6-25-99

Reviewer: Brenda MacDonald, D.V.M. **Date:** June 25, 1999

STUDY TYPE: 13-Week Subchronic Neurotoxicity in the rat [feeding] OPPTS 870.6200; [§82-7]; OECD 424.

TEST MATERIAL (PURITY): CGA 293343 Technical, 98.7%

SYNONYMS: Thiamethoxam

CITATION: Minnema, Daniel J., (1998). 13-Week Dietary Subchronic Neurotoxicity Study with CGA -293343 TECH in Rats. Covance Laboratories Inc., Vienna, Virginia, Study no. Covance 6117-363. June 23, 1998. Unpublished. MRID 44703325.

SPONSOR: Novartis Crop Protection, Inc., NC, USA

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study, CGA 293343 technical (purity 98.7%), was administered to 10 Sprague-Dawley Crl:CD BR rats/sex/dose in the diet at dose levels of 0, 10, 30, 500 and 1500 ppm for males (equal to 0, 0.7, 1.9, 31.8 and 95.4 mg/kg bw/day, respectively); and dose levels of 0, 10, 30, 1000 and 3000 ppm for females (equal to 0, 0.7, 2.1, 73.2 and 216.4 mg/kg bw/day, respectively), for 13 weeks. Special neurological examinations included a Functional Observational Battery (FOB) and locomotor activity (LMA) testing, conducted prior to treatment and during weeks 4, 8 and 13 of treatment. In addition, a detailed histopathological examination of perfused central and peripheral nervous system tissues was conducted.

There were no treatment-related systemic or neurotoxicological effects observed at any dose level tested. **A LOAEL for neurotoxicological and systemic effects was not established. The NOAEL for males is 1500 ppm (95.4 mg/kg bw/day) and for females is 3000 ppm (216.4 mg/kg bw/day) since there were no treatment-related effects observed at any dose level tested.**

This study is classified acceptable and satisfies the guideline requirement for a subchronic neurotoxicity study in rats (§82-7).

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** CGA 293343

Description:	Technical material, light beige crystalline powder
Lot/Batch #:	9600110
Purity:	98.7 % a.i.
Compound Stability:	Not given; stated to be the responsibility of the sponsor
CAS #:	53719-23-4

2. **Vehicle:** N/A; test material administered in the diet.

3. **Test Animals:**

Species:	Rat	
Strain:	Sprague-Dawley Crl:CD BR	
Age at start of dosing:	~6 weeks old	
Wt. at start of dosing :	Males: 176 g to 220 g; Females: 150 g to 181 g	
Source:	Charles River Laboratories, Inc., Raleigh, NC.	
Housing:	Individually housed in suspended wire-mesh cages	
Diet:	PMI Certified Rodent Diet #5002, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	18.7 to 25.6°C
	Humidity:	38.8 to 65.8%
	Air changes:	≥ 10/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least one week	

B. STUDY DESIGN:

1. **In-Life Dates**: May 2, 1997 to August 7, 1997

2. **Animal Assignment**: Animals were randomly assigned to the test groups shown in Table 1 using a computerized weight-randomization program.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Males	Females
Control	0	10	10
Low Dose	10	10	10
Mid Dose	30	10	10
Mid-high Dose	500	10	
Mid-high Dose	1000		10
High Dose	1500	10	
High Dose	3000		10

3. **Dose Selection Rationale**: The high dose levels were chosen based on the results of a 3-month dietary

study, study no. 942089. For males, body weights and food consumption were lower by 15% and 11%, respectively, at the dietary level of 1250 ppm. For females, there was an increase in the severity of nephrocalcinosis and an increase in the severity and incidence of chronic tubular lesions at the dietary level of 2500 ppm. Based on these results, the dose levels chosen for the 13-week neurotoxicity study appear to be satisfactory to the reviewer.

4. Diet Preparation and Analysis: Fresh diets were prepared every week throughout the study period, and were stored at room temperature. The test material was passed through a number 60 sieve, then premix was prepared by mixing the appropriate amount of test substance with 200 g of feed in a Waring blender for ~2 minutes. This premix was then added to the calculated amount of feed to achieve the desired dietary concentrations, and mixed in a Hobart blender for 10 minutes. Stability after storage at room temperature for 7 and 10 days, and homogeneity of mixing were determined for test diets at dose levels of 10 and 3000 ppm, prepared prior to study initiation. Samples were taken in duplicate from the top, middle and bottom of the mixer. Actual test material concentration in the diets was determined for all dose levels, from duplicate samples of test diets taken each week for the first 4 weeks, and then from diet prepared for weeks 8 and 13.

Results:

Stability Analysis: The actual concentrations of CGA 293343 in the 10 and 3000 ppm test diets prepared prior to study initiation, expressed as percentage of the nominal concentration, were as follows:

	Dose (ppm)	
Storage Interval	**10**	**3000**
Day 7	108%	106%
Day 10	66.6%	105%

Based on these results, it was concluded that the formulated diets were stable for 7 days this study (i.e., freshly prepared on a weekly basis).

Homogeneity Analysis: Individual samples of the 10 and 3000 ppm test diets varied by up to 18% and 3%, respectively. The variability was relatively high for the 10 ppm group, but it was unlikely to interfere with the results of the study, since this was the lowest dose level tested. Homogeneity analysis was not performed at dietary concentrations of 30, 500, 1000 and 1500 ppm. However, results of homogeneity analyses conducted on test diets prepared for the 2-generation rat reproduction study (study no. 942121) at dietary concentrations of 10, 30, 1000 and 2500 ppm indicated that these diets were homogeneous at all dose levels tested, i.e., maximum variability was 8.8% seen at 30 ppm.

Concentration Analysis: The range of values for the actual concentrations of CGA 293343 in the test diets, and the overall mean values, expressed as percentage of the nominal concentrations, were as follows:

	Dose (ppm)					
	10	30	500	1000	1500	3000

	Dose (ppm)					
Actual concentration						
Range of values, ppm	9.30 - 12.01	26.32 - 31.73	466.1-535.6	951.7-1132.0	1440-1620	2903-3113
Mean value, ppm	10.06	29.75	497.9	1014.8	1532	3042
% of target concentration						
Range of values	93 - 120	88 -106	93 - 107	95.2-113.0	96.0-108.0	96.8-104.0
Mean value	100.6	99.2	99.6	101.5	102.1	101.4

Based on these results, the actual test material concentrations, when compared to the target concentrations, were considered acceptable for the purposes of this study.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics: For mean body weight, body weight change and food consumption data, if variances of untransformed data were heterogenous, a rank transformation of the data was performed to achieve variance homogeneity. If the transformation did not achieve homogeneity, analyses were still performed on the transformed data. Continuous behavioural data were analysed by a factorial analysis of variance with repeated measures using Biomedical Data Processing (BMDP) software. Univariate analysis of dose (at each time point) was conducted if overall significant dose effects or dose-by-time interactions were detected. LMA data were square-root transformed and then analysed by double-repeated measures analysis of covariance, where week and interval were the within factors, treatment (group) was the between factor, and the pretreatment interval was the covariate. These analyses were considered to be appropriate and acceptable by the reviewer.

6. Positive Control Data Base: Results of a separate study, using trimethyltin chloride (TMT-Cl), a known CNS toxicant in rats, were submitted to validate the neurobehavioural and neurohistological assessment procedures used in the acute neurotoxicity study, and the 13-week dietary subchronic study. Based on the results of this study (Neurotoxicity Study of Trimethyltin in Rats", report no. CHV 0001-979, dated July 25, 1996), it was concluded that "the neurohistological and neurobehavioural findings noted in male rats treated with a single dose of 8.5 mg/kg TMT-Cl are consistent with known neurotoxicological effects associated with this neurotoxicant". TMT-Cl resulted in histopathological findings in the central nervous system, thus validating the procedures used for neurohistopathological assessment. However, since neurobehavioural effects of TMT-Cl primarily result in "emotional damage", TMT-Cl did not result in notable changes to the Functional Observational Battery and locomotor activity evaluations. Hence, the reviewer concluded that although findings in this positive control study were consistent with the known neurotoxic effects of TMT-Cl, the results do not allow for an adequate assessment of the neurobehavioural evaluation procedures. However, in the "Acute Neurotoxicity Study of Orally Administered CGA-293343 TECH in Rats", Study no. 6117-364, dated September 23, 1997, CGA 293343 elicited clearly positive results in the FOB and LMA evaluations at 2 hours postdosing, but not at 1 or 2 weeks postdosing. These results indicate that the neurobehavioural assessment procedures used in these studies are valid.

C. METHODS:

1. Clinical Observations: Animals were examined at least once daily for mortality, moribundity and clinical signs of toxicosis. A detailed clinical examination was conducted on a weekly basis.

2. Body Weight: Individual body weights were measured on a weekly basis.

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3. Food Consumption and Compound Intake: Food consumption values were recorded on a weekly basis. From the body weight and food intake data, the mean daily food consumption per animal (g/animal/day) and the mean daily substance intake per animal (mg/kg bw/day) were calculated.

4. Ophthalmological Examination: An ophthalmological examination was conducted on each animal prior to initiation of treatment and during week 13.

5. Neurobehavioural Evaluations:

a. Functional Observational Battery (FOB) Evaluations: A Functional Observation Battery was performed before initiation of treatment and during weeks 4, 8 and 13 of treatment. Evaluations were conducted during the animals' dark cycle. The following parameters were evaluated:

Home Cage Observations

Appearance of fur	Lacrimation
Color of tears, deposits around eyes	Writhing
Convulsions, tremors	Palpebral closure
Ease of handling, body tone	Piloerection
Ease of removal from cage	Respiration
Excessive vocalizations	Salivation
Exophthalmos	Other signs

Open Field Observations (1-minute observation period)

Arousal	Circling
Posture	Gait
Convulsions	Stereotypy
Tremors	Latency to first step
Number of rears	Number of convulsions
Other signs	

At the end of the observation period, number of urine pools and fecal boli were counted, and the presence or absence of polyuria and diarrhea were noted.

Response Observations

Light approach response	Pupil response
Catalepsy time	Righting reflex
Olfactory response	Touch response
Auditory startle response	Other signs

Performance Measures

Forelimb grip strength	Rectal body temperature
Hindlimb grip strength	Tail flick latency
Landing foot splay	

b. Locomotor Activity (LMA) Evaluation: All animals were evaluated for motor activity before initiation of treatment and during weeks 4, 8 and 13 postdosing. Each animal was placed in an automated photocell activity monitoring device (San Diego Instruments, Model PAS) for 40 minutes. Movement was monitored as activity counts (photobeam breaks) in 1-minute intervals. Data were converted into 8 blocks of 5 minutes each for analysis.

6. **Sacrifice and Pathology:** All surviving animals were sacrificed after 13 weeks of treatment, by i.p. injection of sodium pentobarbital. Six/sex/group were then perfused with heparinized saline followed by a 3.0% glutaraldehyde/2.5% paraformaldehyde solution, after which the following organs were collected for microscopic examination:

Brain with brainstem	Gasserian ganglia
Cervical spinal cord	Sciatic nerve
Mid-thoracic spinal cord	Tibial nerve
Lumbar spinal cord	Sural nerve
Cervical and lumbar dorsal root ganglia	Eyes with a portion of the optic nerve attached
Cervical dorsal and ventral root fibres	Anterior tibialis muscles
Lumbar dorsal and ventral root fibres	Gastrocnemius muscles
Pituitary	Kidneys
Gross lesions	

From non-perfused animals, the kidneys and gross lesions were collected.

All animals that died prior to scheduled sacrifice, and those that survived to study termination were necropsied.

II. RESULTS

A. Observations:

1. Mortality: All animals survived the duration of the study period.

2. Clinical Signs: There were no overt clinical signs of treatment-related toxicity.

B. Body Weight and Body Weight Gain: There were no treatment-related effects. Mean body weight and mean body weight gain values were comparable amongst all groups throughout the study period.

C. Food Consumption and Compound Intake:

1. Food Consumption: There were no treatment-related effects. Mean food intake values were comparable amongst all groups throughout the study period.

2. Compound Consumption : Based on food consumption, the nominal dietary concentrations and body weight, the doses expressed as mean daily mg test substance/kg body weight during the study period were as follows:

i) For males: 0, 0.7, 1.9, 31.8 and 95.4 mg/kg bw/day for the 0, 10, 30, 500 and 1500 ppm groups, respectively; and,

ii) For females: 0, 0.7, 2.1, 73.2 and 216.4 mg/kg bw/day for the 0, 10, 30, 1000 and 3000 ppm groups, respectively.

D. Ophthalmological Examination: There were no treatment-related findings.

E. Neurobehavioural Evaluations:

1. FOB Evaluations:

a) Pretreatment: There were no observations that were out of normal expectations for untreated animals.
b) Weeks 4, 8 and 13 postdosing: There were no findings considered to be related to treatment with CGA 293343.

2. LMA Evaluations:
a) Pretreatment: There were no observations that were out of normal expectations for untreated animals.
b) Weeks 4, 8 and 13 postdosing: There were no findings considered to be related to treatment with CGA 293343.

F. Sacrifice and Pathology:

1. Gross Pathology: There were no findings considered to be related to treatment with CGA 293343.

2. Microscopic Pathology: There were no findings considered to be related to treatment with CGA 293343.

III. DISCUSSION

A. Investigators' Conclusions: "There were no treatment-related findings noted during the clinical observations, ophthalmoscopic examinations, neurobehavioural assessment (FOB and LMA), or macro- and microscopic examinations.
In conclusion, the NOEL for neurotoxicity associated with dietary administration of CGA-293343 TECH to Sprague-Dawley rats for at least 13 weeks is ≤ 1500 ppm in the diet for males and ≤ 3000 ppm for females."

B. Reviewer's Comments: Male and female Sprague-Dawley Crl:CD BR rats were fed test diets containing CGA 293343, purity 98.7%, at dietary concentrations of 0, 10, 30, 500 and 1500 ppm for males (equal to 0, 0.7, 1.9, 31.8 and 95.4 mg/kg bw/day, respectively); and at dietary concentrations of 0, 10, 30, 1000 and 3000 ppm for females (equal to 0, 0.7, 2.1, 73.2 and 216.4 mg/kg bw/day, respectively), 10 rats per sex per group, for 13 weeks. Special neurological examinations included a Functional Observational Battery (FOB) and locomotor activity (LMA) testing, conducted prior to treatment and during weeks 4, 8 and 13 of treatment. In addition, a detailed histopathological examination of perfused central and peripheral nervous system tissues was conducted.

The NOAEL was determined to be 1500 ppm for males (equal to 95.4 mg/kg bw/day) and 3000 ppm for females (equal to 216.4mg/kg bw/day) since there were no treatment-related systemic or neurotoxicological effects observed at any dose level tested.

C. Study Deficiencies: No scientific deficiencies were noted in the study. The animals were not subjected to FOB or LMA evaluations during the first or second week of exposure, as the guidelines recommend. However, there were no treatment-related effects observed during the week 4, 8, and 13 FOB and LMA evaluations, and there were no other neurotoxicological effects observed throughout the study. Hence, the omission to conduct FOB and LMA assessments within the first 2 weeks of the study is not considered to affect the final conclusions of this study.