Joint Exhibit 6

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

WASHINGTON, D.C. 20460

Office of Chemical Safety & Pollution Prevention

MEMORANDUM

Date: November 19, 2013

SUBJECT: **DCPA:** HED Review of the Comparative Thyroid Toxicity Study Protocols.

PC Code: 078701	**DP Barcode:** D413170
Decision No.: NA	**Registration No.:** GDCI-078701-1140
Petition No.: NA	**Regulatory Action:**
Risk Assessment Type: NA	**Case No.:** NA
T**XR No.:** 0056835	**CAS No.:** 1861-32-1
MRID No.: NA	**40 CFR:** 180.185

FROM: Linda Taylor, Ph.D.
Risk Assessment Branch VII
Health Effects Division (7509P)

THRU: Michael Metzger, Branch Chief
Risk Assessment Branch VII
Health Effects Division (7509P)

TO: Jill Bloom, Risk Manager Reviewer
Risk Management and Implementation Branch 2
Pesticide Re-evaluation Division (7508P)

I. CONCLUSIONS: The protocols are inadequate. A new protocol for a range-finding study for juvenile (PND 11) rats should be drafted and submitted for Agency review before any thyroid assays are performed. The range-finding study on young adult rats, referred to in the repeat dose comparative protocol, should be submitted for Agency review also. The Agency recommends that the registrant consider the Agency's 2005 guidance document: Thyroid Assays in Pregnant Animals, Fetuses and Postnatal Animals, and Adult Animals and consult with the Agency prior to revision of the protocols.

II. ACTION REQUESTED: Please review the protocols for the required comparative thyroid

toxicity test.

III. BACKGROUND: In the DCPA 90 day DCI response regarding the HED toxicology data requirements included in the Human Health Assessment Scoping Document in Support of Registration Review on DCPA (D386637), AMVAC Chemical Corporation submitted the following comparative thyroid toxicity protocols.

- 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.
- 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.
- 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.
- 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.

IV. RESULTS/DISCUSSION: The protocols were presented to the HED's Toxicology Science Advisory Council (ToxSAC) for review on September 19, 2013. Based on serious concerns identified in the protocol for the range-finding study for juvenile rats (see Table 1), further review of the other protocols was not warranted. The following concerns were identified in the range-finding study protocol by ToxSAC.

Single/Repeat Exposure Dose Range Finding Study in Male and Female Juvenile Rats. The study protocol is unclear and not adequate. The stated objective is to determine suitable doses for single and repeat (11 days) oral administration of DCPA to 11-day old juvenile male and female CD rats for use in the definitive single dose and repeat dose comparative thyroid and thyroid hormone studies in 11-day old juvenile CD rats.

A summary of the Agency's concerns for the range finding protocol and general comments applicable to all the protocols are outlined below:

1) The Agency questions the utility of this range-finding study, as described in the protocol, based on the limited observations to be performed. Since thyroid hormone measurements are not among the parameters to be assessed, it is not clear what parameter will be used for dose selection. The range-finding study needs to include thyroid hormone measurements.

2) The dose levels to be used should be specified.. The doses referred to in the protocol are not appropriate. See Table 1 for specific recommendations.

3) Regarding the 6 time-mated females to be used, shipping should not be close to delivery date of the dams; the same age of dams should be used for all studies (range-finding, acute,

and repeat studies); and the gestation day on arrival/at time of receipt should be documented.

4) Litters should not be pooled (no cross fostering). Natural litters should be used.

5) There needs to be a discussion of the time of dosing relative to the time of sample collection (hormone measurements).

6) The method of sacrifice is an important aspect of these studies and should be consistent across studies, with justification provided as to why the method will not impact hormone levels.

7) The purity of the test material should be specified with the same test material (purity of a.i.), consistent with the database, used across all studies.

8) The various sections of the range-finding protocol should be separated into an acute component and a repeat component.

The individual sections of the range-finding protocol are discussed in the appended Table 1, which highlights the issues that need to be addressed prior to any further assessment of the data requirement for the comparative thyroid assays.

The range-finding study in PND 11 rats and the range-finding study performed in young adult rats (referenced in the range-finding protocol) should be submitted to the Agency, along with revised protocols for the other thyroid studies. The Agency recommends that the registrant consider the Agency's 2005 guidance document: Thyroid Assays in Pregnant Animals, Fetuses and Postnatal Animals, and Adult Animals and consult with the Agency prior to revision of the protocols.

V. CONCLUSION: The protocols are inadequate. A new protocol for a range-finding study for juvenile (PND 11) rats should be drafted and submitted for Agency review before any thyroid assays are performed. The range-finding study on young adult rats, referred to in the repeat dose comparative protocol, should be submitted for Agency review also. The Agency recommends that the registrant consider the Agency's 2005 guidance document: Thyroid Assays in Pregnant Animals, Fetuses and Postnatal Animals, and Adult Animals and consult with the Agency prior to revision of the protocols.

Table 1: DCPA - Comparative Thyroid Toxicity Protocol Review

	Single/Repeat Exposure Dose **Range Finding** Study in Male and Female Juvenile Rats.
1.2.	Stated Objective: To determine suitable doses of DCPA for single and repeat (11 day) oral administration to 11-day old juvenile male and female CD rats for use on subsequent single and repeat dose comparative thyroid and thyroid hormone studies in young adult and 11-day old juvenile CD rats. **(a) This range-finding protocol is for PND 11 rats only; young adult rats are not included; however, a range-finding study in young adult rats is referred to in the repeat dose comparative protocol and that range-finding study should be submitted to the Agency.** (b) **The Agency questions the utility of this range-finding study in PND 11 pups since thyroid hormone measurements are not being performed. What will be the basis for the dose selection for subsequent studies?**
1.8.	Treatment groups: one control and 4 treatment groups are listed; but actual doses not listed here: ‡indicates that initial dose levels selected by Sponsor. (a) **Need to add proposed doses specifically for the PND 11 pups; significant effects on thyroid hormone levels were observed in the 90-day rat study at the 100 mg/kg/day, which suggests that the dose levels noted below in protocol section 1.9 are too high.**
1.9.	Rationale for dose level selection: 0, 100, 300, 600, 1000 mkd; pubertal development/thyroid function in juvenile CD rats; marked ↓ serum T_4 at 500 mkd (♂↓78%/♀↓47%) and 1000 mkd (♂↓81%/♀↓55%); no change in thyroid weight or histopath; no overt toxicity (MRID 48615905); rat developmental toxicity study (dams dosed on GD 6-15 at 2000 mkd: ↓BWG on GD 9-12; no embryotoxicity or teratology). **(a) Registrant should consider lower doses, based on totality of data (T_{max}, ADME, etc.)** **(b) The 1000 mg/kg/day dose level should not be used** **(c) In 90-day study, significant reduction in thyroid hormones was observed at 100 mg/kg/day (diet) as early as two weeks** (d) **Given that the objective of the comparative assays is to determine not only whether there is sensitivity but at what dose level there is no adverse effect, dose levels below 100 mg/kg/day are recommended.**
2.1.	Duration of treatment: states Minimum period: once; Maximum period: 11 consecutive daily doses **Since there are two phases in this range-finding study; i.e., an acute phase and a repeat phase, it is recommended that each section of this protocol specify the particular aspect being addressed:** **(a) acute phase: one dose;** (b) **repeat phase: 11 consecutive daily doses**
2.2.	Study Structure: total of 3 litters (comprising 5 males and 5 females) allocated to study; one/sex from each litter allocated to each study group (within litter design). If impractical then natural litters will be used. As noted above, it is recommended that this section be divided into the two phases and specify that **(a) Acute phase: a total of 3 litters (comprising 5 males and 5 females) allocated to study; one/sex from each litter allocated to each study group.** **(b) Repeat phase: a total of 3 litters (comprising 5 males and 5 females) allocated to study; one/sex from each litter allocated to each study group.** (c) **Also, the Agency recommends that litters not be pooled and that natural litters be used (no cross fostering).**
2.4.	Identity of treatment groups: to be derived from 6 time-mated females ordered to be delivered during gestation. There is one table that shows 3/sex/group for 1 control and 4 treated. As above, it is recommended the section specify that each phase will include 3/sex/group and there will be a control group and 4 treated groups for each phase.

	(a) The table does not list the dose levels, although under protocol section 1.9 the suggested doses are discussed. The symbol ‡ in the table under dose is not identified, but the symbol § in the table legend indicates that initial dose levels selected by the Sponsor. (b) **The dose levels should be included in the protocol. As discussed above under protocol section 1.9, the 1000 mg/kg/day should not be used; dose levels below 100 mg/kg/day are recommended, based on previous studies.**
4.1.1.	Animals (# ordered): 6 time-mated females to provide 3 randomized litters for each phase of the study. **Regarding the 6 time-mated females to be used, shipping should not be close to delivery date; same age of dam should be used for all studies (range-finding, acute and repeat studies); gestation day at time of receipt should be documented.**
4.1.5.	Selection procedure: On Lactation Day 2, where possible, offspring from suitable litters will be pooled and redistributed evenly to provide randomized litters with at least 6 male and 6 female offspring. If this is impractical then natural litters will be used. **Agency recommends that litters not be pooled; no cross-fostering.**
4.3.	Administration: Frequency: Once daily at ≈the same time each day. **(a) This should indicate that this is for the repeat dose phase.** **(b) There should be a statement regarding the single dose phase; *e.g.*, administration of a single dose will be made to coincide with the time of day dose administration occurs in the repeat dose phase.** **(c) Need to consider the time of dosing; should report time of sample collection vs time of dosing** (d) **Should consider ADME, t_{max}, critical window in morning**
5.1.	Clinical observations: In addition observations will be performed on Days 1-11 of **treatment (repeat phase); and on day of treatment (acute phase).** Dose observations listed: pre-dose observation; as each animal is returned to home cage; at end of dosing each group; as late as possible in working day. **Same comment as before: should specify for each phase.**
5.3.	Body weight: Individual weights: Day 10, Day 11 of age (day that treatment commences), Days 13, 15, 17, 19, and 21 of age, and before necropsy. **This should indicate that this is for the repeat dose component.** **The single dose component: Individual weights: Day 10, Day 11 of age (day of treatment), and before necropsy.**
6.1.	Time for kill: Juveniles: Day 22 of age; Day 12 of study. **(a) As above: *e.g.*, Juveniles: Day 22 of age (repeat phase); Day 12 of study (acute phase).** (b) **For acute exposure, sacrifice should not be more than 24 hours after dose**
6.2	Method of kill: carbon dioxide asphyxiation. **(a) Agency notes that there are different methods listed in the other protocols.** **(b) Need to justify why method will not impact hormone levels. In the gestational protocol, killing pups by putting on cool plate would impact thyroid hormone levels (stress response). Would need a control to compare cold plate to no cold plate.** (c) **Agency recommends decapitation should be used for all studies, all phases, and all ages.**
6.5	Histology and pathology. None scheduled. **The Agency questions the utility of this range-finding study, as described in the protocol, based on the limited observations to be performed. Since thyroid hormone measurements are not among the parameters to be assessed, it is not clear what parameter will be used for dose selection. The range-finding study needs to include thyroid hormone measurements (at least) and histopathology to justify the doses in the definitive study.**

7.2	Statistical analysis: will not be performed due to small group size. **This is inappropriate rationale. Agency recommends that the sample size be increased in order to analyze hormone data.**
8.0	Reporting. **(a) It is recommended that preliminary data from the range-finding study be provided to the Agency *via* WORD or spreadsheet for our input.** (b) **Revised protocols for the acute, repeat, and gestational components should be submitted also; it is recommended that the registrant come in to talk to Agency personal regarding these thyroid assays prior to initiation of additional studies. A copy of the Agency's 2005 Guidance Document for Thyroid Assays in Pregnant Animals, Fetuses and Postnatal Animals, and Adult Animals is attached.**