a Definitive Comparative Thyroid Assay

**PC Code:** 078701
**Decision No.:** 581460
**Petition No.:** N/A
**Risk Assessment Type:** N/A
**TXR No.:** 0058250
**MRID No.:** N/A

**DP Barcode:** D464520
**Registration No.:** N/A
**Regulatory Action:**
**Case No.:** N/A
**CAS No.:** 1861-32-1
**40 CFR:** N/A

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**TO**: James Douglass, Chemical Review Manager
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## I. CONCLUSIONS:

The Agency has reviewed the existing thyroid toxicity data for dimethyl tetrachlorophthalate (DCPA) and concludes that a scientifically robust and defensible human health risk assessment cannot be conducted in the absence of data from the definitive comparative thyroid assay (CTA) which has not yet been completed and submitted to the Agency. While HED continues to have concerns related to the potentially greater toxicity to fetuses suggested by the range finding CTA, without a comprehensive definitive CTA substantiating and clarifying these results, drawing scientifically defensible conclusions about the degree of concern for these potential effects is not possible.

## II. ACTION REQUESTED:

The Pesticide Reevaluation Division (PRD) requested that the Health Effects Division (HED) summarize the available thyroid toxicity data for DCPA and describe the remaining uncertainties and concerns regarding lifestage sensitivities in the absence of a definitive CTA.

## III. BACKGROUND:

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). In several guideline studies (subchronic and chronic) in which DCPA was administered to rats, the affected target organs included the liver, thyroid, and kidney. While thyroid hormones were not examined following subchronic exposure, thyroid toxicity including follicular cell hypertrophy, increased clumped colloid, increased cystic follicles, and follicular cell hyperplasia was observed in adult Sprague-Dawley rats at dose levels above the established lowest-adverse-effect-level (LOAEL). Following chronic exposure to DCPA, thyroid toxicity including decreased ($p<0.01$) thyroid hormone (T4), increased basophilic clumped colloid, increased follicular cell hyperplasia, and increased follicular cell hypertrophy was observed in adult Sprague-Dawley rats. Furthermore, chronic administration of DCPA led to the development of thyroid follicular cell adenomas/carcinomas, hepatocellular adenomas/carcinomas, and hepatocholangiocarcinomas in rats, as well as hepatic adenomas in mice. In the rat multigeneration reproductive toxicity study, thyroid toxicity (increased basophilic clumped colloid and increased follicular cell hypertrophy) was observed in the parental Sprague-Dawley rats at the LOAEL.

In 2002, HED recommended the requirement of a CTA which evaluates the impact of DCPA on thyroid hormone levels, and thyroid toxicity in adults and offspring (fetal and post-natal lifestages) (D281320, T. Dole, 08-JUL-2002). In 2011, HED identified the CTA as a data gap and affirmed its 2002 recommendation that the study be required (D386637, C. Olinger, 27-MAY-2011). Furthermore, the Endocrine Disruptor Screening Program (EDSP) Tier I Assay Weight of Evidence Review Committee (T1WoERC) of the Office of Pesticide Programs (OPP) and the Office of Science Coordination and Policy (OSCP) concluded based on a weight-of evidence (WoE) approach that DCPA demonstrated a potential for interaction with the thyroid hormone pathway and recommended that the registrant conduct a CTA (TXR 0057165, G. Akerman, 29-JUN-2015). Since 2013, HED has provided the registrant with feedback on submitted CTA protocols and the results from two dose range finding studies (MRID 50663603 and 51591701) to inform dose selection for the definitive CTA (see Appendix I for a complete timeline of communication between the Agency and the registrant).

## IV. RESULTS AND DISCUSSION

In 2018, the registrant completed an oral gavage dose range finding CTA that measured thyroid toxicity following gestation (MRID 50663603). During gestation days (GD) 6-20, ten female Sprague-Dawley rats were administered DCPA in aqueous 0.5% (w/v) methylcellulose solution via oral gavage at dose levels of 0 or 100 mg/kg/day, and five female Sprague-Dawley rats were

administered DCPA in aqueous 0.5% (w/v) methylcellulose solution via oral gavage at dose levels of 0.1, 1, or 10 mg/kg/day. In the maternal animals on GD 20, the following parameters were examined: mortality, clinical observations, bodyweight, organ weights (liver and thyroid/parathyroid), uterine contents, gross pathology, histopathology, and the concentration of thyroid hormones in serum (T3 and T4) and plasma (TSH). In the fetuses on GD 20, fetal/litter parameters, gross pathology, and the concentration of thyroid hormones in serum (T3 and T4) and plasma (TSH) were examined.

In maternal animals on GD 20, there were no adverse effects of treatment on mortality, body weight, organ weights, gross pathology, or cesarean section parameters. Regarding the impact of treatment on the concentration of thyroid hormones, no treatment-related effects on the serum T3 concentrations were observed. Serum T4 concentrations were decreased ($p<0.01$) by 25% and 50% at 10 and 100 mg/kg/day, respectively, and no treatment-related effects on serum T4 concentrations were observed at ≤1 mg/kg/day. Between 0.1 and 10 mg/kg/day, plasma TSH concentrations decreased in a non-dose response manner between 36-56%, and at 100 mg/kg/day, an increase of 23% in plasma TSH concentration was observed.

In the fetuses on GD 20, there were no effects of treatment on gross pathology and fetal/litter parameters. However, changes in thyroid hormone concentrations were observed in the male and female fetuses. At 0.1 mg/kg/day, T3 concentrations were increased by 16- 20% and TSH concentrations were decreased by 21-25%. Decreases in T3 (13-18%), T4 (17-25%) and TSH (25-36%) were noted at 1 mg/kg/day. At dose levels ≥10 mg/kg/day, T3 concentrations were below the limit of quantification (BLQ; <5.00 pg/mL) in males and decreased ($p<0.01$) 65-67% in females, and T4 concentrations were decreased ($p<0.01$) by 74-88%, while TSH was decreased between 10-57%.

Based on the results from this study, the dose levels of DCPA that were selected for a range-finding CTA that investigated milk transfer and thyroid hormone levels in dams and offspring following lactation included 0.01, 0.1, 1, or 10 mg/kg/day.

In 2021, the registrant completed a dose range-finding CTA examining milk transfer and thyroid hormone levels following lactation (MRID 51591701). In this study, groups of six pregnant Sprague-Dawley female rats per dose level were administered DCPA in aqueous 0.5% (w/v) methylcellulose solution via oral gavage at dose levels of 0, 0.01, 0.1, 1, or 10 mg/kg/day (target concentrations) during gestation days (GD) 6-20 and lactation days (LD) 1-21. A positive control group containing six rats was administered 6-propyl-2-thiouracil (6-PTU) at 2.0 mg/kg/day from GD 6 to LD 21. In the study, there were issues with dose formulation preparation and a lack of homogeneity data making quantitative use of the concentration data difficult; however, the study results qualitatively showed the presence of DCPA in maternal milk samples.

Taken together, the preliminary data suggests that the fetus is potentially more sensitive to DCPA-induced alterations in thyroid hormone levels compared to maternal animals. At 0.1 mg/kg/day, alterations in fetal thyroid hormones were observed (↑16-20% serum T3 and ↓21-25% plasma TSH in both sexes) in the absence of alterations in thyroid hormone levels in GD 20 dams at this dose level, which provides evidence of a potential lifestage sensitivity. For the

definitive CTA, HED recommended that this dose level (0.1 mg/kg/day) be repeated with an increased sample size (TXR 0058205, D462323, J.P. Rogers, 15-JUL-2021). In the 2005 guidance on thyroid assays in pregnant animals, fetuses, postnatal animals, and adult animals, HED recommended that a sufficient number of pregnant animals be exposed to the test substance to ensure an adequate sample size for hormonal and histological evaluations. Furthermore, HED recommended at least forty litters per dose level. Twenty litters per treatment group should be assigned to the prenatal testing subset and twenty litters per treatment should be assigned for the postnatal evaluations. In addition, HED recommended that a lower dose be included in the definitive study to determine a clear no-observed-adverse-effect-level (NOAEL) (TXR 0058205, D462323, J.P. Rogers, 15-JUL-2021).

The potentially adverse thyroid hormone variations were shown to occur in the fetal lifestage at a dose level lower than the points of departure (PODs) used in the most recent risk assessment conducted in 2002 (D281320, T. Dole, 08-JUL-2002). In the 2002 human health risk assessment, the 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 lowest-observed-adverse-effect-level (LOAEL) of 10 mg/kg/day and a NOAEL of 1 mg/kg/day. The endpoint for both incidental oral and inhalation exposures was based on hepatocellular hypertrophy in the subchronic rat toxicity study at the LOAEL of 100 mg/kg/day and a NOAEL of 50 mg/kg/day. Therefore, the thyroid hormone perturbations in the fetal lifestage were observed at dose levels 10-500X lower than the current PODs used for assessing DCPA risk. In addition, a dermal endpoint was not selected in the 2002 assessment because there was no systemic toxicity occurring in the dermal toxicity study at the high dose of 1000 mg/kg/day (D281320, T. Dole, 08-JUL-2002); however, given the concern for potential lifestage sensitivity, the need for a dermal endpoint would be reassessed after the submission of the definitive CTA data. This suggests that the last human health risk assessment may not be protective of a potentially sensitive lifestage (i.e., the growing fetus).

In conclusion, the preliminary DCPA data evaluated by HED (MRID 51591701 and 50663603) provides evidence that the fetus is potentially more sensitive to thyroid function perturbations due to DCPA exposure compared to the maternal animals. Given the potential fetal sensitivity, HED has concerns for exposures to pregnant females. Currently, the Agency does not have the ability to properly assess all sensitive lifestages to DCPA exposure, including the fetal compartment, due to the outstanding CTA data. Because thyroid hormone perturbations in the fetal lifestage were observed at dose levels significantly lower than the current PODs, applying a standard uncertainty factor of 10X to account for this missing data may not be health protective. In the absence of the required definitive CTA, HED cannot make a reliable determination of "reasonable certainty of no harm" for aggregate exposures under the Food Quality Protection Act (FQPA), or a safety finding for occupational exposures under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

## V. APPENDIX I

| Year | Actions Taken by Registrant | Actions Taken by EPA |
|---|---|---|
| 2002 | | EPA publishes Human Health Risk Assessment recommending CTA be required (D281320, T. Dole, 08-JUL-2002). |
| 2011 | | EPA publishes DCPA scoping document where the CTA is identified as a data gap and recommended that the study be required (D386637, C. Olinger, 27-MAY-2011). |
| 2013 | | EPA issues 90-day Data Call In (DCI) requiring the CTA. |
| 2013 | The registrant submitted the following four protocols to the Agency.<br>• Huntingdon Life Sciences Protocol. HLS Enquiry # 53284 dated 4/9/13. DCPA (Chlorthal Dimethyl): Single and Repeat Exposure Dose Range Finding Study in Male and Female Juvenile Crl:CD(SD) Rats by Oral Gavage Administration.<br><br>• Huntingdon Life Sciences Protocol. HLS Enquiry # 53284/34, dated 4/24/13. DCPA (Chlorthal Dimethyl): Single Dose Comparative Thyroid and Thyroid Hormone Study in Young Adult and 11 Day Old Juvenile CD Rats by Oral Gavage Administration.<br><br>• Huntingdon Life Sciences Protocol. HLS Enquiry # 53284/35, dated 4/24/13. DCPA (Chlorthal Dimethyl): Repeat Dose Comparative Thyroid and Thyroid Hormone Study in Young Adult and 11 Day Old Juvenile CD Rats by Oral Gavage Administration.<br><br>• Huntingdon Life Sciences Protocol. HLS Enquiry # 53284/36, dated 4/24/13. DCPA (Chlorthal Dimethyl): Gestational Exposure Comparative Thyroid and Thyroid Hormone Study in the CD Rat by Oral Administration. | HED recommended that the registrant submit for review a new range-finding study protocol incorporating HED's previous recommendations.<br>(TXR 0056835, D413170, L. Taylor, 19-NOV-2013). |
| 2015 | The registrant submitted a revised CTA dose range-finding study protocol with a phased approach (HLS Study # HLS1095; HLS Study BDG0204; HLS Study BDG0202). | HED 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, D424915, L. Taylor, 16-APR-2015). |
| 2015 | | EDSP Tier I Assay Weight of Evidence Review Committee (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). |

| Year | Actions Taken by Registrant | Actions Taken by EPA |
|---|---|---|
| 2017 | The registrant submitted a positive control study and pre-natal developmental thyroid dose range finding study.<br><br>• MRID 50357301: PTU (Propylthiouracil): positive control pre- and post-natal developmental thyroid study in Sprague-Dawley or Han Wistar Rats by oral administration or when untreated.<br><br>• MRID 50663603: Dose range finding prenatal developmental thyroid study in Sprague-Dawley rats by oral administration. | HED recommended that the new range-finding study 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). |
| 2019 | The registrant submitted a draft study plan for a dose range finding and definitive CTA.<br><br>• Study Plan: JW36WK<br>DCPA: Dose range finding pre- and post-natal developmental thyroid study (including a PTU positive control group and milk investigation) in Sprague-Dawley rats by oral administration | HED recommended a tiered approach to conducting the studies proposed in the submitted study plan. HED recommended that the registrant submit a detailed study protocol that includes only the phase one portion (range finding study) with the DCPA measurements in milk and if possible, some thyroid hormone measurements in serum. Also, HED recommended that the final study report contain internal standards and calibration curves. In reference to the immunoassay, HED 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. HED recommended that the phase one range-finding study be used to optimize doses and sampling times, and to determine whether direct dosing of pups will be necessary for the definitive study (TXR 0057935, D420813, O. Triplett, 17-SEP-2019). |
| 2020 | The registrant submitted an updated study protocol (containing data from a dose-range finding study) for a pre-natal and post-natal developmental thyroid dose range finding study.<br><br>• Study Plan: PM86YP<br>DCPA (Chlorthal Dimethyl): Dose Range Finding Comparative Thyroid Assay Investigating Milk Transfer and Thyroid Hormone Levels in Dams and Pups (Including a PTU Positive Control Group) in Sprague-Dawley Rats by Oral Administration | HED concluded that the updated protocol submitted by the registrant for the range-finding study was adequate with additional recommendations made by HED at that time. For maternal and offspring milk sampling, HED recommended that the LC-MS/MS methods were appropriately validated before beginning the study and any validation methodology and data be included in the final report. For measuring TSH using an immunoassay kit, HED recommended that the sensitivity limit of the assay should be tested and determined within the lab with the standards provided in the kit in addition to serum samples from the positive control animals or serum with a known hormone profile using internal quality control practices. (TXR 0057999, D456384, O. Triplett, 19-MAR-2020). |

| Year | Actions Taken by Registrant | Actions Taken by EPA |
|---|---|---|
| 2021 | The registrant submitted the pre-natal and post-natal developmental thyroid toxicity study report and a protocol for the definitive CTA to the Agency.<br><br>• MRID 51591701: Dose range-finding comparative thyroid assay investigating milk transfer and thyroid hormone levels in dams and pups (including a PTU positive control group) in Sprague-Dawley rats by oral administration.<br><br>• Study Plan: Covance Study Number 8432592. DCPA: Main Pre and Post Natal Developmental Comparative Thyroid Study in CD Rats by Oral Administration | HED provided the registrant with comments on dose selection and recommendations for the definitive CTA (J.P. Rogers, TXR 0058205, D462323, 15-JUL-2021). |