TXR NO. 0051713

DATE: **March 31, 2003**

MEMORANDUM

SUBJECT: CLOTHIANIDIN (TI-435) -2nd Report of the Hazard Identification Assessment Review Committee.

FROM: Pamela M. Hurley
Reregistration Branch 3
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Yan Donovan, Risk Assessor
Health Effects Division (7509C)

PC Code: 044309

On November 14, 2002 the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Clothianidin (TI-435) with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to Clothianidin was evaluated as required by the Food Quality Protection Act (FQPA) of 1996 according to the 2002 OPP 10x Guidance Document. On March 18, 2003, the HIARC evaluated the results of a dermal absorption study in monkeys (MRID No. 45868001) and its impact on the previously selected dermal absorption value. The conclusions drawn at both of these meetings are presented in this report.

Committee Members in Attendance

Members present were: Ayaad Assaad, Jonathan Chen, Donna Davis (RARC representative) Paula Deschamp (RARC representative), Elizabeth Doyle, William Dykstra (alternate), Pamela Hurley, John Liccione, Susan Makris, Elizabeth Mendez, Jess Rowland, PV Shah and Brenda Tarplee

Member(s) in absentia: William Burnam

Data evaluation prepared by: Robert Zendzian, Pharmacologist

Also in attendance were: Scott Hancock (PMRA) via telephone

Data Evaluation / Report Presentation

Pamela M. Hurley
Toxicologist

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INTRODUCTION

On November 14, 2002 the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Clothianidin (TI-435) with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to Clothianidin was evaluated as required by the Food Quality Protection Act (FQPA) of 1996 according to the 2002 OPP 10x Guidance Document. On March 18, 2003, the HIARC evaluated the results of a dermal absorption study in monkeys (MRID No. 45868001) and its impact on the previously selected dermal absorption value. The conclusions drawn at both of these meetings are presented in this report.

I. FQPA HAZARD CONSIDERATIONS

1. Adequacy of the Toxicity Data Base

The HIARC concluded that the toxicology database for Clothianidin is not complete. A complete complement of acceptable developmental, reproduction, developmental neurotoxicity, mammalian neurotoxicity and special neurotoxicity studies are available; however, due to evidence of decreased absolute and adjusted organ weights of the thymus and spleen in multiple studies in the Clothianidin data base, and since juvenile rats in the two-generation reproduction study appear to be more susceptible to these effects, the HIARC recommended that testing be conducted to assess immune system function in adults and in young animals following developmental exposures.

2. Evidence of Neurotoxicity

The HIARC concluded that there is not a concern for neurotoxicity resulting from exposure to Clothianidin.

a. In an acute oral neurotoxicity study (MRID 45422801), TI-435 (Clothianidin; ≥95.2% a.i., Batch # 30037120) in 0.5% methylcellulose/0.4% Tween 80 solution was administered in a single dose by gavage (10 mL/kg) to fasted Fischer 344 rats (12/sex/group) at doses of 0, 100, 200, or 400 mg/kg. The time of peak effect was estimated to be 4 hours post-dosing. After two weeks, 6 animals/sex/group were perfused *in situ* for neurohistological examination and tissues from the control and high-dose groups were examined microscopically. Functional observational battery (FOB) and motor activity were evaluated during Week -1 and on Days 0 (approximately 4 hours post-dosing), 7, and 14. Acceptable positive control data were provided that verify the ability of the observers of the performing lab to conduct the FOB and to assess motor activity, neurotoxicity and behavioral effects.

No treatment-related deaths occurred. Body weights, body weight gains, gross pathology, histopathology, and brain weights were unaffected by treatment.

The following treatment-related FOB effects (# affected/12 vs 0/12 controls, unless otherwise noted) were noted on Day 0: (i) **home cage**; slight to moderate tremors at 400 mg/kg (8 males; 11 females) and decreased activity at 200 (1 male) and 400 (8 males; 11 females) mg/kg; (ii) **open field**; slight to moderate tremors at 400 mg/kg (11 each sex), decreased arousal (sluggish, with some exploratory movement to minimal movement) at 100 (4 treated vs 1 control in males), 200 (5 treated vs 1 control in males; 3 females), and 400 (9 treated vs 1 control in males; 11 females) mg/kg, and slightly uncoordinated gait in one 400 mg/kg male; (iii) **reflex/physiologic**; pupil response (pin point) at 200 (1 male) and 400 (8 males; 9 females) mg/kg, righting reflex (slightly uncoordinated to landing on side) at 200 (2 males) and 400 (2 males; 4 treated vs 1 control in females), and body temperature was decreased ($p \leq 0.05$) in both sexes at 200 (decr 1.3-1.7°C) and 400 (decr 3.7°C) mg/kg. Additionally, clinical signs were observed at 400 mg/kg on Day 0 and included tremors, decreased activity, and ataxia in both sexes.

Total motor activity was decreased (NS) compared to controls at 100 (23%, males only), 200 (43-59%), and 400 (72-81%) mg/kg with decreases ($p<0.05$) observed during intervals 1-3 at 100 (32-41%, males only), 200 (38-80%), and 400 (77-97%) mg/kg. Total locomotor activity was similarly decreased (NS) at 100 (37%, males only), 200 (45-62%), and 400 (83-88%) mg/kg with decreases ($p<0.05$) observed during intervals 1-2 at 100 (40-50% males only), 200 (44-78%), and 400 (81-94%) mg/kg.

The LOAEL for this study is 100 mg/kg based on FOB effects (decreased arousal) and decreased motor and locomotor activity on Day 0 in males. The NOAEL was not established. Neurotoxicity was evidenced by FOB effects (decreased arousal) and decreased motor and locomotor activity on Day 0 in males.

The submitted study is classified as **acceptable/guideline** in conjunction with the supplemental acute neurotoxicity study (MRID 45422802; executive summary below with study summary provided in Appendix III of this DER). Together these studies satisfy the requirements (OPPTS 870.6200a; OECD 424) for an acute neurotoxicity screening battery in rats.

<u>Special Acute Neurotoxicity Study</u> (to establish a No-Observed-Effect-Level of TI-435 in male Fischer 344 rats)

In this acute neurotoxicity study (MRID 45422802), TI-435 (Clothianidin; ≥95.3% a.i., Batch # 30037120) in 0.5% methylcellulose/0.4% Tween 80 solution was administered in a single dose by gavage (10 mL/kg) to fasted male Fischer 344 rats (12/dose) at doses of 0, 20, 40, or 60 mg/kg. Functional observational battery (FOB) and motor activity were evaluated during week -1 and on Day 0 (approximately 4 hours post-dosing). Dose analyses indicated that nominal doses were achieved.

No unscheduled deaths occurred during the study. Clinical signs, FOB, and motor activity were unaffected by treatment.

The LOAEL was not observed. The NOAEL is 60 mg/kg.

The submitted study is classified as **acceptable/guideline** in conjunction with the acute neurotoxicity study (MRID 45422801). Together these studies satisfy the requirements (OPPTS 870.6200a; OECD 424) for an acute neurotoxicity screening battery in rats.

Therefore, LOAEL for the combined studies is 100 mg/kg based on FOB effects (decreased arousal) and decreased motor and locomotor activity on Day 0 in males. The NOAEL is 60 mg/kg.

b. In a subchronic neurotoxicity study (MRID 45422803), TI-435 (clothianidin; ≥95.3% a.i., Batch # 30037120) was administered in the diet for 13 weeks to 12 Fischer 344 rats/sex/group at doses of 0, 150, 1000, or 3000 ppm (equivalent to 0/0, 9.2/10.6, 60.0/71.0, or 177.0/200.1 mg/kg/day in males and females respectively). At termination, 6 animals/sex/group were perfused *in situ* and tissues from the control and 3000 ppm groups were examined microscopically. Functional observational battery (FOB) and motor activity were evaluated during Weeks -1 (prior to dosing), 4, 8, and 13. Acceptable positive control data were provided that verified the ability of the observers of the performing lab to conduct the FOB and to assess motor activity, neurotoxicity and behavioral effects.

No treatment-related mortalities were observed. Clinical signs, ophthalmoscopic observations, FOB, motor activity, absolute brain weights, gross necropsy, and neurohistology were unaffected by treatment.

At 3000 ppm, food consumption was decreased ($p \leq 0.05$) throughout most of the study (5-20%). These findings corresponded to the decreased overall body weight gains (11-13%). Body weights were decreased compared to controls ($p \leq 0.05$) in the males during Weeks 10-12 (4-5%), and in females during Weeks 8 and 13 (4% each).

The LOAEL is 3000 ppm (equivalent to 177.0/200.1 mg/kg [M/F]) based on slightly decreased food consumption, body weights, and body weight gains. The NOAEL is 1000 ppm (equivalent to 60.0/71.0 mg/kg [M/F]).

There was no evidence of neurotoxicity at any dose tested.

The submitted study is classified as **acceptable/guideline** and satisfies the requirements (OPPTS 870.6200b) for a subchronic neurotoxicity screening battery in rats.

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c. In a non-guideline study that addressed some aspects of neurotoxicity and pharmacology (MRID 45422823), groups of three male CD-1 mice were tested for acute neurobehavioral effects following single gavage doses of TI-435 (95.5% a.i.; vehicle, 5% arabic gum) at doses of 0 (5% arabic gum), 12.5, 25, 50, 100, 200, or 400 mg/kg bw. Clinical signs and mortality were monitored from 0.5 hours to 24 hours post-treatment. The following tests were also conducted with groups of eight or ten male CD-1 mice: prolongation of hexobarbital-induced sleeping time following doses of 25, 75, or 225 mg/kg bw; induction of tonic flexor and tonic extensor convulsions with sub-threshold electroshock following doses of 6.25, 12.5, 25, 75, or 225 mg/kg bw; synergistic effect of pentylenetetrazol on convulsions following doses of 25, 75, or 225 mg/kg bw; effect on intestinal transport following doses of 25, 75, or 225 mg/kg bw; and effect on muscle strength following doses of 25, 75, or 225 mg/kg bw. Groups of six male CD rats were tested for effects on body temperature following gavage doses of 30, 100, 300, 1000, or 3000 mg/kg bw. In other tests, groups of four male CD rats were tested for effects on blood pressure and heart rate following doses of 300, 1000, or 3000 mg/kg bw, and groups of six male CD rats were tested for effects on prothrombin and activated thromboplastin times following doses of 300, 1000, or 3000 mg/kg bw. In an *in vitro* study, effects of agonist-induced contraction (acetylcholine, histamine, and barium) on the isolated ileum of guinea pigs was tested in the presence of 10^{-6}, 10^{-5}, or 10^{-4} mol/L of TI-435. Cholinesterase activity was not determined. Animals were not examined grossly or microscopically for neuropathological lesions.

In the clinical and neurobehavioral assessment, no effects were observed in mice at doses of 12.5 or 25 mg/kg. At 50 mg/kg, decreased spontaneous motor activity was observed from all three mice at the 0.5-hour observation and tremors and deep respirations were observed in a single mouse at the 0.5- or 1-hour observations. All of these effects were graded slight. Additional clinical signs including decreases in reactivity, grooming, and muscle tone; prone position; staggering gait; mydriasis, and hypothermia were observed at the higher doses, with effects becoming more severe with increased dose. These signs had disappeared in the lower dose groups at the 24-hour observation, but tremor, staggering gait and a decrease in spontaneous locomotor activity were observed in one mouse in the 400 mg/kg group at the 24-hour observation. Cyanosis was observed in another mouse at 400 mg/kg prior to death at 3 hours posttreatment.

Hexobarbital-induced sleeping time was prolonged at 225 mg/kg, tonic flexor and extensor convulsions with sub-threshold shock were induced at ≥25 mg/kg, intestinal transport was suppressed at ≥75 mg/kg, and muscle strength was suppressed at ≥225 mg/kg. There was no effect of treatment on pentylenetetrazol-induced convulsions. In studies with rats, body temperature was decreased at ≥300 mg/kg. There was no effect of treatment on blood coagulation parameters or on blood pressure and heart rate in rats. Treatment with TI-435 had no dose-related effect on contractile response to various agonists in isolated guinea pig ileum preparations. A NOAEL for acute neurotoxicity (for acute reference dose consideration during subsequent risk assessment) is 25 mg/kg bw.

Based on the effects seen in this study, the LOAEL for acute neurotoxicity of TI-435 in male CD-1 mice was 50 mg/kg bw (based on transient signs of decreased spontaneous motor activity, tremors, and deep respirations), with a NOAEL of 25 mg/kg bw.

This neurotoxicity/pharmacology study is classified as **Acceptable/Non-guideline** and does *not* satisfy the guideline requirement for an acute neurotoxicity study in rats (870.6200a; OECD 424). The study was not intended to fulfill 870.6200a guidelines. Major deviations from 870.6200a guidelines include failure to use the established protocol for neurotoxicity and motor activity testing, the use of the mouse as the test species, use of a single sex, and use of only 3 mice/test group.

d. In a developmental neurotoxicity study (MRID 45422804) TI 435 (clothianidin, 95.5-95.9% a.i.; Batch # LOT30037120-97) was administered in the diet to pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats (25/dose) from gestation day (GD) 0 to lactation day (LD) 22 at doses of 0, 150, 500, or 1750 ppm (0, 12.9, 42.9, and 142 mg/kg/day during gestation; 0, 27.3, 90.0, and 299.0 mg/kg/day during lactation). Dams were allowed to deliver naturally and were killed on LD 22. On postnatal day (PND) 5, ten pups/litter were randomly selected in order to reduce variability among the litters; the remaining offspring were weighed and euthanized. On PND 12, twenty litters/dose were randomly selected for continued examination, and the litters were standardized to 8 pups/litter. Subsequently, ten pups/sex/group were selected for neurobehavioral testing (Subsets 2 and 3) and neuropathological examination (Subsets 1 and 4). Pups not selected for behavioral evaluations (Subset 5) were killed on PND 22. Acceptable positive control data that validated the procedures and observers of the performing lab to assess motor activity, neurotoxicity and behavioral effects were provided.

No unscheduled parental deaths occurred during the study. Clinical signs, gross pathology, pregnancy rate, number of implantations/dam, gestation length, and sex ratio were unaffected by treatment. It was stated that no treatment-related FOB findings were observed; however, no FOB data were provided.

In the 1750 ppm dams, body weights were consistently decreased ($p<=0.05$ or NS) throughout gestation and lactation (decr 2-8%). Body weight gains were decreased ($p<=0.05$) during GDs 0-3 (decr 63%) and LDs 4-7 (decr 67%). These decreases corresponded with the reductions ($p<=0.05$) noted in absolute and relative (decr 6-23%) food consumption during the gestation and lactation periods. It should be noted that body weight gains increased ($p<=0.05$) during LDs 14-22 (incr 215%) and that overall body weight gains were similar to controls for the gestation (GD 0-20) and lactation (LD 1-22) periods.

No treatment-related findings were observed in the 500 or 150 ppm groups.

The maternal LOAEL is 1750 ppm based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 500 ppm.

No treatment-related differences in live litter size, postnatal survival (through PND 22), sex ratios, clinical signs, food consumption, sexual maturation, or physical landmarks were observed in any treated group. Learning, memory, brain weights, gross pathology, neuropathology, and morphometric measurements were unaffected by the test substance. It was stated that no treatment-related FOB findings were observed; however, no FOB data were provided.

During the pre-weaning period, decreased ($p<=0.05$) body weight was noted in the females at 500 ppm (decr 6-7%) and in both sexes at 1750 ppm (decr 6-16%). Body weight gains were decreased ($p<=0.05$) in the females at 500 ppm (decr 8-24%) and in both sexes at 1750 ppm (decr 11-48%). Overall pre-weaning (PND 5-22) body weight gains were decreased ($p<=0.05$) by 7% in the 500 ppm females and by 18% at 1750 ppm in both sexes.

At 1750 ppm, two male and three female rats were found dead on PNDs 25-27. This finding was considered related to a failure to thrive post-weaning, based on the patterns of body weight gains and losses. During post-weaning, decreased ($p<=0.05$) body weights were noted in both sexes (decr 4-15%) and body weight gains were decreased by 21% in the females.

A slight retardation in surface righting reflex was observed on PND 3 at >=150 ppm (decr 34-47%) compared to controls; however, the toxicological significance of this finding is equivocal.

On PND 22, mean total motor activity (number of movements) was decreased (NS) in the 500 ppm females (decr 21%) and in both sexes at 1750 ppm (decr 10-24%). Additionally at 1750 ppm, mean motor activity counts were decreased ($p<=0.05$) compared to controls during one to four intervals on PND 22 (decr 17-32%) and on PND 62 (↓11-13%).

On PND 23, the average (over all 5 blocks) magnitude of the acoustic startle response was decreased in the females at 500 (decr 27%, NS) and 1750 (decr 48%, $p<=0.01$) compared to controls.

The offspring LOAEL is 500 ppm, based on decreased body weights, body weight gains, motor activity, and acoustic startle response in the females. The offspring NOAEL is 150 ppm.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats.

3. Developmental Toxicity Study Conclusions

a. In a developmental toxicity study (MRID 45422711) TI-435 (95.2% a.i., Lot # 30037120) was administered to 25 Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats/dose by gavage (in a vehicle of 0.5% methyl cellulose) at dose levels of 0(vehicle), 10, 40, or 125 mg/kg bw/day from days 6 through 19 of gestation (dosage volume of 10ml/kg adjusted daily on the basis of individual weights before intubating). On gestation day (GD) 20, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. The total numbers of fetuses examined (number of litters) for the 0, 10, 40, and 125 mg/kg bw/day groups were 299 (23), 294 (22), 330 (24), and 340 (25), respectively. Approximately one-half of the fetuses were examined viscerally, and the other one-half of the fetuses were examined for skeletal malformations/variations.

No statistically significant decreases in food consumption, body weight, or body weight gain were observed in the 10 mg/kg/day group. Dams in the 40 mg/kg/day group had decreased mean body weight gain ($p<0.01$) and decreased food consumption ($p<0.05$) over GDs 6-9. Mean body weight gain, body weight and food consumption values of the 40 mg/kg/day group were comparable to controls for the other treatment intervals including the treatment interval of GDs 6-20. The 125 mg/kg/day group had statistically significant decreased mean absolute body weights during GD 7-20 ($p<0.05$; 0.01), as well as decreased ($p<0.01$) corrected final body weights (93% of control), decreased ($p<0.01$) body weight gains over the intervals GD 6-9 and GD 6-20, and decreased corrected body weight gains for GDs 6-20 (55% of controls). With the exception of GDs 18-20, the highest dose group had statistically significant decreased food consumption at all treatment intervals ($p<0.01$). Overall food consumption of the 125 mg/kg/day group during the interval GDs 6-20 was 83% of controls.

Treatment with TI-435 did not adversely affect clinical signs or gross necropsy findings.

The maternal LOAEL is 40 mg/kg bw/day based on decreased body weight gain and food consumption. The maternal NOAEL is 10 mg/kg bw/day.

No treatment-related, statistically significant effects on pregnancy rates, number of corpora lutea, pre- or post-implantation losses, resorptions/dam, fetuses/litter, fetal body weights, or fetal sex ratios were observed in the treated groups as compared with the controls.

No treatment-related external, visceral, or skeletal malformations/variations were observed in any groups.

The developmental NOAEL is ≥ 125 mg/kg bw/day, and a developmental LOAEL could not be established.

The developmental toxicity study in the rat is classified Acceptable/Guideline, and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat.

b. In a developmental toxicity study (MRID 45422713), TI-435 (95.2% a.i., Lot # 30037120) dissolved in aqueous 0.5% methylcellulose was administered to 23 female New Zealand White [Hra:(NZW)SPF] rabbits/dose by gavage at dose levels of 0, 10, 25, 75, or 100 mg/kg bw/day from days 6 through 28 of gestation. On gestation day (GD) 29, does were sacrificed, subjected to gross necropsy, and all fetuses examined externally. The total numbers of fetuses examined (number of litters) was 150 (18), 201 (23), 179 (22), 133 (17), and 85 (11) for the 0, 10, 25, 75, and 100 mg/kg bw/day groups, respectively. All of the fetuses were examined viscerally and for skeletal malformations/variations.

Maternal toxicity was evident at doses of 75 mg/kg/day and higher. Increased incidences of scant feces in cage and orange urine were observed in does treated with 75 mg/kg/day and higher. At the 75 mg/kg/day dose, does additionally exhibited mortality (2/23), early delivery (2/23), abortion (1/23), and decreased body weight gain (53% of controls; n.s.) and food consumption (83% of controls; $p<0.05$) for the treatment interval of GDs 6-29. At 100 mg/kg/day, maternal toxicity was additionally indicated by: mortality (3/23); abortions (6/23**); early deliveries (2/23); increased incidence of red substance in the cage pan (8/4 vs. 0/0 for controls; $p<0.05$); decreased mean absolute body weights starting at GD 14 and continuing throughout the study (90-94% of controls; $p<0.05$; 0.01), loss in body weight over treatment intervals GDs 9-12, 12-15, and 6-29; and decreased food consumption at all treatment intervals (34-90% of controls). No dose-related gross necropsy findings were noted.

The maternal LOAEL is 75 mg/kg/day based on increased incidences of clinical signs (scant feces and orange urine) and mortalities and decreased food consumption. The maternal NOAEL is 25 mg/kg/day.

Developmental toxicity was evident at doses of 75 and 100 mg/kg/day. Two does in each of these dose groups delivered early, six 100 mg/kg/day does aborted, and dose-related decreases in gravid uterine weights were observed (89 and 81% of controls, respectively; n.s.). Examination of cesarean section data revealed a number of differences in the 100 mg/kg/day group, including: an increase in the total number of resorptions (15 vs. 5 for controls; n.s.) and resorptions/doe (1.4 vs. 0.3 for controls; n.s.); total number of early resorptions (10 vs. 2 for controls; n.s.) and early resorptions/dose (0.9 vs. 0.1 for controls; n.s.), and post implantation loss (18.3% vs. 3.3% for controls), and decreased mean fetal body weights (84% of controls; $p<0.01$). One doe from the 100 mg/kg/day group had complete litter resorption, along with two 100 mg/kg/day group does that died early.

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External examination of fetuses did not reveal any treatment-related alterations. Visceral examination revealed a statistically increased ($p<0.01$) litter incidence of an absent lung lobe in fetuses from the 75 and 100 mg/kg/day groups [fetus(litter) incidence: 3(3) and 8(5), respectively, vs. 0(0) for controls; 18 and 45% of litters affected, respectively]. Skeletal examination found a statistically decreased ($p<0.01$) litter average for ossified sternal centra per fetus in the 75 and 100 mg/kg/day groups [3.83 and 3.76, respectively, vs. 3.99 for controls]. In the 100 mg/kg/day group, other findings included a statistically increased ($p<0.01$) litter incidence of incomplete ossification of the sternal centra and absent hindpaw phalanges [fetus(litter) incidence for both: 2(2) vs. 0(0) for controls; 18% of litters affected].

The developmental LOAEL is 75 mg/kg bw/day, based on premature deliveries, decreased gravid uterine weights, an increased litter incidence of a missing lobe of the lung and decreased litter average for ossified sternal centra per fetus. The developmental NOAEL is 25 mg/kg bw/day.

The developmental toxicity study in the rabbit is classified **Acceptable/Guideline**, and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits.

4. <u>Reproductive Toxicity Study Conclusions</u>

In a 2-generation reproduction study (MRID 45422715) TI-435 (Clothianidin; 95.3-96.0% a.i., lot/batch #300371200) was administered to 30 Sprague-Dawley rats/sex/dose in the diet at concentrations of 0, 150, 500, or 2500 ppm. One litter was produced by each generation. Pre-mating doses were 0, 9.8, 31.2, or 163.4 mg/kg bw/day, respectively, for F_0 males, 0, 11.5, 36.8, or 188.8 mg/kg bw/day, respectively, for F_0 females, 0, 10.7, 34.3, or 195.7 mg/kg bw/day, respectively, for F_1 males, and 0, 12.2, 39.0, or 237.0 mg/kg bw/day, respectively, for F_1 females; the values for the F_0 generation were considered to be representative of the test substance intake for the entire study. F_0 and F_1 male and female parental animals were administered test or control diet for at least 70 days prior to mating, throughout mating, gestation, and lactation, and until necropsy. Additional information pertaining to diet analyses was obtained from two separately provided reports (MRIDs 45422825 and 45422826), and historical control data were also provided (MRID 45422716).

There were no treatment-related effects on parental clinical signs or mortality. High-dose F_0 males had decreased absolute body weights during weeks 4-10 of pre-mating (87-92% of controls) and decreased cumulative body weight gain during pre-mating (80% of controls), and high-dose F_1 males had decreased absolute body weights throughout pre-mating (79-83% of controls). High-dose F_0 females had decreased absolute body weights during weeks 2-10 of pre-mating (86-91% of controls), throughout gestation (86-88% of controls), and throughout lactation (82-89% of controls), with decreased body weight

gains during pre-mating and gestation (61 and 83% of controls, respectively). High-dose F_1 females had decreased absolute body weights throughout pre-mating (83-84% of controls), throughout gestation (84-87% of controls), and throughout lactation (85-88% of controls), with decreased body weight gains during pre-mating and gestation (82 and 92% of controls, respectively). These groups generally also had significantly increased food consumption. Absolute thymus weights were decreased in high-dose F_0 and F_1 males and females (84, 71, 65, and 59% of controls for F_0 males, F_1 males, F_0 females, and F_1 females, respectively) and relative (to body) thymic weights were also decreased in high-dose F_1 males and high-dose females of both generations. **The parental systemic toxicity LOAEL is 2500 ppm (163.4 mg/kg bw/day in males, 188.8 mg/kg bw/day in females), based on decreased absolute body weights and body weight gains with decreased absolute and relative thymus weights in both sexes. The parental systemic NOAEL is 500 ppm (31.2 mg/kg bw/day in males, 36.8 mg/kg bw/day in females).**

Mean absolute body weights of F_1 high-dose male and female pups were significantly decreased from LD 4 through the end of weaning (74-84% of controls; $p<0.01$), and mean absolute body weights of F_2 high-dose male and female pups were significantly decreased from LD 7 through the end of weaning (79-87% of controls; $p<0.01$). Pup body weight gains of high-dose males and females of both generations were significantly decreased throughout lactation (F_1 combined sexes: 67-76% of controls, $p<0.01$; F_2 combined sexes: 72-81% of controls, $p<0.05$ or $p<0.01$), and the body weight gains of F_1 mid-dose pups of both sexes were slightly decreased during LD 7-14 (90% of controls for both sexes and 89% of controls for combined sexes; $p<0.05$). Decreased mean absolute thymus weights were noted in high-dose pups of both generations (71-72% of controls for high-dose F_1 males and females; 75-76% of controls for high-dose F_2 males and females) as well as in mid-dose F_1 pups (87-90% of controls). The spleen weights (relative and absolute) were reduced ($p<0.01$) in females of high dose in the F_1 generation and in males and females of high dose in the F_2 generation as well. Mid- and high-dose F_1 males had increased mean ages of acquisition of balanopreputial separation (42.5 [$p<0.05$] and 47.9 [$p<0.01$] vs. 41.2 days for controls) and high-dose F_1 females had increase mean age of vaginal perforation (34.7 vs. 32.4 days for controls; $p<0.01$). A dose-related increased number of stillbirths was noted in the mid- and high-dose groups of both generations in the absence of effects on related parameters such as live birth index, live litter size, and/or numbers of implantations. **Therefore, the offspring toxicity LOAEL is 500 ppm (31.2 mg/kg bw/day in males, 36.8 mg/kg bw/day in females), based on decreased body weight gains, delayed sexual maturation (males), and decreased absolute thymus weights in F_1 pups of both sexes and an increase in stillbirths in both generations. The offspring toxicity NOAEL is 150 ppm (9.8 mg/kg bw/day in males, 11.5 mg/kg bw/day in females).**

There were no treatment-related effects on mating performance, fertility, estrous cyclicity, gestation length, or precoital intervals. There were no treatment-related abnormal gross or microscopic findings related to the reproductive organs of the parental animals or

offspring. The mean primordial follicle, antral follicle, and corpora lutea counts of high-dose F_1 adults were similar to controls. The percentage of progressively motile sperm was decreased in high-dose F_0 males (88% of controls; $p<0.01$), in the absence of an effect on the percent motile, while in high-dose F_1 males decreases were noted in both the percent motile (90% of controls; $p<0.01$) and the percent progressively motile (77% of controls; $p<0.01$). Males of both generations also had increased percentages of sperm with detached heads (F_0: 2.1 vs. 0.6% for controls; F_1: 4.0 vs. 0.7% for controls). **The reproductive toxicity LOAEL for males is 2500 ppm (163.4 mg/kg bw/day) in males, based on decreased sperm motility, and increased number of sperm with detached heads in both generations. The reproductive toxicity NOAEL for males is 500 ppm (31.2 mg/kg bw/day). The reproductive toxicity LOAEL for females is not identified, and the reproductive toxicity NOAEL for females is 2500 ppm (188.8 mg/kg bw/day).**

This study is classified **Acceptable/Guideline** and satisfies the guideline requirements for a 2-generation reproductive study in the rat [OPPTS 870.3800; OECD 416]. However, it must be noted that significant variation in the analytical dietary concentrations at all dose levels make the actual dosages to the animals uncertain.

5. Additional Information from Literature Sources

No additional information is available from the literature.

6. Pre-and/or Postnatal Toxicity

The HIARC concluded that there is a concern for pre- and/or postnatal toxicity resulting from exposure to Clothianidin in the reproduction and developmental neurotoxicity studies.

a. Determination of Susceptibility

No quantitative or qualitative susceptibility was observed in either of the developmental rat or rabbit studies. No developmental toxicity was observed in the rat at dose levels which induced decreases in body weight gain and food consumption in the dams. In the rabbit, premature deliveries, decreased gravid uterine weights, an increase in litter incidence of a missing lobe of the lung and a decrease in the litter average for ossified sternal centra per fetus were noted at a dose level in which maternal death, a decrease in food consumption and clinical signs (scant feces and orange urine) were observed. The developmental effects are not considered to be qualitatively more severe than the maternal effects.

Quantitative susceptibility was observed in both the reproduction and developmental neurotoxicity studies. In the 2-generation reproduction study, the NOAEL for offspring toxicity is 9.8/11.5 mg/kg/day (M/F) based on decreased body weight gains, delayed sexual maturation (males), decreased absolute thymus weights in F_1 pups of both sexes and an increase in stillbirths in both generations at the LOAEL of 31.2/36.8 mg/kg bw/day (M/F). The parental systemic NOAEL is 31.2/36.9 mg/kg/day (M/F) based on decreased absolute body weights and body weight gains with decreased absolute and relative thymus weights in both sexes at the LOAEL of 163.4/188.8 mg/kg bw/day (M/F).

In the developmental neurotoxicity study, the NOAEL for offspring toxicity is 12.9 mg/kg/day based on decreased body weight gains, motor activity and acoustic startle response at the LOAEL of 42.9 mg/kg/day. The parental NOAEL is 42.9 mg/kg/day based on decreased body weights, body weight gains and food consumption at the LOAEL of 142 mg/kg bw/day.

b. Degree of Concern Analysis and Residual Uncertainties

Since there is quantitative evidence of increased susceptibility of the young following exposure to Clothianidin in the 2-generation reproduction and developmental neurotoxicity studies in rats, HIARC performed a Degree of Concern Analysis to: 1) determine the level of concern for the effects observed when considered in the context of all available toxicity data; and 2) identify any residual uncertainties after establishing toxicity endpoints and traditional uncertainty factors to be used in the risk assessment of this chemical. If residual uncertainties are identified, HIARC examines whether these residual uncertainties can be addressed by a special FQPA safety factor and, if so, the size of the factor needed. The results of the HIARC Degree of Concern analysis for Clothianidin follow.

The degree of concern for the 2-generation reproduction study is low because the observed effects are well characterized and there are clear NOAELs/LOAELs. In addition, the endpoint of concern is the one that is being used for short, intermediate and long term dietary and non-dietary exposure risk assessments. The degree of concern for the developmental neurotoxicity study is also low because the observed effects are well characterized and there are clear NOAELs/LOAELs. There are no residual uncertainties.

c. Special FQPA Safety Factor(s): The special FQPA safety factor is reduced to 1x because there are no/low concerns and no residual uncertainties with regard to pre- and/or postnatal toxicity.

The Special FQPA Safety Factor recommended by the HIARC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.

7. Recommendation for a Developmental Immunotoxicity Study

The HIARC concluded that there is a concern for immunotoxicity following exposure of clothianidin during the period of organogenesis. This concern was based on the decreases in absolute and adjusted thymus and spleen weights observed in several species in various studies. In addition, the available data indicate that the juvenile rats appeared to be more sensitive/susceptible to these effects than adults in the two-generation reproduction study. Therefore, the HIARC is recommending that testing be conducted to assess immune system function in adults and young animals following exposure during the period of organogenesis. The protocol for this testing should be developed following discussion with OPP/HED scientists (see Appendix A).

HIARC determined that a 10X database uncertainty factor (UF_{DB}) is needed to account for the lack of a developmental immunotoxicity study when assessing acute (single dose) and repeated dose exposure scenarios since the available data provide no basis to support reduction or removal of the default 10X factor. The following points were considered in this determination:

a. It is assumed that the developmental immunotoxicity study will be conducted at dose levels similar to those used in the rat reproduction study with clothianidin (9.8, 31.2, 163.4 mg/kg/day) wherein the offspring NOAEL / LOAEL was 9.8 /31.2 mg/kg/day, respectively.

b. It is possible that the results of the developmental immunotoxicity study could impact the current selected acute regulatory doses since the NOAELs used to establish the acute Reference doses for dietary risk assessment (25 mg/kg/day for both Females 13-50 and for the General Population) are approximately 2.5 times greater than the offspring NOAEL in the rat reproduction study conducted with with clothianidin (9.8 mg/kg/day).

c. It is also possible that the results of the developmental immunotoxicity study could impact the current selected repeated dose exposure scenerios because the NOAEL used to establish these endpoints is based on the offspring NOAEL from the rat reproduction study conducted with clothianidin (9.8 mg/kg/day).

Given these circumstances, HIARC does not have sufficient reliable data justifying selection of an additional safety factor for the protection of infants and children lower than the default value of 10X for both single and repeated dose exposure scenarios. Therefore, a UF_{DB} of 10X will be applied to both single and repeated dose exposure scenarios (i.e., acute and chronic RfDs, short- and intermediate-term incidental oral exposures and short-, intermediate-, and long-term dermal and inhalation exposures) to account for the lack of the developmental immunotoxicity study with clothianidin.

II. HAZARD IDENTIFICATION

1. Acute Reference Dose (aRfD) - Females 13-50

Study Selected: Developmental Toxicity Study in Rabbits § 870.3700

MRID No.: 45422712

Executive Summary: In a developmental toxicity study (MRID 45422713), TI-435 (95.2% a.i., Lot # 30037120) dissolved in aqueous 0.5% methylcellulose was administered to 23 female New Zealand White [Hra:(NZW)SPF] rabbits/dose by gavage at dose levels of 0, 10, 25, 75, or 100 mg/kg bw/day from days 6 through 28 of gestation. On gestation day (GD) 29, does were sacrificed, subjected to gross necropsy, and all fetuses examined externally. The total numbers of fetuses examined (number of litters) was 150 (18), 201 (23), 179 (22), 133 (17), and 85 (11) for the 0, 10, 25, 75, and 100 mg/kg bw/day groups, respectively. All of the fetuses were examined viscerally and for skeletal malformations/variations.

Maternal toxicity was evident at doses of 75 mg/kg/day and higher. Increased incidences of scant feces in cage and orange urine were observed in does treated with 75 mg/kg/day and higher. At the 75 mg/kg/day dose, does additionally exhibited mortality (2/23), early delivery (2/23), abortion (1/23), and decreased body weight gain (53% of controls; n.s.) and food consumption (83% of controls; $p<0.05$) for the treatment interval of GDs 6-29. At 100 mg/kg/day, maternal toxicity was additionally indicated by: mortality (3/23); abortions (6/23**); early deliveries (2/23); increased incidence of red substance in the cage pan (8/4 vs. 0/0 for controls; $p<0.05$); decreased mean absolute body weights starting at GD 14 and continuing throughout the study (90-94% of controls; $p<0.05$; 0.01), loss in body weight over treatment intervals GDs 9-12, 12-15, and 6-29; and decreased food consumption at all treatment intervals (34-90% of controls). No dose-related gross necropsy findings were noted.

The maternal LOAEL is 75 mg/kg/day based on increased incidences of clinical signs (scant feces and orange urine) and mortalities and decreased food consumption. The maternal NOAEL is 25 mg/kg/day.

Developmental toxicity was evident at doses of 75 and 100 mg/kg/day. Two does in each of these dose groups delivered early, six 100 mg/kg/day does aborted, and dose-related decreases in gravid uterine weights were observed (89 and 81% of controls, respectively; n.s.). Examination of cesarean section data revealed a number of differences in the 100 mg/kg/day group, including: an increase in the total number of resorptions (15 vs. 5 for controls; n.s.) and resorptions/doe (1.4 vs. 0.3 for controls; n.s.); total number of early resorptions (10 vs. 2 for controls; n.s.) and early resorptions/doe (0.9 vs. 0.1 for controls; n.s.), and post implantation loss (18.3% vs. 3.3% for controls), and decreased mean fetal body weights (84% of controls; $p<0.01$). One doe from the 100 mg/kg/day group had complete litter resorption, along with two 100 mg/kg/day group does that died early.

Ue

External examination of fetuses did not reveal any treatment-related alterations. Visceral examination revealed a statistically increased ($p<0.01$) litter incidence of an absent lung lobe in fetuses from the 75 and 100 mg/kg/day groups [fetus(litter) incidence: 3(3) and 8(5), respectively, vs. 0(0) for controls; 18 and 45% of litters affected, respectively]. Skeletal examination found a statistically decreased ($p<0.01$) litter average for ossified sternal centra per fetus in the 75 and 100 mg/kg/day groups [3.83 and 3.76, respectively, vs. 3.99 for controls]. In the 100 mg/kg/day group, other findings included a statistically increased ($p<0.01$) litter incidence of incomplete ossification of the sternal centra and absent hindpaw phalanges [fetus(litter) incidence for both: 2(2) vs. 0(0) for controls; 18% of litters affected].

The developmental LOAEL is 75 mg/kg bw/day, based on premature deliveries, decreased gravid uterine weights, an increased litter incidence of a missing lobe of the lung and decreased litter average for ossified sternal centra per fetus. The developmental NOAEL is 25 mg/kg bw/day.

The developmental toxicity study in the rabbit is classified **Acceptable/Guideline**, and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits.

Dose and Endpoint for Establishing aRfD: The developmental NOAEL of 25 mg/kg/day based on an increased litter incidence of a missing lobe of the lung at the LOAEL of 75 mg/kg/day. Other effects observed at 75 mg/kg/day were premature deliveries, decreased gravid uterine weights and decreased litter average for ossified sternal centra per fetus. These are not considered to be single dose effects.

Uncertainty Factor (UF): 1000 (10x for interspecies extrapolation, 10x for intraspecies variations and a 10x database factor for the lack of a developmental immunotoxicity study).

Comments about Study/Endpoint/Uncertainty Factor: This endpoint is considered appropriate for the population subgroup, Females 13-50 because the observed developmental effects may occur following a single dose. The route of administration is appropriate for dietary considerations.

Acute RfD (Females 13-50) = $\frac{\text{25 mg/kg (NOAEL)}}{\text{1000 (UF)}}$ = **0.025 mg/kg**

2. Acute Reference Dose (aRfD) - General Population

Study Selected: Special Neurotoxicity/Pharmacology Study in Mice and Rats § N/A

MRID No.: 45422823

Executive Summary: In a non-guideline study that addressed some aspects of neurotoxicity and pharmacology (MRID 45422823), groups of three male CD-1 mice were tested for acute neurobehavioral effects following single gavage doses of TI-435 (95.5% a.i.; vehicle, 5% arabic gum) at doses of 0 (5% arabic gum), 12.5, 25, 50, 100, 200, or 400 mg/kg bw. Clinical signs and mortality were monitored from 0.5 hours to 24 hours post-treatment. The following tests were also conducted with groups of eight or ten male CD-1 mice: prolongation of hexobarbital-induced sleeping time following doses of 25, 75, or 225 mg/kg bw; induction of tonic flexor and tonic extensor convulsions with sub-threshold electroshock following doses of 6.25, 12.5, 25, 75, or 225 mg/kg bw; synergistic effect of pentylenetetrazol on convulsions following doses of 25, 75, or 225 mg/kg bw; effect on intestinal transport following doses of 25, 75, or 225 mg/kg bw; and effect on muscle strength following doses of 25, 75, or 225 mg/kg bw. Groups of six male CD rats were tested for effects on body temperature following gavage doses of 30, 100, 300, 1000, or 3000 mg/kg bw. In other tests, groups of four male CD rats were tested for effects on blood pressure and heart rate following doses of 300, 1000, or 3000 mg/kg bw, and groups of six male CD rats were tested for effects on prothrombin and activated thromboplastin times following doses of 300, 1000, or 3000 mg/kg bw. In an *in vitro* study, effects of agonist-induced contraction (acetylcholine, histamine, and barium) on the isolated ileum of guinea pigs was tested in the presence of 10^{-6}, 10^{-5}, or 10^{-4} mol/L of TI-435. Cholinesterase activity was not determined. Animals were not examined grossly or microscopically for neuropathological lesions.

In the clinical and neurobehavioral assessment, no effects were observed in mice at doses of 12.5 or 25 mg/kg. At 50 mg/kg, decreased spontaneous motor activity was observed from all three mice at the 0.5-hour observation and tremors and deep respirations were observed in a single mouse at the 0.5- or 1-hour observations. All of these effects were graded slight. Additional clinical signs including decreases in reactivity, grooming, and muscle tone; prone position; staggering gait; mydriasis, and hypothermia were observed at the higher doses, with effects becoming more severe with increased dose. These signs had disappeared in the lower dose groups at the 24-hour observation, but tremor, staggering gait and a decrease in spontaneous locomotor activity were observed in one mouse in the 400 mg/kg group at the 24-hour observation. Cyanosis was observed in another mouse at 400 mg/kg prior to death at 3 hours posttreatment.

Hexobarbital-induced sleeping time was prolonged at 225 mg/kg, tonic flexor and extensor convulsions with sub-threshold shock were induced at ≥25 mg/kg, intestinal transport was suppressed at ≥75 mg/kg, and muscle strength was suppressed at ≥225 mg/kg. There was no effect of treatment on pentylenetetrazol-induced convulsions. In studies with rats, body temperature was decreased at ≥300 mg/kg. There was no effect of

treatment on blood coagulation parameters or on blood pressure and heart rate in rats. Treatment with TI-435 had no dose-related effect on contractile response to various agonists in isolated guinea pig ileum preparations. A NOAEL for acute neurotoxicity (for acute reference dose consideration during subsequent risk assessment) is 25 mg/kg bw.

Based on the effects seen in this study, the LOAEL for acute neurotoxicity of TI-435 in male CD-1 mice was 50 mg/kg bw (based on transient signs of decreased spontaneous motor activity, tremors, and deep respirations), with a NOAEL of 25 mg/kg bw.

This neurotoxicity/pharmacology study is classified as **Acceptable/Non-guideline** and does *not* satisfy the guideline requirement for an acute neurotoxicity study in rats (870.6200a; OECD 424). The study was not intended to fulfill 870.6200a guidelines. Major deviations from 870.6200a guidelines include failure to use the established protocol for neurotoxicity and motor activity testing, the use of the mouse as the test species, use of a single sex, and use of only 3 mice/test group.

Dose and Endpoint for Establishing aRfD: The NOAEL of 25 mg/kg in mice based on transient signs of decreased spontaneous motor activity, tremors and deep respirations at 50 mg/kg.

Uncertainty Factor (UF): 1000 (10x for interspecies extrapolation, 10x for intraspecies variations and a 10x database factor for the lack of a developmental immunotoxicity study).

Comments about Study/Endpoint/Uncertainty Factor: This endpoint is considered appropriate for the general population because the effects were observed following a single dose and the route of administration (oral) is appropriate for dietary considerations.

Acute RfD (General Population) = $\frac{25 \text{ mg/kg (NOAEL)}}{1000 \text{ (UF)}}$ = **0.025 mg/kg**

3. Chronic Reference Dose (cRfD)

Study Selected: 2-Generation Reproduction Study in the Rat §870.3800

MRID No.: 45422714 through -16

Executive Summary: In a 2-generation reproduction study (MRID 45422715) TI-435 (Clothianidin; 95.3-96.0% a.i., lot/batch #300371200) was administered to 30 Sprague-Dawley rats/sex/dose in the diet at concentrations of 0, 150, 500, or 2500 ppm. One litter was produced by each generation. Pre-mating doses were 0, 9.8, 31.2, or 163.4 mg/kg bw/day, respectively, for F_0 males, 0, 11.5, 36.8, or 188.8 mg/kg bw/day, respectively, for F_0 females, 0, 10.7, 34.3, or 195.7 mg/kg bw/day, respectively, for F_1 males, and 0, 12.2, 39.0, or 237.0 mg/kg bw/day, respectively, for F_1 females; the values for the F_0 generation were considered to be representative of the test substance intake for the entire study. F_0 and F_1 male and female parental animals were administered test or control diet for at least 70 days prior to mating, throughout mating, gestation, and lactation, and until necropsy. Additional information pertaining to diet analyses was obtained from two separately provided reports (MRIDs 45422825 and 45422826), and historical control data were also provided (MRID 45422716).

There were no treatment-related effects on parental clinical signs or mortality. High-dose F_0 males had decreased absolute body weights during weeks 4-10 of pre-mating (87-92% of controls) and decreased cumulative body weight gain during pre-mating (80% of controls), and high-dose F_1 males had decreased absolute body weights throughout pre-mating (79-83% of controls). High-dose F_0 females had decreased absolute body weights during weeks 2-10 of pre-mating (86-91% of controls), throughout gestation (86-88% of controls), and throughout lactation (82-89% of controls), with decreased body weight gains during pre-mating and gestation (61 and 83% of controls, respectively). High-dose F_1 females had decreased absolute body weights throughout pre-mating (83-84% of controls), throughout gestation (84-87% of controls), and throughout lactation (85-88% of controls), with decreased body weight gains during pre-mating and gestation (82 and 92% of controls, respectively). These groups generally also had significantly increased food consumption. Absolute thymus weights were decreased in high-dose F_0 and F_1 males and females (84, 71, 65, and 59% of controls for F_0 males, F_1 males, F_0 females, and F_1 females, respectively) and relative (to body) thymic weights were also decreased in high-dose F_1 males and high-dose females of both generations. **The parental systemic toxicity LOAEL is 2500 ppm (163.4 mg/kg bw/day in males, 188.8 mg/kg bw/day in females), based on decreased absolute body weights and body weight gains with decreased absolute and relative thymus weights in both sexes. The parental systemic NOAEL is 500 ppm (31.2 mg/kg bw/day in males, 36.8 mg/kg bw/day in females).**

Mean absolute body weights of F_1 high-dose male and female pups were significantly decreased from LD 4 through the end of weaning (74-84% of controls; $p<0.01$), and mean absolute body weights of F_2 high-dose male and female pups were significantly decreased from LD 7 through the end of weaning (79-87% of controls; $p<0.01$). Pup body weight

gains of high-dose males and females of both generations were significantly decreased throughout lactation (F_1 combined sexes: 67-76% of controls, $p<0.01$; F_2 combined sexes: 72-81% of controls, $p<0.05$ or $p<0.01$), and the body weight gains of F_1 mid-dose pups of both sexes were slightly decreased during LD 7-14 (90% of controls for both sexes and 89% of controls for combined sexes; $p<0.05$). Decreased mean absolute thymus weights were noted in high-dose pups of both generations (71-72% of controls for high-dose F_1 males and females; 75-76% of controls for high-dose F_2 males and females) as well as in mid-dose F_1 pups (87-90% of controls). The spleen weights (relative and absolute) were reduced ($p<0.01$) in females of high dose in the F_1 generation and in males and females of high dose in the F_2 generation as well. Mid- and high-dose F_1 males had increased mean ages of acquisition of balanopreputial separation (42.5 [$p<0.05$] and 47.9 [$p<0.01$] vs. 41.2 days for controls) and high-dose F_1 females had increase mean age of vaginal perforation (34.7 vs. 32.4 days for controls; $p<0.01$). A dose-related increased number of stillbirths was noted in the mid- and high-dose groups of both generations in the absence of effects on related parameters such as live birth index, live litter size, and/or numbers of implantations. **Therefore, the offspring toxicity LOAEL is 500 ppm (31.2 mg/kg bw/day in males, 36.8 mg/kg bw/day in females), based on decreased body weight gains, delayed sexual maturation (males), and decreased absolute thymus weights in F_1 pups of both sexes and an increase in stillbirths in both generations. The offspring toxicity NOAEL is 150 ppm (9.8 mg/kg bw/day in males, 11.5 mg/kg bw/day in females).**

There were no treatment-related effects on mating performance, fertility, estrous cyclicity, gestation length, or precoital intervals. There were no treatment-related abnormal gross or microscopic findings related to the reproductive organs of the parental animals or offspring. The mean primordial follicle, antral follicle, and corpora lutea counts of high-dose F_1 adults were similar to controls. The percentage of progressively motile sperm was decreased in high-dose F_0 males (88% of controls; $p<0.01$), in the absence of an effect on the percent motile, while in high-dose F_1 males decreases were noted in both the percent motile (90% of controls; $p<0.01$) and the percent progressively motile (77% of controls; $p<0.01$). Males of both generations also had increased percentages of sperm with detached heads (F_0: 2.1 vs. 0.6% for controls; F_1: 4.0 vs. 0.7% for controls). **The reproductive toxicity LOAEL for males is 2500 ppm (163.4 mg/kg bw/day) in males, based on decreased sperm motility, and increased number of sperm with detached heads in both generations. The reproductive toxicity NOAEL for males is 500 ppm (31.2 mg/kg bw/day). The reproductive toxicity LOAEL for females is not identified, and the reproductive toxicity NOAEL for females is 2500 ppm (188.8 mg/kg bw/day).**

This study is classified **Acceptable/Guideline** and satisfies the guideline requirements for a 2-generation reproductive study in the rat [OPPTS 870.3800; OECD 416]. However, it must be noted that significant variation in the analytical dietary concentrations at all dose levels make the actual dosages to the animals uncertain.

Dose and Endpoint for Establishing cRfD: Offspring NOAEL of 9.8 mg/kg/day from the

2-generation reproduction study based on decreased body weight gains and delayed sexual maturation, decreased absolute thymus weights in F_1 pups and an increase in stillbirths in both generations at 31.2 mg/kg/day.

Uncertainty Factor(s): 1000 (10x for interspecies extrapolation, 10x for intraspecies variations and a 10x database factor for the lack of a developmental immunotoxicity study).

Comments about Study/Endpoint/Uncertainty Factor: This endpoint is considered appropriate for chronic dietary exposure because the route of administration (oral) is appropriate for dietary considerations. The study and endpoint were selected because it is protective of effects observed in all the other available studies.

Chronic RfD =	$\frac{\text{9.8 mg/kg/day (NOAEL)}}{\text{1000 (UF)}}$ =	**0.0098 mg/kg/day**

4. Incidental Oral Exposure: Short- and Intermediate - Term (1-30 days and 1-6 Months)

Study Selected: 2-Generation Reproduction Study in the Rat §870.3800

MRID No.: 45422714 through -16

Executive Summary: See chronic dietary endpoint

Dose and Endpoint for Risk Assessment: Offspring NOAEL of 9.8 mg/kg/day from the 2-generation reproduction study based on decreased body weight gains and delayed sexual maturation, decreased absolute thymus weights in F_1 pups and an increase in stillbirths in both generations at 31.2 mg/kg/day.

Comments about Study/Endpoint: This endpoint is based on an oral study, which is the route of interest for an incidental oral risk estimate. The study and endpoint were selected because it is protective of effects observed in all the other available studies. The endpoint is appropriate for all durations as the effect may be a result of either short- and/or longer-term exposure. In addition, it is appropriate for incidental oral exposure because it is based on offspring effects from the reproduction study.

5. Dermal Absorption

Dermal Absorption Factor: 1%

A dermal absorption study with monkeys is available. In a dermal penetration study (MRID 45868001) TI-435 [Clothianidin] as the FS 600 formulation (10% a.i.) [nitroimino- ^{14}C] TI-435) was administered to 5 male Rhesus monkeys. Test material was applied to a shaved area (4 cm x 6 cm) of skin on the back of each animal. The total dose was contained in 100 ml of test substance and was applied at a dose of 6.13 ug/cm^2. Exposure duration was 8 hours at the end of which time the application site was washed. Subjects were monitored for 120 hours. Urine and feces were collected for the exposure period and the subsequent monitoring period.

Mean Dermal Data as Percent of a Dermal Dose of 6.13 ug/cm^2

Sample	% Recovery
Urine	0.05 (± 0.04)
Feces	0.13 (±0.21)
Cage/Pan/Chair Wash, Debris	0.06 (±0.09)
Duoderm/Dome	2.99 (±4.75)
Skin Wash	94.77 (varied with sample)
Totals	98.00 (±0.66)

Dermal absorption as the sum of urinary and fecal excretion and Cage/Pan/Chair Wash, Debris was 0.24 (± 0.11) as percent of dose. Adjustment of the direct absorption determination was not necessary because recovery from the dermal dose was >90%. This study in the monkey was acceptable.

The HIARC concluded that a value of 1% dermal absorption is appropriate for use in risk assessment. This estimation takes into account any variability that would have likely occurred with testing several dose levels.

The dermal absorption value assumes that the 10% formulation used in the monkey study is representative of products that will be available on the market. If there are significant changes in future formulated products, then the dermal absorption value of 1% will be re-evaluated.

6. Dermal Exposure: All Durations

Study Selected: 2-Generation Reproduction Study in the Rat §870.3800

MRID No.: 45422714 through -16

Executive Summary: See chronic dietary endpoint

Dose and Endpoint for Risk Assessment: Offspring NOAEL of 9.8 mg/kg/day from the 2-generation reproduction study based on decreased body weight gains and delayed sexual maturation, decreased absolute thymus weights in F_1 pups and an increase in stillbirths in both generations at 31.2 mg/kg/day.

Comments about Study/Endpoint: This endpoint is based on an oral study. A dermal study is available; however, the selected endpoint addresses potential effects on offspring, which are not examined in the dermal study. Therefore, the study and endpoint were selected because it is protective of effects observed in all the available studies. The mouse single dose and rat single and multiple dose metabolism studies indicate that oral absorption is in the range of 90% or greater. Therefore, extrapolation from the oral to the dermal route is not likely to grossly underestimate anticipated adverse effects. The endpoint is appropriate for all durations as the effect may be a result of either short- and/or longer-term exposure. A 1% dermal absorption factor should be used for route-to-route extrapolation.

7. Inhalation Exposure: All Durations

Study Selected: 2-Generation Reproduction Study in the Rat §870.3800

MRID No.: 45422714 through -16

Executive Summary: See chronic dietary endpoint

Dose/Endpoint for Risk Assessment: Offspring NOAEL of 9.8 mg/kg/day from the 2-generation reproduction study based on decreased body weight gains and delayed sexual maturation, decreased absolute thymus weights in F_1 pups and an increase in stillbirths in both generations at 31.2 mg/kg/day.

Comments about Study/Endpoint: This endpoint is based on an oral study. No inhalation studies are available. Therefore, an oral study is selected to estimate risk using a route-to-route extrapolation. The study and endpoint were selected because it is protective of effects observed in all the available studies. The endpoint is appropriate for all durations as the effect may be a result of either short- and/or longer-term exposure. Absorption via inhalation is assumed to be equivalent to absorption via the oral route.

8. Margins of Exposure

Summary of target Margins of Exposure (MOEs) for risk assessment.

Route / Duration	Short-Term (1-30 Days)	Intermediate-Term (1 - 6 Months)	Long-Term (> 6 Months)
Occupational (Worker) Exposure			
Dermal	100	100	100
Inhalation	100	100	100
Residential (Non-Dietary) Exposure			
Oral	1000	1000	N/A
Dermal	1000	1000	1000
Inhalation	1000	1000	1000

For residential exposure (dermal and inhalation) risk assessments, a MOE of 1000 is required and includes the conventional 100 and an additional 10x FQPA safety factor for the data gap for a developmental immunotoxicity study.

9. Recommendation for Aggregate Exposure Risk Assessments

As per FQPA, 1996, when there are potential residential exposures to the pesticide, aggregate risk assessment must consider exposures from three major sources: oral, dermal and inhalation exposures. The toxicity endpoints selected for these routes of exposure may be aggregated as follows: short-, intermediate- and long-term exposures (incidental oral, dermal and inhalation exposure) can be aggregated because of the use of a common endpoint for oral, dermal (oral equivalent) and inhalation (oral equivalent) routes of exposure.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID Nos. 45422719 and 45422720

Executive Summary: In a combined chronic/carcinogenicity study (MRIDs 45422719 and 45422720), TI-435 (clothianidin, 95.2-95.5% a.i., Lot No. 30037120) was administered daily in the diet to 80 Crl:CD®(SD)BR VAF/Plus® rats/sex/dose at dose levels of 0, 150, 500, 1500, or 3000 ppm (equivalent to 0/0, 8.1/9.7, 27.4/32.5, 82.0/97.8, and 156.5/193.4 mg/kg/day in males and females respectively) for 24 months. At 12 months, 20 rats/sex/dose were sacrificed.

There were no compound-related effects on mortality, clinical signs, functional observational battery, food efficiency, water consumption, ophthalmology, hematology, clinical chemistry, or urinalysis.

Treatment-related decreases ($p<=0.05$) in body weights and food consumption were observed in females dosed at 1500 and 3000 ppm and in males dosed at 3000 ppm; decreases ($p<=0.01$) of 25-31% in cumulative body weight gains were observed at Weeks 1-13, 13-50, and 1-102 in high dose animals.

In the kidney in the 3000 ppm males, granular material or calculus was observed grossly in the males when all animals were combined. The incidences of pelvic mineralization and transitional cell hyperplasia were increased at the terminal ($p\leq0.01$) and interim sacrifices in males. Pelvic angiectasis and tubular ectasia were increased ($p\leq0.05$) in the females when all animals were combined. Relative (to body) kidney weight was increased ($p\leq0.05$) in the females at the interim and terminal sacrifices. All these abnormalities occurred at an increase of 8-23% beyond the concurrent controls, and exceeded the historical control values. The observed microscopic lesions were of minimal to slight severity on average. In conclusion, slight signs of nephrotoxicity were observed.

In the liver in the 3000 ppm group, mottled livers were observed grossly in males when all animals were combined. Altered hepatocellular eosinophilic foci were observed at the terminal sacrifice in males and females ($p\leq0.05$), as well as the interim sacrifice in females. Altered hepatocellular eosinophilic focus in females (21% vs 11%; NS) were also observed at 1500 ppm when all animals were combined. Increased ($p\leq0.01$) relative (to body) liver weights were observed in the 3000 ppm females at the interim and terminal sacrifices. The incidence of liver lymphohistiocytic infiltrate exceeded the concurrent controls at 1500 (69% treated vs 53% concurrent controls) and 3000 (69%) ppm in females. Minor increases in liver congestion in males at terminal sacrifice were observed at $\geq$1500 ppm. All abnormalities observed at termination occurred at an increase incidence of 12-25% beyond the concurrent controls, and exceeded the historical control values. In conclusion, slight signs of hepatotoxicity were observed.

In the lung, increased ($p \leq 0.05$) relative (to body) lung weights were observed in the 3000 ppm females at the interim and terminal sacrifices (incr 23-27%), and the incidence of chronic lung inflammation was increased 11% ($p \leq 0.01$). The incidence of these lesions exceeded the historical control values. Other corroborating signs of lung toxicity were not observed.

Ovary interstitial gland hyperplasia was observed ($p \leq 0.01$) at 1500 (↑19%) and 3000 (↑30%) ppm when all animals were combined. The incidence of this lesion exceeded the historical control values.

Other minor effects were observed at 3000 ppm when all animals were combined ($p \leq 0.05$ by pair-wise comparison and dose-effect trend) as follows: (i) stomach edema and hemorrhage in males; (ii) thymus epithelial hyperplasia in females; (iv) pituitary angiectasis females; (v) stomach glandular erosion and edema in females; and (vi) uterus congestion. Thyroid follicular cyst(s) were observed at 3000 ppm in females (38% treated vs 21% controls).

The LOAEL is 1500 ppm in females and 3000 ppm in males (equivalent to 156.5/97.8 mg/kg/day in males/females), based on decreased body weight, and food consumption, ovary interstitial gland hyperplasia, increased lymphohistiocytic infiltrate and altered hepatocellular eosinophilic focus of the liver in females; and decreased body weight, and food consumption and slightly increased incidences of pelvic mineralization and transitional cell hyperplasia in the kidney, mottled livers, and altered hepatocellular eosinophilic foci in the livers of males. The NOAEL for this study is 500 ppm in females and 1500 ppm in males (equivalent to 82.0/32.5 mg/kg/day in males/females).

At the doses tested, there was a treatment-related increase in thyroid adenoma incidence in females when compared to controls. When all animals were considered, the incidence of C-cell adenoma in the thyroid was increased ($p<=0.05$) in the 1500 and 3000 ppm females (incr 11-12%), a positive ($p<=0.05$) dose-effect trend was established, and the incidence exceeded the historical controls (20-21% treated vs 12% historical controls). However, a dose-dependent effect was not present in the 3000 ppm females that survived until terminal sacrifice (n=12-30): 150 (38%), 500 (25%), 1500 (35%) and 3000 (20%) ppm vs 20% controls. The combined incidence of C-cell adenoma and carcinoma was increased in the females at 1500 (incr 12%; $p \leq 0.05$) and 3000 (incr 10%; NS) ppm females. A statistically significant effect on combined C-cell adenoma and carcinoma was not demonstrated in the 3000 ppm females by pair-wise comparison, and a dose-effect trend was not established. Furthermore, a dose-dependent effect on combined C-cell adenoma and carcinoma was not present in the 3000 ppm females that survived until terminal sacrifice: 150 (51%), 500 (33%), 1500 (35%), and 3000 (20%) ppm vs 27% in the concurrent controls. However, in the absence of scientific evidence or rationale to discount the thyroid tumors, this finding is considered to be treatment related. Dosing was considered adequate based on body weight, body weight gain, and food consumption decreases, and slight abnormalities in the kidney and liver.

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This study is **acceptable/guideline** and does satisfy the guideline requirement for a chronic/ carcinogenicity study (OPPTS 870.4300; OECD 453) in rats.

Discussion of Tumor Data : At the doses tested, there was a treatment-related increase in thyroid adenoma incidence in females when compared to controls. When all animals were considered, the incidence of C-cell adenoma in the thyroid was increased ($p<=0.05$) in the 1500 and 3000 ppm females (incr 11-12%), a positive ($p<=0.05$) dose-effect trend was established, and the incidence exceeded the historical controls (20-21% treated vs 12% historical controls). However, a dose-dependent effect was not present in the 3000 ppm females that survived until terminal sacrifice (n=12-30): 150 (38%), 500 (25%), 1500 (35%) and 3000 (20%) ppm vs 20% controls. The combined incidence of C-cell adenoma and carcinoma was increased in the females at 1500 (incr 12%; $p\leq0.05$) and 3000 (incr 10%; NS) ppm females. A statistically significant effect on combined C-cell adenoma and carcinoma was not demonstrated in the 3000 ppm females by pair-wise comparison, and a dose-effect trend was not established. Furthermore, a dose-dependent effect on combined C-cell adenoma and carcinoma was not present in the 3000 ppm females that survived until terminal sacrifice: 150 (51%), 500 (33%), 1500 (35%), and 3000 (20%) ppm vs 27% in the concurrent controls.

In the males, hepatocellular carcinoma incidence was increased at 500 (4% treated vs 0% controls; NS) and 3000 (5% treated; $p\leq0.05$) ppm when all animals were combined (Table 8). A significant ($p\leq0.05$) dose-effect trend was also reported. The increased incidence of hepatocellular carcinoma only slightly exceeded the maximum historical control values in the conducting laboratory (4% treated vs 3.3% historical controls), and the Sponsor stated that observed incidence did not exceed the historical control values reported by other laboratories (6%).

Dosing was considered adequate based on body weight, body weight gain, and food consumption decreases, and slight abnormalities in the kidney and liver.

A statistical analysis provided by the Health Effects Division statistician provided the following:

Thyroid C-Cell Adenomas and/or Carcinomas Combined in Females:

Without Interim Sacrifice Animals

	0	150	500	1500	3000
Nos.	9[a]/56	14/54	9/52	16/55	14/57
(%)	(16)	(26)	(17)	(29)	(25)
p =	0.4895	0.1260	0.3575	0.1303	0.2331

With Interim Sacrifice Animals

Nos.	9/78	14/76	10[a]/76	18[a]/76	17[a]/78	
(%)	(12)	(18)	(13)	(24)	(22)	
p =	0.2223	0.1432		0.3068	0.0646	0.0896

Based on the statistical analysis conducted by HED, the increase in thyroid c-cell adenomas and/or carcinomas are not considered to be biologically significant.

Hepatocellular Carcinomas in Males

ppm	0	150	500	1500	3000
Nos.	0/37	0/31	3/33	0/36	4[a]/51
(%)	(0)	(0)	(9)	(0)	(8)
p =	0.0278*	-	0.0231*	-	0.0231*

There were no liver adenomas and no hepatocellular hyperplasia in any of the treated groups. There was an increase in hepatocellular eosinophilic foci; however, according to the pathology report these did not appear to be related to the carcinomas. Historical control data from the same testing laboratory indicate 2/60 or 3.8% of male rats with hepatocellular carcinomas. Historical control data from Charles River Laboratories indicate a range of 1.43 to 8.00% with a mean of 2.42% of male rats with these tumors in the 2002 database. Based on the lack of a continuum (no indication of preneoplastic lesions or hepatocellular adenomas), no dose response and the fact that the percentage of these tumors are within the Charles River historical control range (slightly above at the low dose), the increase in these tumors are not considered to be biologically significant.

Adequacy of the Dose Levels Tested: Dosing was considered adequate based on body weight, body weight gain, and food consumption decreases, and slight abnormalities in the kidney and liver.

2. Carcinogenicity Study in Mice

MRID Nos. 45422721, 45422722, and 45422709

Executive Summary: In this mouse oncogenicity study (MRIDs 45422721, 45422722, and 45422709), TI-435 (Clothianidin; 95.2-95.5% a.i.; Lot #: 30037120) was administered in the diet to 50 Crl:CD-1®(ICR)BR VAF/Plus® mice/sex/group for up to 18 months at doses of 0, 100, 350, or 1250 ppm (equivalent to 0, 13.5/17.0, 47.2/65.1, and 171.4/215.9 mg/kg/day in males/females, respectively). In addition, 50 mice/sex were treated with 700 ppm for weeks 1-4, 2000 ppm for weeks 5-10, 2500 ppm for weeks 11-34, and 2000/1800 ppm (males/females, respectively) (equivalent to 254.1/322.3

mg/kg/day in males/females, respectively) from week 35 until study termination at week 79 and will be referred to as the 2000/1800 ppm group.

No treatment-related effects on hematology, organ weights, or gross pathology were observed. There was no adverse effect at 100 or 350 ppm.

At week 78 in the 1800 ppm females, survival was significantly ($p \leq 0.01$) decreased (↓43%) compared to the concurrent controls, and a dose-related trend ($p \leq 0.01$) was demonstrated. In addition, at week 65 survival was 54% in the 1800 ppm females vs 88-92% in the other groups at week 65 (statistical analyses not reported). Decreases in survival began at week 20 and persisted throughout the study.

Clinical signs were restricted to an increased incidence of vocalization, observed at ≥1250 ppm in both sexes (22-46 treated animals/dose group/sex vs 0 controls, n=50).

Body weights were decreased ($p \leq 0.05$) in the males at 2000/1800 ppm (↓4-18%), and in the females at 1250 (↓5-13%) and 2000/1800 ppm (↓9-17%). Overall (weeks 1-78) body weight gains decreased ($p \leq 0.01$) in the 2000/1800 ppm group (↓24% and 35% in males and females respectively) and in the 1250 ppm females (↓26%). The greatest reduction in body weight gain occurred at the high dose during weeks 11-33 (↓85% and 73% in males and females respectively), when the animals were being treated at 2500 ppm.

Food consumption was frequently decreased ($p \leq 0.05$) in the 2000/1800 ppm group (↓ 8-21% in males and ↓11-24% in females), beginning at week 5 (when the dose was increased to 2000 ppm). Decreases ($p \leq 0.05$) in food efficiency in the 2000/1800 ppm group lead to cumulative decreases of 33-82% at weeks 5-10 (2000 ppm) and 117-151% at weeks 11-33 (2500 ppm).

In the 1800 ppm females, increased ($p \leq 0.01$) incidences of pulmonary congestion (26% treated vs 0% controls; 0%, historical controls), adrenal cortex congestion (22% treated vs 2% controls; 0-2%, historical controls), and cervix fibromuscular hyperplasia (58% treated vs 28% controls; 0-28.0%, historical controls) were observed; however, these effects were considered equivocal without further evidence of an adverse effect in these organs.

The LOAEL is 1250 ppm (215.9 mg/kg/day) based on decreased body weights and body weight gains in females. The NOAEL is 350 ppm (65.1 mg/kg/day).

There was no treatment-related increase in tumor incidences in the treated animals when compared to controls. Dosing was considered adequate based on decreased body weights, body weight gains, food consumption, and food efficiency in both sexes and decreased survival in females.

Under the conditions of this study, the carcinogenic potential of TI-435 is considered negative.

This study is **acceptable/guideline** and satisfies the requirements for a carcinogenicity study [OPPTS 870.4200; OECD 451] in mice.

Discussion of Tumor Data: There was no treatment-related increase in tumor incidences in the treated animals when compared to controls.

Adequacy of the Dose Levels Tested: Dosing was considered adequate based on decreased body weights, body weight gains, food consumption, and food efficiency in both sexes and decreased survival in females.

3. Classification of Carcinogenic Potential: In accordance with the Draft 1999 Carcinogen Risk Assessment Guidelines, the HIARC classified clothianidin as "not likely to be carcinogenic to humans". A statistical analysis showed that the increase in thyroid c-cell tumors in female rats was not significant, especially when carcinomas and adenomas are combined. The increased incidence of hepatocellular carcinomas in male rats at the low and high doses are just outside historical control incidences for the same testing laboratory (only 2 studies) but are within the historical control range for the animal supplier. In addition, there was no dose-response and there is no continuum (i.e. no preneoplastic lesions and no adenomas). Based on these factors, it was determined that there is no evidence of carcinogenicity in rats. There is no evidence of carcinogenicity in mice.

IV. MUTAGENICITY

The HIARC concluded that there is a concern for mutagenicity resulting from exposure to clothianidin. Some of the batches of test material tested positively and some tested negatively. The HIARC has requested that the composition of the test materials used in the mutagenicity studies be investigated to determine whether or not the differences in composition may have affected the results from the studies.

In four acceptable bacterial gene mutation studies, technical clothianidin (TI-435) was mutagenic in only one formulation (Batch/Lot No. 30034708, and only in one strain of *Salmonella typhimurium*, TA1535, but consistently negative in three other bacterial assays using different batches/Lot Nos. of the technical (see following table). Negative results were also obtained with the more sensitive base-substitution strains TA100 and *E. coli* WP2 μvrA$^-$. The lot inducing the positive response was negative in an independently performed study using both the plate incorporation protocol and the preincubation modification of the plate incorporation assay. In addition, this formulation (batch) proved to be a clastogen at toxic dose levels in *in vitro* Chinese hamster lung (CHL) cultures as well as in mouse lymphoma L5178Y (TK $^{+/-}$) cells, but negative in another mammalian cell test system (V79/HGPRT). Limited *in vivo* assays (rat UDS, mouse micronucleus) were negative.

Acceptable Mutagenicity Assays with Clothianidin:

MRID	BATCH/LOT NO. (PURITY)	TEST	RESULT
45422740	30034708 (96% a.i.)	Mouse Micronucleus	Negative
45422739	30034708 (96% a.i.)	In Vivo UDS (Rat)	Negative
45422738	30034708 (96% a.i.)	V79 (HGPRT)	Negative
45422737	30034708 (96% a.i.)	L5178Y ($TK^{+/-}$)	Positive [Small Colony Clastogenicity]
45422736	30034708 (96% a.i.)	CHL (CA)	Positive
45423734	30034708 (96% a.i.)	Ames[1]	Negative
	NLL 6100-3 (98.6% a.i.)	(TAIS 1535 only)	Negative
45422733	30037120 (95.2% a.i.)	Ames	Negative
45422732	12256321 (99% a.i.)	Ames/*E. coli*[2]	Negative
45422731	30034708 (96% a.i.)	Ames/*E. coli*[2]	Positive (TA1535)

[1]Test material assayed in the standard plate incorporation procedure and the preincubation modification to the plate incorporation test.

[2]Two independent plate incorporation assays were performed.

V. HAZARD CHARACTERIZATION

Clothianidin has low toxicity in the rat via the oral, dermal and inhalation route (Toxicity Category III and IV). In an acute oral study in mice, it is more toxic (Toxicity Category II). It is only slightly irritating to the eye and is not irritating to the skin (both Toxicity Category IV). It is not a sensitizer in the Guinea Pig (Magnusson-Kligman Maximization test) under the conditions of the study.

In subchronic studies in rats and dogs, decreases in body weight and body weight gain were observed in both species. Dogs also displayed some anemia and decreased white blood cells, albumin, and total protein and appear to be more sensitive to the effects of clothianidin. In the dog study, males are more sensitive than females. No effects were observed up to the limit dose in the 29-day dermal study in rats. Thus, the oral route of administration is more toxic in the rat than the dermal route.

In the chronic feeding studies in the dog, rat and mouse, again the dog appears to be the most sensitive species followed by the rat and females appear to be more sensitive than males in all three species. Anemia was observed in the dog. In the rat, decreased body weight and food consumption, ovary interstitial gland hyperplasia, increased lymphohistiocytic infiltrate and altered hepatocellular eosinophilic focus of the liver were observed in females; and decreased body weight and food consumption, slightly increased incidences of pelvic mineralization and transitional cell hyperplasia in the kidney, mottled livers, and altered hepatocellular eosinophilic foci in the liver were observed in males. In the mouse, decreases in body weight and body weight gain in females and increases in vocalization in both sexes were the only observed effects.

In the rat, although in terms of ppm the NOAELs and LOAELs for the subchronic and chronic feeding studies were similar, more effects were observed in the chronic study. In the dog, administration of clothianidin for a longer period of time does not appear to have any additional effects or effects at lower dose levels.

In an acute neurotoxicity study in rats via gavage, FOB effects were observed, which included decreased arousal and decreased motor and locomotor activity on Day 0 in males. Effects at dose levels above the LOAEL included tremors, slightly uncoordinated gait, effects on pupil response and righting reflex, decrease in body temperature and ataxia. Mice appear to be more sensitive to the acute neurotoxic effects of clothianidin when administered by gavage. Effects were also observed on day 0 in males (no female mice were tested) at lower dose levels than in rats which included transient signs of decreased spontaneous motor activity, tremors, and deep respirations. At higher dose levels, decreases in reactivity, grooming, and muscle tone; prone position; staggering gait; mydriasis, and hypothermia were observed.

In the subchronic feeding neurotoxicity study in rats, no indications of neurotoxicity were observed. Slightly decreased food consumption, body weights, and body weight gains were the only observed effects. The LOAEL was similar to the subchronic feeding study in rats. The NOAEL was higher because the selected dose levels were different between the two studies.

In the developmental neurotoxicity study, there was evidence of quantitative susceptibility in pups. The NOAEL for offspring toxicity is based on decreased body weight gains, motor activity and acoustic startle response at the same dose level as the NOAEL for the parents. The parental LOAEL is based on decreased body weights, body weight gains and food consumption.

In the developmental and reproduction studies, no quantitative or qualitative susceptibility was observed in either of the development rat or rabbit studies. No developmental toxicity was observed in the rat at dose levels which induced decreases in body weight gain and food consumption in the dams. In the rabbit, premature deliveries, decreased gravid uterine weights, an increase in litter incidence of a missing lobe of the lung and a decrease in the litter average for ossified sternal centra per fetus were noted at a dose level in which maternal death, a decrease in food consumption and clinical signs (scant feces and orange urine) were observed. The developmental effects are not considered to be qualitatively more severe than the maternal effects. Quantitative susceptibility was observed in the reproduction study. At the parental NOAEL, the LOAEL for offspring toxicity is based on decreased body weight gains, delayed sexual maturation (males), decreased absolute thymus weights in F_1 pups of both sexes and an increase in stillbirths in both generations. The parental systemic LOAEL is based on decreased absolute body weights and body weight gains with decreased absolute and relative thymus weights in both sexes.

In the rat chronic feeding/carcinogenicity study, an apparent increase in thyroid c-cell tumors was observed in females. In addition, an increased incidence of hepatocellular carcinomas in males was examined more closely. A statistical analysis revealed that the increase in thyroid c-cell tumors did not appear to be significant, especially when carcinomas and adenomas are combined. The increased incidence of hepatocellular carcinomas at the low and high doses were just outside historical control incidences for the same testing laboratory (only 2 studies) but were within the historical control range for the animal supplier. In addition, there was no dose-response. Finally, there was no continuum (i.e. no preneoplastic lesions and no adenomas). There was no evidence of an increase in tumors in mice. Therefore, the HIARC classified clothianidin as not likely to be carcinogenic in humans.

The mutagenicity studies gave mixed results. Some of the batches of test material tested positively and some tested negatively. The HIARC has requested that the composition of the test materials used in the mutagenicity studies be investigated to determine whether or not the differences in composition may have affected the results from the studies.

In some of the toxicological studies, there was evidence of possible effects on the immune system. Decreased absolute and adjusted thymus and spleen weights were observed in multiple studies. In addition, juvenile rats in the two-generation reproduction study appeared to be more susceptible to these effects. Therefore, the HIARC recommends that testing be conducted to assess immune system function in adults and in young animals following developmental exposures.

In rats, clothianidin is readily absorbed and excreted within 96 hours following a single low dose or repeated low doses, but at a high dose, absorption became biphasic and was saturated. The studies suggest that a multiple exposure regimen did not affect the absorption/excretion processes. There was rapid absorption and distribution of administered radioactivity to all organs

and tissues followed by rapid excretion with reduction to background levels in most tissues and organs within 24 hours. There was a somewhat greater rate of absorption and elimination in females. Excretory patterns did not exhibit gender-related variability but reflected the delayed absorption in the high-dose group. The metabolites identified (primarily oxidative demethylation products and cleavage products of the nitrogen-carbon bond between the nitroimino and thiazolyl moieties) were consistent with Phase I processes.

In mice, clothianidin is readily absorbed and excreted within 168 hours following a single low dose. Urine was the major route of excretion. Neither TI-435 nor its metabolites appeared to exhibit potential for bioaccumulation. Excretory patterns did not exhibit gender-related variability. The major metabolites in both urine and feces were the parent compound (TI-435) and TZNG [N-(2-chlorothiazol-5-ylmethyl)-N'-nitroguanidine] which resulted from N-demethylation of TI-435.

VI. DATA GAPS / REQUIREMENTS

- The HIARC has requested that the composition of the test materials used in the mutagenicity studies be investigated to determine whether or not the differences in composition may have affected the results from the studies.

- A developmental immunotoxicity study with comparative measures between the pups and the parents (see Attachment A and consult with HED scientists to develop a protocol).

VII. ACUTE TOXICITY

Acute Toxicity of Clothianidin (TI-435) PC Code 044309

GDLN	Study Type	MRID	Results	Tox Category
870.11	Acute Oral - rat	45422621	LD_{50} > 5000 mg/kg	IV
870.11	Acute Oral - mouse	45422622	LD_{50} (M): 389 mg/kg bw (95% c.i. = 380-475) LD_{50} (F): 465 mg/kg bw (95% c.i. = 384-561) LD_{50} Combined: 425 mg/kg bw (95% c.i. = 380-475)	II
870.12	Acute Dermal - rat	45422634	LD_{50} > 2000 mg/kg	III
870.13	Acute Inhalation	45422636	LC_{50} (M & F): > 5.538 mg/L	IV
870.24	Primary Eye Irritation	45422701	Slightly irritating to the eye	IV
870.25	Primary Skin Irritation	45422703	Not irritating to the skin	IV
870.26	Dermal Sensitization	45422705	Is not a sensitizer under conditions of study.	N/A

VIII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

Summary of Toxicological Dose and Endpoints for Clothianidin

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (Females 13-50 years of age)	Developmental NOAEL = 25 UF = 1000[a] **Acute RfD** = 0.025 mg/kg	FQPA SF = 1 **aPAD** = acute RfD / FQPA SF = 0.025 mg/kg	Developmental rabbit study Developmental LOAEL = 75 mg/kg/day based on an increased litter incidence of a missing lobe of the lung.
Acute Dietary (General population)	NOAEL = 25 UF = 1000[a] **Acute RfD** = 0.025 mg/kg	FQPA SF = 1 **aPAD** = acute RfD / FQPA SF = 0.025 mg/kg	Special Neurotoxicity/Pharmacology Study in Mice and Rats LOAEL = 50 mg/kg based on transient signs of decreased spontaneous motor activity, tremors and deep respirations.
Chronic Dietary (All populations)	Offspring NOAEL= 9.8 UF = 1000[a] **Chronic RfD** = 0.0098 mg/kg/day	FQPA SF = 1 **cPAD** = chronic RfD / FQPA SF = 0.0098 mg/kg/day	2-Generation Reproduction Study Offspring LOAEL = 31.2 mg/kg/day based on decreased mean body weight gain and delayed sexual maturation, decreased absolute thymus weights in F_1 pups and an increase in stillbirths in both generations.
Incidental Oral (All Durations)	NOAEL= 9.8 mg/kg/day	**Residential** LOC for MOE = 1000[a] **Occupational** = NA	2-Generation Reproduction Study Offspring LOAEL = 31.2 mg/kg/day based on decreased mean body weight gain and delayed sexual maturation, decreased absolute thymus weights in F_1 pups and an increase in stillbirths in both generations.
Dermal (All Durations)	Oral study NOAEL= 9.8 mg/kg/day (dermal absorption rate = 1 %)	**Residential** LOC for MOE = 1000[a] **Occupational** LOC for MOE = 100	2-Generation Reproduction Study Offspring LOAEL = 31.2 mg/kg/day based on decreased mean body weight gain and delayed sexual maturation, decreased absolute thymus weights in F_1 pups and an increase in stillbirths in both generations.
Inhalation (All Durations)	Oral study NOAEL= 9.8 mg/kg/day (inhalation absorption rate = 100%)	**Residential** LOC for MOE = 1000[a] **Occupational** LOC for MOE = 100	2-Generation Reproduction Study Offspring LOAEL = 31.2 mg/kg/day based on decreased mean body weight gain and delayed sexual maturation, decreased absolute thymus weights in F_1 pups and an increase in stillbirths in both generations.
Cancer (oral, dermal, inhalation)	**Classification: Not Likely**		

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

[a] Additional 10x database uncertainty factor for lack of a developmental immunotoxicity study.

NOTE: The Special FQPA Safety Factor recommended by the HIARC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.

Attachment A
Immunotoxicity Testing for Clothianidin

Due to evidence of decreased absolute and adjusted organ weights of the thymus and spleen in multiple studies in the clothianidin data base, and since juvenile rats in the two-generation reproduction study appeared to be more susceptible to these effects, the HIARC recommended that testing be conducted to assess immune system function in adults and in young animals following developmental exposures. For the adult animals, a study that meets the specifications of a guideline immunotoxicity study (OPPTS 870.7800) should be conducted. In this study, young adult rats (6 - 8 weeks of age) are exposed to the test substance for 28 days, at which time they are terminated. In addition, an enumeration of splenic or peripheral blood total B cells, total T cells, and T cell subpopulations should be conducted. The spleen and thymus are examined macroscopically, and organ weights are recorded. Assessments of immune system function include an evaluation of the response to a T-cell-dependent antigen, sheep red blood cells (SRBC). The SRBC antigen response assays can be conducted either by an antibody plaque-forming cell (PFC) assay or by immunoglobulin quantification by enzyme-linked immunosorbent assay (ELISA).

Although there is currently no formal EPA guideline for a developmental immunotoxicity study, a number of sources can be consulted to form the basis for recommendations on study conduct. For example, studies have been conducted in numerous laboratories to assess the developmental immunotoxic potential for a myriad of pharmaceuticals, pesticides, and environmental pollutants, and the results of many of these studies are reported in the literature (e.g., Chapin et al., 1997; Smialowicz et al., 2001). Additionally, the National Toxicology Program (NTP) has devised an immunotoxicity testing battery (in adult mice) which has demonstrated the value of a combination of two or three immune system tests to achieve >90% predictability of immunotoxic compounds in rodents (Holladay and Luster, 1994); the use of multiple tests, both structural and functional, is an approach that is also applicable in assessing immunotoxicity in juvenile animals following developmental exposures. Scientific workshops on immunotoxicity conducted by ILSI in 2000 and NIEHS in 2001 (Luster et al., 2002) have discussed the most appropriate experimental design and tests for evaluating the potential developmental immunotoxic effect of chemicals in experimental models.

The registrant is asked to consult with the agency regarding the most appropriate study design for a developmental immunotoxicity testing with clothianidin. The following general study characteristics should ensure that the study design accommodates for temporal differences in immune system maturation between rodents and humans, assesses the immune system during critical windows of vulnerability, includes an array of standardized sensitive assays in order to maximize the potential for detecting a treatment-related response. As always, it is recommended that pharmacokinetic data and information on the mode of action of the chemical should be used to design the study and select dose levels.

Species: The rat is generally regarded as the species of choice for developmental immunotoxicity testing for the purpose of risk assessment, since there is extensive information available on the immune system of the rat, a large historical data base of reproductive data, and a low background incidence of malformations and stress effects.

Number of test subjects: As with all guideline studies that examine developmental toxicity, the litter is considered the primary unit for evaluation. Each test group should contain at least 20 pregnant dams, and from the resulting litters, at least one pup/sex per litter should be selected for immunotoxicological assessment.

Exposure period: The exposure period should cover the entire period of immunological

ontogenesis, ranging in the rat approximately from the time of implantation through to the time of sexual maturation, e.g., gestation (GD) 6 through postnatal (PND 42).

Route of exposure: The route of exposure should be based upon the primary known or potential route of human exposure, in this case oral. The extent of exposure duration through several early life stages in the rodent would require test substance administration to the maternal animal during gestation and in early lactation, direct administration to the offspring during late lactation and postweaning ages, and to the dam and/or directly to pups during mid to late lactation.

Sampling schedule: Blood and other tissue samples would be taken at necropsy, on approximately PND 42, when the antibody response in rats approximates that of a mature animal.
Endpoints: As with other studies that include developmental exposures, a developmental immunotoxicity study should include standard measurements of growth (body weight), survival, clinical response, and macroscopic pathology. Additionally, a number of research efforts in the field of immunotoxicology have demonstrated the sensitivity of the following morphological and functional endpoints in detecting effects on the immune system of young animals following developmental exposures:

- Complete total and differential blood cell count (CBC)
- Thymus, spleen, and lymph node weights
- Evaluation of primary antibody response to a T-dependent antigen (e.g., sheep red blood cell [SRBC])
- Functional test of Th1 immunity (e.g., cytotoxic T lymphocyte [CTL] or delayed hypersensitivity response [DHR])
- An enumeration of splenic or peripheral blood total B cells, total T cells, and T cell subpopulations
- Histopathology of lymphoid organs/tissues

References:

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