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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: 8/5/09

SUBJECT: Dinotefuran: Review of the range finding study in rats for developmental immunotoxicity and developmental neurotoxicity study

PC Code: 044312
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0055238
MRID No.: 47677501

DP Barcode: 366688
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: NA
40 CFR: 158.500

FROM: **Whang Phang, PhD**
Toxicologist
RAB 3/HED (7509P)

THROUGH: **Paula Deschamp, Branch Chief**
RAB 3/HED (7509P)

TO: **Rita Kumar, Risk Manager Reviewer**
Registration Division (7505P)

CONCLUSION: The results of this dose-range finding study provide information regarding the potential immunotoxic effects in offspring rats after exposure to dinotefuran during prenatal, postnatal, and post-weaning periods. Dinotefuran did not affect the various parameters examined. The parameters examined are comparable to those measured in a definitive immunotoxicity study. The study is considered acceptable/non-guideline. When the results of this study are considered with the entire toxicity data base of dinotefuran, this study fulfills the requirement for a developmental immunotoxicity study, at this time. For dose-range finding of a developmental neurotoxicity study (DNT), this diet concentration (10000 ppm) is considered suitable as a high dose level for the DNT study.

ACTION REQUESTED: Review the submitted dose-range-finding developmental immunotoxicity study developmental neurotoxicity study in rats.

RESULTS/DISCUSSION: the registrant submitted a dose-range finding study (MRID 47677501) for developmental neurotoxicity and developmental immunotoxicity study on dinotefuran in rats. This study has been reviewed, and the Data Evaluation Report is attached. The results of this range finding study showed that under the conditions of the study, dinotefuran did not affect the distribution of splenocyte subpopulations (total B cell, total T cells, helper/DTH T cells, cytotoxic T cells, and natural killer cells) in the weanlings of F_1 generation. It did not affect the anti-SRBC antibody forming cell response (humoral immunity) and NK cell activity (innate immunity). Therefore, it was concluded that dinotefuran showed no evidence of an effect on the functionality of the immune system in rats which were exposed to dinotefuran during the prenatal, postnatal, and post-weaning periods. Although, this study was a dose-range-finding study, it examined all the parameters which would have been required in a regular developmental immunotoxicity study and the highest tested dose (1035 mg/kg) was slightly greater than the limit dose (1000 mg/kg). Considering the results and conduct of the study, HED believes that this range-finding study provides sufficient data for understanding the immunotoxic potential of dinotefuran in the test animals, which were exposed to this chemical during prenatal, postnatal, and weaning periods and satisfies the data requirement for a developmental immunotoxicity study in rats.

For dose-range finding of a developmental neurotoxicity study, the maternal toxicity LOAEL was not observed. The NOAEL for maternal toxicity is 10,000 ppm. The offspring toxicity LOAEL was 10,000 ppm based on decreased pup body weights in both sexes. The NOAEL for offspring toxicity is 3000 ppm. Therefore, this diet concentration (10,000 ppm) is considered suitable as a high dose level for the DNT study.

MRID Summary Table Example

Study Type	MRID	Comments
Dose-range-finding study in rats for developmental immunotoxicity and developmental neurotoxicity of dinotefuran.	47677501 Acceptable/non-guideline 1000, 3000, & 10,000 ppm (105, 318, & 1035 mg/kg/day)	The results demonstrate that dinotefuran did not affect any of the parameters examined for an immunotoxicity study in test animals exposed to dinotefuran during, prenatal, postnatal, and weaning periods at doses as high as 1035 mg/kg/day.

DATA EVALUATION RECORD

DINOTEFURAN (MTI-446)

Study Type: Non-Guideline; Developmental Immunotoxicity Study in Rats

Work Assignment No. 6-01-212 (MRID 47677501)

Prepared for
Health Effects Division
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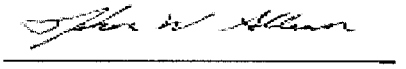
Signature: 
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Yung G. Yang, Ph.D. **Signature:** Yung G. Yang
Toxicology and Epidemiology Branch, HED (7509P) **Date:** 7/30/2009
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** [illegible]
Risk Assessment Branch 3, HED (7509P) **Date:** 7/30/09

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Developmental Immunotoxicity [feeding] - rats; Non-Guideline
& Dose-Range Finding for Developmental Neurotoxicity Study

PC CODE: 044312 **DP BARCODE:** D364521, D366688

TXR #: 0055238 **SUBMISSION:** S845031

TEST MATERIAL (PURITY): Dinotefuran (94.7% a.i.)

SYNONYMS: MTI-446; *N*-methyl-*N'*-nitro-*N''*-[(tetrahydro-3-furanyl)methyl]guanidine

CITATION: Hoberman, A. M. (2009) Oral (diet) dosage-range finding developmental neurotoxicity and immunotoxicity study of MTI-446 (Dinotefuran) in Crl:CD(SD) rats. Charles River Laboratories Preclinical Services, Horsham, PA. Laboratory Project ID.: SRY00001, January 16, 2009. MRID 47677501. Unpublished.

SPONSOR: Mitsui Chemical, Inc., 1-5-2 Higashi-Shimbashi, Minato-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In a developmental immunotoxicity study and a dose-range finding for a developmental neurotoxicity study (DNT) (MRID 47677501), MTI-446 (dinotefuran; 94.7%; Lot # 2200210) was administered in the diet to presumed pregnant Sprague Dawley (Crl:CD[SD]) rats (10/dose) at dietary levels of 0, 1000, 3000, or 10,000 ppm (equivalent to 0, 105.4, 317.8, or 1035.4 mg/kg/day, respectively) beginning on gestation day (GD) 6, and continuing through lactation day (LD) 21. The F1 generation were potentially exposed through the maternal milk and the dam's dose formulation through weaning. On PND 21, the F1 generation were selected (up to three pups/sex/litter) randomly for immunotoxicity assays. F1 pups (20/sex/dose) were fed the same dietary concentrations as their dams beginning on PND 21 until termination (PND 42). Systemic toxicity parameters were evaluated in the P and F1 generations, and immunotoxicity was evaluated in the F1 generation. Ten F1 pups/sex/dose group were examined for humoral immune response by measuring IgM antibody forming cell responses following immunization with sheep red blood cell (SRBC); the remaining ten F1 pups/sex/dose group were examined for innate immune response by performing a natural killer (NK) cell assay. The splenocytes of the pups used for the NK cell assay were also phenotyped by flow cytometry.

For maternal toxicity, no treatment-related adverse effects were observed on mortality, clinical signs, body weights, body weight gains, food consumption, reproductive performance, or gross pathology.

The maternal toxicity LOAEL was not observed. The NOAEL for maternal toxicity is

10,000 ppm (equivalent to 1035.4 mg/kg bw/day).

For offspring toxicity, no treatment-related effects were observed on the birth, live birth, viability, or lactation indices or on sex ratio on PND 21. There were no clinical signs of toxicity, and all pups sacrificed on PND 4 and PND 21 appeared normal at necropsy. At 10,000 ppm, pup body weights were decreased ($p \leq 0.01$) by 13-18% during PND 13-21. After weaning, no treatment-related adverse effects were observed on mortality, clinical signs, food consumption, or gross pathology in the post-weaning F1 generation. At 10,000 ppm, post-weaning body weights were decreased ($p \leq 0.05$) by 7-22% during PND 22-57 in the males, and by 7-11% during PND 22-36 and PND 57-64 in the females.

The offspring toxicity LOAEL was 10,000 ppm (equivalent to 1035.4 mg/kg bw/day), based on decreased body weights in both sexes. The NOAEL for offspring toxicity is 3000 ppm (equivalent to 317.8 mg/kg bw/day).

For dose-range finding of a developmental neurotoxicity study, this diet concentration (10000 ppm) is considered suitable as a high dose level for the DNT study.

For developmental immunotoxicity, there were no treatment-related effects on antibody forming cell response (humoral immunity) and Natural Killer Cell activity (innate immunity). No differences that attributable to treatment were noted in the distribution of splenocyte subpopulations. Increased spleen weights were observed in the 1000 and 3000 ppm males, but this finding was not dose-dependent and was considered incidental.

Under conditions of this study, there were no immunologically adverse effects on antibody forming cell response or Natural Killer cell activity in male and female rats that exposed to dinotefuran during the prenatal, postnatal and post-weaning period. The LOAEL for developmental immunotoxicity was not observed. The NOAEL is 10,000 ppm (equivalent to 1035.4 mg/kg bw/day).

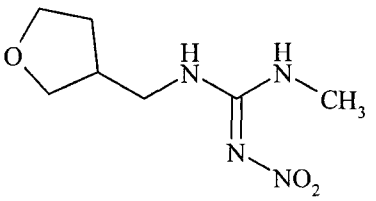
This study is classified **acceptable/non-guideline** and provides information regarding the potential immunotoxic effect in offspring rats after exposure to dinotefuran during the prenatal, postnatal, and post-weaning periods. Additionally, it is a range-finding study for a definitive developmental neurotoxicity study.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: MTI-446 (Dinotefuran)

Description:	White powder
Lot #:	2200210
Purity:	94.7% a.i.
Compound Stability:	Stable in the diet for up to 23 days when stored at 22°C or -70°C
CAS # of TGAI:	165252-70-0
Structure:	

2. Vehicle: Diet

3. Test animals:

Species:	Rats (females only)
Strain:	Sprague Dawley (Crl:CD[SD])
Age/weight at study initiation:	Approximately 10 weeks; 219-242 g
Source:	Charles River Laboratories (Raleigh, NC)
Housing:	Individually in stainless steel, wire-bottomed cages during gestation (mating pairs were housed in the male's cage during cohabitation); beginning no later than GD 20, dams were housed in nesting boxes until delivery or termination on GD 25.
Diet:	Certified Rodent LabDiet® 5002 (PMI® Nutrition International, LLC, St. Louis, MO), *ad libitum*
Water:	Reverse osmosis-filtered tap water, *ad libitum*
Environmental conditions:	**Temperature:** 18-26 °C **Humidity:** 30-70% **Air changes:** ≥10/h **Photoperiod:** 12 h light/12 h dark
Acclimation period:	Five days

B. STUDY DESIGN

1. In life dates: Start: 01/27/08 End: Approximately 04/23/08

2. Purpose: The purpose of this study was to: (1) determine suitable dose levels of the test substance for use in a developmental neurotoxicity study; (2) evaluate specific aspects of the functional immunological status of F1 progeny exposed *in utero*, during lactation via maternal milk, and for five weeks following weaning; and (3) determine if there were sufficient evidence of an effect on functional immunological status of F1 progeny to warrant inclusion of immunological end-points in a subsequent formal developmental neurotoxicity/immunotoxicity study.

3. Mating procedure: Non-pregnant, nulliparous females were cohabited with males of the same strain on a one-to-one basis for a maximum of five days. Vaginal smears were

collected daily, and mating was confirmed by the presence of a copulatory plug or sperm in the vaginal smear. The day mating was confirmed was designated as gestation day (GD) 0.

4. **Animal assignment:** Healthy, mated (presumed pregnant) female rats were randomly assigned, stratified by GD 0 body weight, to the test groups noted in Table 1. Male rats were only used for the purpose of breeding. On PND 21, 20 F1 pups/sex/dose level (up to three pups/sex/litter) were randomly selected for immunotoxicity assays; one or two pups/sex/litter were selected for each of the two assays, when possible.

TABLE 1: Study design[a]

Test group	Dietary concentration (ppm)	Intake (mg/kg/day)[b]	# P Females	# F1 Rats/sex
Control	0	0	10	20
Low	1000	105.4	10	20
Mid	3000	317.8	10	20
High	10,000	1035.4	10	20

a Data were obtained from pages 28 and 37 of the study report. Dietary formulations were adjusted for test compound purity (94.7% a.i.).

b Calculated by the reviewers as the average of the achieved intake for the overall gestation and lactation periods.

5. **Dose selection:** It was stated that the doses were selected based on the results of a previously performed 2-generation reproduction toxicity study in rats (RCC Study Report #775192; 45639913). In this study, pups exposed to 10,000 ppm exhibited reduced body weights (decreased approximately 10%) during lactation. The high dose of 10,000 ppm was selected for the present study as a comparable dose level.

6. **Test diet preparation and analysis:** Dietary formulations were prepared by mixing the appropriate amount of the test substance (adjusted for purity) for each concentration with basal diet to form a premix. The premix was then diluted with additional basal diet to yield the desired dietary concentration. Diets were prepared at least biweekly and were stored at room temperature. Homogeneity (top, middle, bottom) was determined in all dose levels of the initial dietary preparation; stability analyses were performed on the 1000 and 10,000 ppm formulations of the initial preparation. Concentration analyses were performed on all dose levels of the first, second, fifth, and ninth preparations. Stability was determined following storage at 22°C or -70°C for up to 23 days.

Results

Homogeneity analysis (%RSD): 0.9-1.5%

Stability analysis (% of Day 0): 86.8-99.8% after storage at 22°C for 23 days
98.2-102.1% after storage at -70°C for 23 days

Concentration analysis (% of nominal): 91.8-99.4%

The analytical data indicate that the mixing procedure was adequate, and that the variation

between the target and actual dosage to the study animals was acceptable. It was noted that the stability of the 1000 ppm preparation stored at 22°C was slightly below the acceptable limits (±10%).

7. **Dosage administration:** Maternal rats were given continuous access to the dietary formulations from GD 6 through lactation day (LD) 21. F1 pups selected for immunotoxicity assays were provided continuous access to the same dietary concentration as their dam from post-natal day (PND) 21 until termination (PND 36-42 for males; PND 43-49 for females).

C. OBSERVATIONS

1. **Parental females:** All dams were examined at least twice each day for mortality, and clinical observations and general appearance on GD 0. The dams were also examined for clinical observations, abortions, premature deliveries, and deaths on GD 6, 9, 12, 15, 18, 20, and 25 (rats that did not deliver a litter), and on LD 0, 4, 7, 13, and 21. Body weights were recorded prior to initiation of dosing, and on GD 0, 6, 9, 12, 15, 18, 20, and 25 (if necessary), and LD 0, 4, 7, 13, and 21; body weight gains were reported for each interval, for GD 6-20 and GD 0-20, and for LD 0-21. Food consumption (g/animal/day and g/kg/day) was reported on the same intervals as body weight gains up to LD 13, as pups begin to consume maternal food on or about LD 13. Dams were also evaluated for adverse clinical signs during parturition.

2. **Litter observations:** Litters were examined at least twice daily for mortality, and were counted daily. Litter sizes (all pups delivered), live litter size (live born pups only), and pup viability at birth were recorded. Clinical observations were recorded daily during the pre-weaning period. Pup body weights were recorded on PND 0, 4, 7, 11, 13, 17, and 21. On PND 4, litters were randomly culled to twelve pups each (six pups/sex when possible).

3. **Post-weaning observations:** The selected F1 pups were observed at least twice daily for mortality and weekly for clinical signs of toxicity and general appearance during the post-weaning period. Body weights were recorded weekly beginning on PND 22 and prior to termination; body weight gains were reported for each interval and for the post-weaning period. Food consumption (g/animal/day and g/kg/day) was reported weekly and for the post-weaning period.

D. POSTMORTEM OBSERVATIONS

1. **Parental animals:** After weaning (PND 21), dams were euthanized by carbon dioxide asphyxiation and subjected to a gross necropsy. The number and distribution of implantation sites were recorded. Rats that did not deliver a litter were killed on GD 25 and examined for gross lesions. The uteri of these animals were examined while being pressed between glass plates to confirm the absence of implantation sites. Gross lesions were retained in neutral buffered 10% formalin for possible future examination.

2. **Offspring:** Pups that died before initial examination of the litter for viability were evaluated for vital status at birth. The lungs were removed and immersed in water. Pups

with lungs that sank were considered stillborn; pups with lungs that floated were considered live born and to have died shortly after birth. Pups found dead were necropsied for the cause of death; pups found dead on PND 0-4 were preserved in Bouin's solution for possible future examination. Pups culled on PND 4 were euthanized by an intraperitoneal injection of sodium pentobarbital and necropsied. On PND 21, all pups not selected for immunotoxicity assays were killed by carbon dioxide asphyxiation and necropsied.

c. **Post-weaning offspring:** The F1 pups selected for immunotoxicological evaluations were killed between eight and ten weeks of age. These animals were subjected to a gross necropsy, and the spleens were excised and retained for immunological evaluations.

E. IMMUNOTOXICITY

1. **Antibody-forming cell (AFC) assay:** Ten F1 rats/sex/dose group were immunized by an intravenous injection of 2×10^8 sheep red blood cells (SRBC) in a volume of 0.5 mL. The F1 rats were killed four days following sensitization and completion of the exposure period (PND 36-40 for males; PND 43-47 for females). The rats were weighed, euthanized by carbon dioxide asphyxiation, bled via the inferior vena cava, and examined grossly. The spleens were removed, weighed, and shipped on ice to the testing laboratory (ImmunoTox®, Inc, VA). Single cell suspensions were prepared from each spleen by mashing the spleens using a Stomacher® blender. Cell suspensions were then centrifuged and resuspended in 6 mL of Earle's Balanced Salt Solution with HEPES. Cell viability was determined using propidium iodide. Dilutions of 1:50 and 1:150 were prepared from each suspension, and a 0.1 mL aliquot of each dilution was combined with 25 µL of guinea pig complement, 25 µL of SRBC, and 0.5 mL of warm agar (0.5%) supplemented with DEAE-dextran. Each mixture was then plated on a separate petri dish, covered with a microscope cover slip, and incubated at 36-38°C for 3 h. Spleen cell number was determined on the 6 mL suspensions using a Coulter counter, and the plaques were counted using a Bellco plaque viewer. Each plaque is generated from a single IgM-producing B cell, permitting the number of AFC present in the whole spleen to be calculated. The cells/spleen, specific activity (AFC/10^6 spleen cells), and total spleen activity (AFC/spleen) were reported.

2. **Natural killer (NK) cell assay:** NK cells are large granular lymphocytes that possess anti-tumor activity without prior acquisition of information; therefore, NK cell activity is a measure of the functioning of the innate immune system. YAC-1 cells (ATCC TIB 160), a mouse lymphoma, were used as the target cells. The YAC-1 cells were adjusted to a concentration of 1×10^7 cells/mL and radiolabeled by incubation with 500 µCi of ^{51}Cr for approximately 90 minutes in a 36-38°C incubator with frequent agitation. The cells were then washed four times with RPMI 1640 medium, and resuspended at concentration of 5×10^4 nucleated cells/mL. Single cell suspensions of spleens were obtained as above, with the cells resuspended in RPMI 1640 supplemented with 10% fetal bovine serum. The spleen cell suspensions were adjusted to six concentrations: 2×10^7, 1×10^7, 5×10^6, 2.5×10^6, 1.25×10^6, and 0.625×10^6, yielding effector-to-target ratios of 200:1, 100:1, 50:1, 25:1, 12.5:1, and 6.25:1, respectively. The maximum ^{51}Cr release was determined by adding 0.1 mL of YAC-1 cells and 0.1 mL of 0.1% Triton X-100 to each of 12 replicate wells in a 96-well plate. The spontaneous ^{51}Cr release was determined by adding 0.1 mL of YAC-1 cells and 0.1 mL of medium to 12 replicate wells. Each effector concentration was

tested by adding 0.1 mL of YAC-1 cells and 0.1 mL of diluted spleen cells to four replicate wells. The plates were incubated for approximately 4 h at 36-38°C in a 5-7% CO_2 atmosphere. The plates were then centrifuged at 240-260 x g for approximately 10 minutes, and 0.1 mL of the supernatant was removed from each well and counted for radioactivity. The mean (±SE) percent cytotoxicity at each effector concentration was determined for each treatment group and compared to the respective values for the controls.

3. **Splenocyte phenotyping:** Single cell suspensions of spleens were obtained as above, with the cells resuspended in RPMI 1640 supplemented with 10% fetal bovine serum. Cells were adjusted to 1 x 10^7 cells/mL, and 100 µL of appropriately diluted antibodies against CD45RA (total B cells), CD 5 (total T cells), CD 4 (T helper cells), CD 8 (cytotoxic T cells), and NKR-P1A (NK cells) were combined with 100 µL of cells and incubated for 20 minutes at 4°C in the dark. The cells were processed with an automated system (Coulter® TQ-Prep unit) and analyzed by flow cytometry.

F. DATA ANALYSIS

1. **Statistics:** The following statistical procedures were used:

Parameter	Statistical procedure
Clinical observations and other proportion data	Variance Test for Homogeneity of the Binomial Distribution
Continuous data (body weights, body weight gains, food consumption, and organ weights)	Bartlett's Test of Homogeneity of Variances followed by Analysis of Variance (ANOVA) when appropriate; if the ANOVA was significant ($p \leq 0.05$), Dunnett's Test was used. If Bartlett's Test was significant ($p \leq 0.001$), Kruskal-Wallis Test was used. If the Kruskal-Wallis Test was significant, Dunn's Method of Multiple Comparisons was used. If there were greater than 75% ties, Fisher's Exact Test was used.
Count data	Kruskal-Wallis Test as above.
Immunotoxicity data (splenocyte phenotyping, modified hemolytic plaque assay, natural killer cell assay)	Bartlett's Chi Square Test for homogeneity of variances, followed by parametric one-way ANOVA when appropriate; if the ANOVA was significant, Dunnett's Test was used. Non-homogeneous data were evaluated using a non-parametric ANOVA; if the ANOVA was significant, the Gehan-Wilcoxon Test was used. Additionally, Jonckheere's Test was used to evaluate trends across control and treatment groups.

Statistical significance was denoted at $p \leq 0.05$ and $p \leq 0.01$. The statistical methods were considered appropriate.

2. **Indices**

Reproductive indices: The following reproductive indices were calculated by the performing laboratory from breeding and parturition records of animals in the study:

Fertility index (%) = # of pregnant females/# of females mated x 100

Gestation index (%) = # of females that delivered live pups/# of pregnant females x 100

Offspring viability indices: The following offspring indices were calculated by the performing laboratory from lactation records of litters in the study:

Live birth index (%) = # of pups born alive/# pups born x 100

Viability index (%) = # of live pups on PND 4 (pre-cull)/# of pups born alive x 100

Lactation index (%) = # of live pups at weaning/# of live pups on PND 4 (post-cull) x 100

3. **Historical control data:** Historical control reproductive data were not provided. Historical control ranges for selected immunological (anti-SRBC antibody response, NK cell activity, and splenocyte phenotyping surface marker data) and spleen weight parameters were provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

a. **Mortality:** There were no deaths in the P generation females. All dams survived until scheduled termination.

b. **Clinical signs of toxicity:** Clinical signs observed during gestation and lactation consisted of localized alopecia, sparse hair coat, urine-stained abdominal fur, and chromorhinorrhea. These findings were considered unrelated to treatment because: (i) the incidences were not dose-dependent; (ii) the finding occurred in only one or two rats; and/or (iii) the finding was only observed in the controls.

2. Body weights, body weight gains, and food consumption

a. **Gestation:** There were no adverse, treatment-related effects on body weights, body weight gains or food consumption during gestation (Table 2a). Body weights and overall (GD 0-20) body weight gains were similar to controls in all treatment groups. Absolute and relative food consumption were decreased ($p \leq 0.01$) by 9-10% during GD 6-9 in the 10,000 ppm dams, resulting in a 23% decrease (not significant [NS]) in body weight gains for this period. However, these transient findings were not considered adverse.

TABLE 2a. Mean (±SD) body weights (g), body weight gains (g), and absolute (g/rat/day) and relative (g/kg/day) food consumption during gestation[a]

Observation/gestation day		Dose Group (ppm)			
		0	1000	3000	10,000
Body weight	GD 0	228.9±7.0	229.1±7.3	229.7±7.4	229.9±7.0
	GD 6	256.3±10.6	259.4±17.3	263.1±12.2	259.2±9.6
	GD 20	361.9±10.9	366.3±24.0	378.2±21.3	362.7±17.6
Body weight gain	GD 6-9	13.6±5.6	11.3±6.5	13.0±5.6	10.5±3.2 (↓23)
	GD 0-20	133.0±6.1	137.2±19.3	148.5±16.1	132.8±14.2
Food consumption (absolute)	GD 6-9	21.2±1.6	21.0±3.0	21.2±2.0	19.2±0.5** (↓9)
	GD 0-20	20.5±0.7	20.6±2.3	21.4±1.7	20.0±0.8
Food consumption (relative)	GD 6-9	80.6±7.5	79.0±7.1	78.7±5.4	72.5±2.0** (↓10)
	GD 0-20	70.3±2.5	69.8±4.3	71.0±3.3	68.2±1.4

a Data were obtained from Tables A3, A4, A7, and A8 on pages 49-50 and 53-54 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
** Significantly different from controls; p≤0.01

b. Lactation: There were no effects of treatment on body weights, body weight gains, or food consumption during lactation (Table 2b).

TABLE 2b. Mean (±SD) body weights (g), body weight gains (g), and absolute (g/rat/day) and relative (g/kg/day) food consumption during lactation[a]

Observation/lactation day		Dose Group (ppm)			
		0	1000	3000	10,000
Body weight	LD 0	279.2±10.9	279.4±20.9	288.5±19.6	274.0±9.2
	LD 21	292.1±11.5	286.8±25.0	295.7±17.8	288.5±20.3
Body weight gain	LD 0-21	13.4±11.8	7.3±10.6	7.2±8.9	14.5±15.2
Food consumption (absolute)	LD 0-13	43.0±3.8	40.6±6.0	42.5±3.2	39.9±3.5
Food consumption (relative)	LD 0-13	146.7±12.0	141.2±17.7	141.3±9.7	140.1±9.5

a Data were obtained from Tables A5, A6, A9, and A10 on pages 51-52 and 55-56 of the study report.

3. Test substance intake: Test substance intakes (mg/kg/day) for gestation and lactation are presented in Table 3.

TABLE 3. Mean (±SD) test compound intake (mg/kg/day) during gestation and lactation[a]

Time period	Dose Group (ppm)			
	0	1000	3000	10,000
Gestation – GD 6-20	0.0±0.0	69.5±4.3	211.8±9.0	669.9±22.1
Lactation – LD 1-13	0.0±0.0	141.2±17.7	423.9±29.0	1400.8±94.6
Mean[b]	0.0	105.4	317.8	1035.4

a Data were obtained from Table A1 on pages 46-47 of the study report.
b Calculated by reviewers

4. Reproductive performance: There were no effects of treatment on fertility or gestation indices or on the duration of gestation (Table 4).

TABLE 4. Reproductive performance[a]				
Observation/study week	Dose Group (ppm)			
	0	1000	3000	10,000
Number mated	10	10	10	10
Fertility index (%)[b]	90.0	90.0	100	100
Gestation index (%)[c]	100	100	100	100
Gestation duration (days)	22.3±0.5	22.2±0.4	22.4±0.5	22.5±0.5

a Data were obtained from Table A11 on page 57 of the study report.
b Fertility index (%) = # of pregnant females/# of females mated x 100
c Gestation index (%) = # of females that delivered live pups/# of pregnant females x 100

5. **Parental postmortem results:** There were no test substance-related necropsy observations, as these were confined to persistent clinical observations that were not considered to be a result of treatment. No gross lesions were identified at necropsy.

B. OFFSPRING

1. **Viability and clinical signs:** Litter parameters are presented in Table 5. No effects of treatment were observed on the birth, live birth, viability, or lactation indices or on sex ratio on PND 21.

   Clinical signs observed in the F1 pups from birth to PND 21 consisted of scab on the forelimb, ungroomed coat, mild dehydration, pale body, and black tip of tail. These findings were considered unrelated to treatment because: (i) the incidences were not dose-dependent; (ii) the finding occurred in only one litter; and/or (iii) the finding was only observed in the controls.

TABLE 5. Litter parameters[a]				
Parameter	Dose Group (ppm)			
	0	1000	3000	10,000
Implantation sites	123	133	144	149
Mean (±SD) implantation sites	13.7±1.4	14.8±1.1	14.4±1.4	14.9±1.2
Birth index (%)[b, c]	100	100	100	100
Mean (±SD) offspring born	13.1±2.2	14.0±1.4	13.9±1.6	13.9±1.4
Mean (±SD) live pups PND 0	12.9±2.3	14.0±1.4	13.9±1.6	13.9±1.4
Mean (±SD) pups PND 4 pre-cull	12.9±2.3	14.0±1.4	13.9±1.6	13.9±1.4
Mean (±SD) pups PND 4 post-cull	11.4±1.3	12.0±0.0	11.9±0.3	12.0±0.0
Mean (±SD) pups PND 21	11.4±1.3	12.0±0.0	11.9±0.3	12.0±0.0
Live birth index (%)	98.3	100	100	100
Viability index (%)	100	100	100	100
Lactation index (%)	100	100	100	100
Sex ratio (% males on PND 21)	47.4±17.1	49.1±6.5	52.8±15.8	55.0±16.3

a Data were obtained from Tables A11 and A12 on pages 57-59 of the study report.
b Birth index (%) = # pregnant females that delivered live offspring/# pregnant females x 100
c Calculated by reviewers

2. **Body weight:** At 10,000 ppm, pup body weights were decreased ($p \leq 0.01$) by 13-18%

during PND 13-21 (Table 6). Body weights were also decreased ($p \leq 0.05$) by 9-11% during PND 17-21 in the 1000 ppm pups, but since dose-dependency was not observed, this finding was considered incidental. Pup body weights at 3000 ppm were similar to controls.

TABLE 6. Mean (±SD) pup weights (g)[a]

Post-natal day (PND)	Dose Group (ppm)			
	0	1000	3000	10,000
PND 0	6.7±0.8	6.2±0.5	6.5±0.2	6.6±0.3
PND 4[b]	10.6±1.3	9.9±1.0	10.5±0.9	10.3±0.7
PND 4[c]	10.7±1.2	10.0±1.0	10.6±0.9	10.4±0.8
PND 7	15.8±1.4	14.9±1.4	15.6±1.4	15.1±1.3
PND 11	22.6±1.6	21.1±2.5	21.3±2.6	20.7±2.0
PND 13	26.0±1.9	23.9±3.5	23.9±2.6	22.6±1.7** (↓13)
PND 17	33.0±3.1	30.0±4.2* (↓9)	30.6±2.7	28.1±1.7** (↓15)
PND 21	46.7±4.7	41.5±6.3* (↓11)	42.3±3.8	38.2±2.5** (↓18)

a Data were obtained from Table A12 on page 60 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
b Pre-standardization on PND 4
c Post-standardization on PND 4
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

3. **Offspring postmortem results:** All pups sacrificed on PND 4 and PND 21 appeared normal at necropsy.

C. POST-WEANING F1 PUPS

1. Mortality and clinical signs

a. **Mortality:** There were no treatment-related deaths in the post-weaning F1 generation. One 1000 ppm female (#14532) was found dead on PND 25. This rat had no adverse clinical observations prior to death, but its body weight at weaning was much lower than other rats of this dose group (29.0 g vs. group mean of 44.4 g), and all tissues appeared normal at necropsy. Therefore, this rat was considered to have failed to thrive. All other F1 post-weaning rats survived to scheduled termination.

b. **Clinical signs of toxicity:** Clinical signs observed following weaning consisted of sparse hair coat, chromorhinorrhea, chromodacryorrhea, misaligned incisor, and a swollen, red digit with a laceration. These findings were considered unrelated to treatment because: (i) the incidences were not dose-dependent; (ii) the finding occurred in only one rat; (iii) the observation is common in this species and strain; and/or (iv) the finding was only observed in the controls.

2. **Body weights, body weight gains, and food consumption:** At 10,000 ppm, post-weaning body weights were decreased ($p \leq 0.05$) by 7-22% during PND 22-57 in the males, and by 7-11% during PND 22-36 and PND 57-64 in the females (Table 7). However, body weight gains in these animals were generally unaffected during this period, with the exception of

PND 57-64 in the females (↓27%; p≤0.01). This was considered to reflect the higher exposure of the pups to the test compound at or near weaning.

Body weights were also decreased (p≤0.05) in the 1000 and 3000 ppm males and females on PND 22 (↓9-14%) and in the 1000 ppm males on PND 29 (↓7%), and body weight gains were decreased (p≤0.01) in the 3000 ppm females by 32% during PND 57-64. These transient, sporadic decreases were considered incidental.

Food consumption was unaffected by treatment at all dose levels.

TABLE 7. Mean (±SD) body weights (g), body weight gains (g), and absolute (g/rat/day) and relative (g/kg/day) food consumption in F1 pups post-weaning[a]

Observation/gestation day		Dose Group (ppm) 0	1000	3000	10,000
Males					
Body weight	PND 22	53.4±5.6	47.7±7.1** (↓11)	48.8±4.4* (↓9)	41.8±4.2** (↓22)
	PND 29	96.3±9.2	90.0±11.1* (↓7)	93.6±8.7	83.3±7.6** (↓13)
	PND 57	332.0±28.6	327.1±28.5	330.0±27.0	308.1±19.3** (↓7)
Body weight gain	PND 22-57	278.6±26.8	279.4±24.6	281.2±23.9	266.3±15.9
Food consumption (absolute)	PND 23-58	39.3±3.3	37.5±4.7	39.2±2.8	36.5±3.1
Food consumption (relative)	PND 23-58	102.2±4.4	100.3±11.3	103.5±3.9	104.4±5.6
Females					
Body weight	PND 22	51.8±5.0	44.4±9.0** (↓14)	46.8±4.3* (↓10)	42.1±4.8** (↓19)
	PND 29	88.9±8.0	82.8±15.1	86.2±8.1	79.2±7.5** (↓11)
	PND 57	214.2±17.5	206.5±30.1	208.8±9.7	198.6±16.1* (↓7)
	PND 64	235.8±20.2	225.4±34.7	223.4±10.5	214.3±17.7** (↓9)
Body weight gain	PND 57-64	21.6±5.8	18.9±8.2	14.6±3.5** (↓32)	15.8±5.4** (↓27)
Body weight gain	PND 22-64	184.0±19.0	180.3±29.8	176.6±11.1	172.2±16.4
Food consumption (absolute)	PND 23-64	30.0±2.4	30.1±4.6	28.5±2.7	28.6±2.1
Food consumption (relative)	PND 23-64	104.7±5.1	112.3±5.7	105.5±11.3	112.0±10.0

a Data were obtained from Tables B5 through B12 on pages 110-117 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
* Significantly different from controls; p≤0.05
** Significantly different from controls; p≤0.01

3. **Post-weaning offspring postmortem results:** No gross lesions were identified at necropsy.

D. IMMUNOTOXICITY

1. **AFC assay:** The results of the AFC assay are presented in Table 8. There were no statistically significant effects on the antibody-forming cell (humoral immunity) response when the data were evaluated as either specific activity or total spleen activity.

Spleen weights were increased (p≤0.05) by 30-37% in the 1000 and 3000 ppm males, but this finding was not dose-dependent and was considered incidental.

TABLE 8. Mean (±SE) AFC assay parameters in F1 pups post-weaning[a]

Parameter	Dose Group (ppm)			
	0	1000	3000	10,000
Males				
Terminal body weight (g)	343.8±15.3	354.4±8.6	355.7±9.9	340.0±6.7
Spleen weight (mg)	619±81	846±40** (↑37)	803±36* (↑30)	776±44
Spleen cells (x 10^7)	78.50±9.85	82.83±9.32	102.13±8.64	85.39±4.85
IgM AFC/10^7 spleen cells	1172±172	670±152	1027±140	909±143
IgM AFC/spleen (x 10^3)	912±171	611±180	1022±132	788±140
Females				
Terminal body weight (g)	239.3±7.3	238.0±8.0	233.5±3.7	230.4±6.2
Spleen weight (mg)	521±26	546±27	549±29	557±32
Spleen cells (x 10^7)	61.84±3.85	60.14±4.80	61.91±3.99	59.00±6.36
IgM AFC/10^7 spleen cells	2198±601	1673±345	1496±405	1668±260
IgM AFC/spleen (x 10^3)	1400±415	960±211	918±240	929±134

a Data were obtained from Tables 7 and 8 of Appendix 6 on pages 360-361 of the study report. n=10
* Statistically different from controls; p≤0.05
** Statistically different from controls; p≤0.01

2. **NK cell assay:** The results of the NK cell assay are presented in Table 9. Terminal body weights were decreased (p≤0.05) by 8-14% in the 3000 ppm and above females. While there was an increasing trend in NK cell activity in the males for effector:target ratios of 12.5:1 and above, none of the treatment groups attained statistical significance in pair-wise comparisons with controls. There was no effect of treatment on NK cell activity in the females.

TABLE 9. Mean (±SE) NK cell assay parameters in F1 pups post-weaning[a]

Parameter		Dose Group (ppm)			
		0	1000	3000	10,000
Males					
Terminal body weight (g)		369.2±9.5	369.7±9.8	373.7±9.6	341.1±6.6
Absolute spleen weight (mg)		749±39	774±47	733±35	671±27
Relative to body spleen weight (%)		0.202±0.008	0.210±0.012	0.198±0.011	0.197±0.007
Effector:target ratio	200:1	14.3±2.8	16.2±2.1	19.9±1.4	21.6±3.5
	100:1	6.0±1.4	6.8±1.3	8.9±0.7	10.6±2.2
	50:1	1.9±0.7	2.4±0.5	3.2±0.3	4.2±1.1
	25:1	<1	<1	1.2±0.3	1.6±0.6
	12.5:1	<1	<1	<1	<1
	6.25:1	<1	<1	<1	<1
Females					
Terminal body weight (g)		254.2±5.7	246.1±17.0	234.7±4.2* (↓8)	218.0±4.7** (↓14)
Spleen weight (mg)		498±18	512±41	476±21	482±26
Relative to body spleen weight (%)		0.196±0.004	0.209±0.010	0.202±0.010	0.222±0.012
Effector:target ratio	200:1	26.6±3.7	26.0±3.3	26.0±2.8	28.7±2.7
	100:1	14.1±2.5	14.4±2.3	13.5±1.8	15.1±1.8
	50:1	6.9±1.2	7.2±1.3	6.7±0.9	7.5±0.8
	25:1	4.3±0.7	4.0±0.7	3.9±0.5	4.0±0.5
	12.5:1	2.9±0.5	3.0±0.5	2.9±0.5	2.8±0.3
	6.25:1	2.1±0.3	2.0±0.3	1.8±0.2	1.9±0.2

a Data were obtained from Tables 1 and 2 of Appendix 6 on pages 354-355 and Tables 9 and 10 on pages 362-363 of the study report. n=9-10
* Statistically different from controls; p≤0.05
** Statistically different from controls; p≤0.01

3. **Splenocyte phenotyping:** The results of the splenocyte phenotyping are presented in Table 10. There were no effects of treatment on the distribution of phenotypes examined in the splenocytes. Absolute spleen cell number was decreased ($p \leq 0.05$) by 21% in the 10,000 ppm males. The absolute numbers of all of the cell types examined were also decreased in the 10,000 ppm males, achieving statistical significance for the natural killer cells (↓22%; $p \leq 0.05$). These differences were not attributed to treatment with the test compound, but were a reflection of the decreased number of spleen cells, because the percentages of each phenotype in the treated groups were comparable to controls.

TABLE 10. Mean (±SE) splenocyte surface marker differential in F1 pups post-weaning[a]

Parameter	Dose Group (ppm)			
	0	1000	3000	10,000
Males				
Spleen cells (x 10^7)	88.04±5.19	88.58±6.19	74.38±3.76	69.66±5.42* (↓21)
Total B cells ($CD45^+$)	485.2±43.0	503.2±46.5	433.6±30.7	386.3±35.3
Total T cells ($CD5^+$)	251.4±17.2	246.0±14.3	195.9±9.9	209.5±20.4
Helper/DTH T cells ($CD4^+CD5^+$)	139.9±12.5	131.0±8.5	112.2±6.1	124.5±13.1
Cytotoxic T cells ($CD8^+CD5^+$)	109.8±6.8	111.2±7.3	83.3±7.0	83.8±9.2
Natural killer cells ($NKR\text{-}P1A^+CD8^-$)	140.3±8.5	142.4±7.2	110.9±6.7	109.8±11.0* (↓22)
Females				
Spleen cells (x 10^7)	57.27±2.55	62.80±5.49	59.57±5.90	55.67±4.75
Total B cells ($CD45^+$)	310.6±25.3	323.8±36.4	348.5±39.8	316.0±32.7
Total T cells ($CD5^+$)	167.6±13.5	203.7±18.3	162.9±14.2	163.1±12.2
Helper/DTH T cells ($CD4^+CD5^+$)	99.4±7.2	117.9±11.1	97.0±7.2	92.3±5.5
Cytotoxic T cells ($CD8^+CD5^+$)	74.8±7.1	90.8±9.1	68.8±7.4	72.8±8.8
Natural killer cells ($NKR\text{-}P1A^+CD8^-$)	97.8±7.9	114.6±9.2	90.7±9.0	95.8±9.7

a Data were obtained from Tables 3 and 5 of Appendix 6 on pages 356 and 358 of the study report. n=10
* Statistically different from controls; $p \leq 0.05$

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATOR'S CONCLUSIONS:** On the basis of these data, the P generation maternal NOAEL was 10,000 ppm. A transient reduction in food consumption at 10,000 ppm on GD 6-9 was not considered toxicologically adverse. Since there were no adverse effects on litter number and pup viability at this dose, this dietary concentration was considered suitable as a high dose level for a guideline developmental neurotoxicity study. The NOAEL for general toxicity for the F1 generation was 3000 ppm. Direct exposure through the diet and milk resulted in a reduction in body weight in the 10,000 ppm males and females. The NOAEL for the functional assessment of the immune system was 10,000 ppm, the highest dose tested. There were no effects of MTI-446 on the innate and humoral components of the immune system for F1 generation male and female rats exposed in utero and through milk and diet.

As for dose range finding for a developmental neurotoxicity study, since there were no adverse effects on litter number and pup viability at 10000 ppm, this diet concentration is considered suitable as a high dose level for the guideline DNT study.

B. REVIEWER COMMENTS

Maternal animals: Systemic toxicity was not observed. There were no adverse effects of treatment on mortality, clinical signs, body weights, body weight gains, reproductive performance, or gross pathology.

Absolute and relative food consumption were decreased ($p \leq 0.01$) by 9-10% during GD 6-9 in the 10,000 ppm dams, resulting in a 23% decrease (not significant [NS]) in body weight gains for this period. However, these transient findings were not considered adverse.

The LOAEL for maternal toxicity was not observed. The NOAEL for maternal toxicity is 10,000 ppm (equivalent to 1035.4 mg/kg bw/day).

Offspring: No effects of treatment were observed on the birth, live birth, viability, or lactation indices or on sex ratio on PND 21. There were no clinical signs of toxicity, and all pups sacrificed on PND 4 and PND 21 appeared normal at necropsy.

At 10,000 ppm, pup body weights were decreased ($p \leq 0.01$) by 13-18% during PND 13-21. Body weights were also decreased ($p \leq 0.05$) by 9-11% during PND 17-21 in the 1000 ppm pups, but since dose-dependency was not observed, this finding was considered incidental. Pup body weights at 3000 ppm were similar to controls.

The LOAEL for offspring toxicity is 10,000 ppm (equivalent to 1035.4 mg/kg bw/day), based on decreased body weights in both sexes. The NOAEL for offspring toxicity is 3000 ppm (equivalent to 317.8 mg/kg bw/day).

Post-weaning offspring: There were no treatment-related deaths in the post-weaning F1 generation. One 1000 ppm female (#14532) was found dead on PND 25. This rat had no adverse clinical observations prior to death, but its body weight at weaning was much lower than other rats of this dose group (29.0 g vs. group mean of 44.4 g), and all tissues appeared normal at necropsy. Therefore, this rat was considered to have failed to thrive. There were no adverse, treatment related effects on clinical signs, food consumption, or gross pathology.

At 10,000 ppm, post-weaning body weights were decreased ($p \leq 0.05$) by 7-22% during PND 22-57 in the males, and by 7-11% during PND 22-36 and PND 57-64 in the females.

The LOAEL for post-weaning offspring toxicity is 10,000 ppm (equivalent to 1035.4 mg/kg bw/day), based on decreased body weights in both sexes. The NOAEL for offspring toxicity is 3000 ppm (equivalent to 317.8 mg/kg bw/day).

Under the condition of this study, for dose range finding of a developmental neurotoxicity study, this diet concentration (10000 ppm) is considered suitable as a high dose level for the guideline DNT study.

Immunotoxicity: There was no evidence of immunotoxicity. There were no treatment-related effects on the antibody forming cell response (humoral immunity) or Natural Killer

cell activity (innate immunity). No differences that attributable to treatment were noted in the distribution of splenocyte subpopulations. Increased spleen weights were observed in the 1000 and 3000 ppm males, but this finding was not dose-dependent and was considered incidental.

The LOAEL for immunotoxicity was not observed. The NOAEL for immunotoxicity is 10,000 ppm (equivalent to 1035.4 mg/kg bw/day).

C. **STUDY DEFICIENCIES:** No significant deficiencies were noted.

13544

R174638

Chemical Name: Dinotefuran

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