Section A6.3.1-2 **Repeated dose toxicity**

Annex Point IIA6.3 **Mouse**

Oral, 28-day

		unaffected by treatment at all dose levels. See Table A6.3.1.2-2
4.5.3	Urinalysis	Not applicable
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	There were no treatment-related effects on absolute and relative organ weights at any of the dose levels employed.
4.6.2	Gross and histopathology	There were no treatment-related macroscopic or microscopic histopathological alterations at any of the dose levels employed.
4.7	**Other**	One male treated at 50000ppm was found dead on day 3. Macroscopic pathology revealed multiple areas of discoloration in the renal cortex and medulla of both kidneys, mucoid material in the renal pelves and bilateral enlargement of the lumbar and renal lymph nodes. These findings indicated the probable cause of death to be pre-existing renal disease and unrelated to the administration of dinotefuran. The animal was replaced.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Guidelines: OECD guideline no. 407 (1995), which is equivalent to 92/69/EEC (method B.7). Deviations from 92/69/EEC - none; special functional observations and motor activity assessment required by OECD 407 not performed Methods: Groups of 10 male and 10 female mice were treated orally for at least 4 weeks with dinotefuran, by admixture in the diet at constant nominal concentrations of 0, 5000, 25000 and 50000ppm. Mean achieved dose levels were 0, 901, 4612 and 10303mg/kg bw/day (males) and 0, 1043, 5359 and 12289mg/kg bw/day (females). Morbidity/mortality checks were performed twice daily and a detailed clinical examination was performed weekly. Body weights and food consumption were recorded weekly throughout the study. Hematology and serum clinical chemistry analyses were performed in week 5, each on 5 mice/sex/group which had food withdrawn for at least 4 hours prior to blood sampling. Decedents and all surviving animals killed at the end of the study were subjected to necropsy and *post mortem* examination of major organs and tissues. Selected organs were weighed and all preserved tissues from animals treated at 0 or 50000ppm were examined by light microscopy. Gross lesions were also examined from all animals treated at 5000 and 25000ppm.
5.2	**Results and discussion**	No target organs were identified in either sex at the highest dose level employed. A no-observed-effect-level (NOEL) was established as 5000ppm diet in both sexes, equivalent to dose levels of 901mg/kg bw/day (males) and 1043mg/kg bw/day (females), based on the occurrence of reduced weight gain in both sexes at dose levels ≥25000ppm and slightly elevated total serum protein and albumin concentrations in males at 50000ppm.

Section A6.3.1-2 **Repeated dose toxicity**

Annex Point IIA6.3 **Mouse**

Oral, 28-day

5.3	**Conclusion**		
5.3.1	LO(A)EL	Not determined	
5.3.2	NO(A)EL	Since reduced weight gain was a consequence of reduced diet palatability and the minor changes in serum chemistry are considered not to be adverse, a no-observed-adverse-effect-level (NOAEL) was established in both sexes as 50000ppm, equivalent to dose levels of 10303mg/kg bw/day (males) and 12289mg/kg bw/day (females).	X2
5.3.3	Reliability	1	
5.3.4	Deficiencies	Yes, special functional observations and motor activity assessment required by OECD 407 not performed.	

Table A6.3.1.2-1 Treatment related effects on group mean body weight and overall weight gain

Week of study	Males treated at (ppm):				Females treated at (ppm):			
	0	5000	25000	50000	0	5000	25000	50000
1	30.7	32.1	32.6	32.8	25.5	26.1	26.5	26.0
2	33.1	33.7	32.9	30.9	27.0	27.5	27.0	24.9*
3	34.9	35.3	34.0	32.0*	28.4	29.0	27.9	26.0*
4	35.4	35.7	34.1	33.3	28.9	29.0	28.3	27.0
5	36.2	37.0	35.4	33.6	29.4	30.4	28.6	27.8
Overall gain (g)	5.5	4.9	2.8	0.8	3.9	4.3	2.1	1.8

* $p < 0.05$

Table A6.3.1.2-2 Treatment related serum clinical chemistry findings – week 5

Sex	Dose level (ppm)	Mean serum total protein ± SD (g/dL)	Mean serum albumin ± SD (g/dL)
Male	0	4.7 ± 0.23	3.2 ± 0.13
	5000	4.8 ± 0.26	3.2 ± 0.30
	25000	5.0 ± 0.25	3.3 ± 0.18
	50000	5.1* ± 0.18	3.6* ± 0.23
Female	0	4.8 ± 0.15	3.5 ± 0.13
	5000	5.0 ± 0.22	3.4 ± 0.26
	25000	5.1 ± 0.14	3.7 ± 0.09
	50000	4.9 ± 0.20	3.5 ± 0.13

* $p < 0.05$

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*4 Jan 2013*
Materials and Methods	*As described by Applicant.*
Results and discussion	*X1 Section 4.3 Reduced palatability of the test diet may have contributed to the reduced bodyweights at 25000 and 50000 ppm, but in the opinion of the RMS this should conservatively be regarded as a treatment-related adverse effect..*
Conclusion	*X2 Section 5.3.2 As noted above the RMS considers the reduced bodyweight gain and food consumption at to be adverse, and therefore a LOAEL of 25000 ppm and a NOAEL of 5000 ppm are established in this study.*
Reliability	*As described by Applicant.*
Acceptability	*Acceptable*
Remarks	*None*
	COMMENTS FROM ... *(specify)*
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.3.2 **Repeated dose toxicity**

Annex Point IIA6.3 **Dermal**

Rat

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 2001b, 28-day dermal toxicity study with MTI-446 in rats, [redacted], unpublished report no. 6648-149, October 12, 2001.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD guideline no. 410 (1981), which is equivalent to 92/69/EEC (method B.9) US-EPA OPPTS 870.3200 (1998)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	2200210
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White powder
3.1.2.2	Purity	93.0% + 7.6% water, purity of dried material 98.9%
3.1.2.3	Stability	Expiration date: 01 May 2002
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Crl:CD®(SD)IGS BR
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	About 8 weeks old, weighing 247-326 g for males. 166-218 g for females
3.2.6	Number of animals per group	10 males and 10 females per group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of treatment	28 days

Section A6.3.2		Repeated dose toxicity
Annex Point IIA6.3		Dermal Rat
3.3.2	Frequency of exposure	7 days per week
3.3.3	Postexposure period	None
3.3.4	**Dermal**	
3.3.4.1	Area covered	10 % of body surface area
3.3.4.2	Occlusion	Semi occlusive
3.3.4.3	Vehicle	0.5% carboxymethyl cellulose
3.3.4.4	Dose applied	0 (vehicle only), 40, 200 and 1000mg/kg bw/day.
3.3.4.5	Total volume applied	2 mL/ kg dinotefuran
3.3.4.6	Duration of exposure	6 – 7 h per day
3.3.4.7	Removal of test substance	water
3.3.4.8	Controls	Vehicle only
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, on days 1, 8, 15, 22 and 29
3.4.1.2	Mortality	Yes, on days 1, 8, 15, 22 and 29
3.4.2	Body weight	Yes, pre-dose and weekly thereafter starting on the first day of treatment
3.4.3	Food consumption	Yes, weekly
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	Yes, pre-dose and on day 26
3.4.6	Haematology	Yes Number of animals: all animals Time points: end of study Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, clotting time, prothrombin time, thromboplastin time,
3.4.7	Clinical Chemistry	Yes Number of animals: all animals Time points: end of study Parameters: sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, sorbitol dehydrogenase, methaemoglobin, lipids, hormone (specify hormones), acid/base balance, cholinesterase inhibition.
3.4.8	Urinalysis	No
3.5	**Sacrifice and**	

Section A6.3.2 **Repeated dose toxicity**
Annex Point IIA6.3 **Dermal**
Rat

	pathology	
3.5.1	Organ Weights	Yes Organs: liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart;
3.5.2	Gross and histopathology	Yes High dose group and controls Organs: brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, gall bladder (mouse), lymph nodes peripheral nerve, bone marrow, skin, eyes The decedent was also subjected to necropsy, but organ weights were not recorded.
3.5.3	Other examinations	Dermal irritation reactions were scored immediately before application on days 1, 8, 15, 22, 29 and on the day of necropsy. Erythema, edema, atonia, desquamation and fissuring reactions were scored on a 4-point scale from 0 (none) to 3 (severe). The occurrence of eschar and exfoliation were also recorded.
3.5.4	Statistics	Where appropriate, Levene's test was used to test homogeneity of variance. One-way ANOVA was applied to appropriate data followed by Dunnett's t-test if ANOVA was significant.
3.6	**Further remarks**	Changes in posture, reactivity to handling, tonic/clonic movements, stereotypical/bizarre behavior patterns and gait abnormalities were also assessed weekly. On day 24/25, expanded clinical observations (ECO) were performed on all animals comprising an evaluation of reactivity to handling, vocalisation, palpebral closure, exophthalmos, lacrimation, salivation, respiration, appearance of fur, piloerection, muscle tone and pupillary status. Each animal was placed in an open field for 2 minutes and observed for locomotor activity and posture or gait abnormalities. The auditory response to a Galton's whistle, proprioceptive positioning reaction, pinna response, pupillary status, pupillary response, grip strength and nociceptive reflex were also evaluated. The motor activity of all animals was quantitatively assessed on day 24/25 for 40 minutes.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No effects
4.1.2	Mortality	A male animal treated at 40mg/kg bw/day died on day 24 of study. No clinical signs of toxicity were evident prior to death, but at necropsy, congestion of the liver and lungs and hemorrhage in the lungs and thymus were noted. Since no deaths occurred at higher dose levels, the death at 40mg/kg bw/day is considered incidental to treatment with dinotefuran.
4.2	**Body weight gain**	No effects, although females at 40mg/kg bw/day had a significantly ($p < 0.05$) lower weight gain than the female controls in week 3, the overall weight gain was comparable to the controls and effect on weight gain did not occur at higher dose levels.
4.3	**Food consumption and compound**	No effects

	intake	
4.4	**Ophtalmoscopic examination**	No ocular lesions were evident in any animal after 25 days of treatment.
4.5	**Blood analysis**	
4.5.1	Haematology	No effects
4.5.2	Clinical chemistry	No effects
4.5.3	Urinalysis	Not applicable
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No effects
4.6.2	Gross and histopathology	Treatment-related histopathological alterations at 1000mg/kg bw/day were confined to a minimal increase in the incidence and severity of acanthosis / hyperkeratosis in the treated skin of females. The finding is considered not to be an adverse effect or toxicologically relevant because slight to moderate acanthosis / hyperkeratosis occurred in 2 control females and in the untreated skin of a female at 200mg/kg bw/day. Furthermore, all control and 1000mg/kg bw/day males also showed the skin alteration. There were no treatment-related histopathological alterations in the other tissues examined from animals treated at 1000mg/kg bw/day. See Table A6.3.2-1
4.7	**Other**	There were no treatment-related effects at any dose level on any of the ECO parameters evaluated and no statistically significant ($p > 0.05$) effects on quantitative motor activity. One male treated at 40mg/kg bw/day and 2 females at 1000mg/kg bw/day showed slight (grade 1) skin atonia at the application site on one or two occasions during the treatment period. There were no other signs of dermal irritation at any dose level. Since the observed atonia was transient and was not accompanied by other signs of irritation, the finding is considered not to be an adverse effect.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

OECD guideline no. 410 (1981), which is equivalent to 92/69/EEC (method B.9); US-EPA OPPTS 870.3200 (1998)

No relevant deviations from test guidelines

Method:

Four groups of 10 male and 10 female SD rats were treated by dermal application for 29 days with 2mL/kg dinotefuran as a suspension in 0.5% aqueous carboxymethyl cellulose, at nominal dose levels of 0, 40, 200 and 1000mg/kg bw/day. Applications were made to clipped, intact dorsal skin sites (approximately 10% of body surface area) for 6 - 7 hours/day under semi-occluded dressings.

The animals were observed twice daily for morbidity / mortality and detailed clinical examinations outside the home cage were performed on days 1, 8, 15, 22 and 29. Dermal irritation reactions were scored immediately before application on days 1, 8, 15, 22, 29 and on the day of necropsy. Body weights were recorded pre-dose and weekly thereafter starting on the first day of treatment. Food consumption was recorded weekly. Hematology and clinical chemistry analyses were

Section A6.3.2	Repeated dose toxicity
Annex Point IIA6.3	Dermal
	Rat

		performed on blood samples collected from all animals (food deprived) on day 30. All survivors were killed on day 30 and subjected to necropsy, *post mortem* examination of major organs and tissues and organ weight recording. The decedent was also subjected to necropsy. A full range of tissues was preserved from all animals and stained sections from the animals treated at 0 or 1000mg/kg bw/day, and the decedent, were examined microscopically. Gross lesions were examined microscopically from all animals.
5.2	**Results and discussion**	A no-observed-adverse-effect-level (NOAEL) was established as > 1000mg/kg bw/day, based on the absence of systemic and local adverse effects at this dose level.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not determined
5.3.2	NO(A)EL	1000mg/kg bw/day
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Table A6.3.2-1 Incidence of selected histopathological alterations

Organ: finding	Incidence of lesion in:							
	Males treated at (mg/kg bw/day):				Females treated at (mg/kg bw/day):			
	0	40	200	1000	0	40	200	1000
Treated skin:								
- no. examined	10	1	0	10	10	0	0	10
- inflammation	2	0	0	2	3	0	0	2
-acanthosis/ hyperkeratosis	10	1	0	10	2	0	0	8
Untreated skin:								
- no. examined	10	1	0	10	10	0	1	10
- inflammation	8	0	0	4	8	0	1	7
- fibrosis	0	0	0	0	0	0	1	0
- ulceration	0	0	0	0	0	0	0	1
- acanthosis/ hyperkeratosis	0	0	0	0	1	0	1	0

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*8 January 2013*
Materials and Methods	*As described by Applicant.*
Results and discussion	*As described by Applicant.*
Conclusion	*As described by Applicant.*
Reliability	*As described by Applicant.*
Acceptability	*Acceptable*
Remarks	*None*

COMMENTS FROM ... *(specify)*

Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.3.3 **Repeated dose toxicity**

Annex Point IIA6.3 **Inhalation**

Rat, 28-day

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 2002, MTI-446: 28-day inhalation (nose only) toxicity study in the rat, [redacted], unpublished report no. 719/16, February 26, 2002.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD guideline 412 (1981), which is equivalent to 92/69/EEC (method B.8)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	5400610
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.1%
3.1.2.3	Stability	Expiration date: 7 March 2005
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Crl:WI[GlxBRL/Han]BR
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	About 9 weeks old, weighing 173.8-226.6 g for males and 144.9-182.2 g for females
3.2.6	Number of animals per group	10 males and 10 females per group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Inhalation
3.3.1	Duration of treatment	29 or 30 days

Section A6.3.3		**Repeated dose toxicity**	
Annex Point IIA6.3		**Inhalation**	
		Rat, 28-day	
3.3.2	Frequency of exposure	7 days per week	
3.3.3	Postexposure period	None	
3.3.4	**Inhalation**		
3.3.4.1	Concentrations	Target concentrations	0.22 mg/L 0.66 mg/L 2.08 mg/L
		Nominal concentrations	2.89 mg/L 16.02 mg/L 61.24 mg/L
		Analytical concentrations	0.22 mg/L 0.66 mg/L 2.08 mg/L
3.3.4.2	Particle size	The calculated MMAD ± GSD were 2.03 ± 3.31 µm, 1.80 ± 3.60 µm and 1.55 ± 2.96 µm, respectively.	
3.3.4.3	Type or preparation of particles	Dust in the air	
3.3.4.4	Type of exposure	Nose only	
3.3.4.5	Vehicle	Air	
3.3.4.6	Concentration in vehicle	Nominal concentrations of 0 (air only), 2.89, 16.02 and 61.24 mg/L	
3.3.4.7	Duration of exposure	6 hours per day	
3.3.4.8	Controls	Air only	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, daily - generally immediately after exposure and several times up to 2 hours after exposure.	
3.4.1.2	Mortality	Yes, twice daily	
3.4.2	Body weight	Yes, before exposure on day 1, at weekly intervals and at necropsy	
3.4.3	Food consumption	Yes, weekly	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	Yes, examinations were performed on all animals pre-exposure and on all animals treated at 0 or 2.08mg/L during week 4.	
3.4.6	Haematology	Yes Number of animals: all animals Time points: during week 4 Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, clotting time,	

		prothrombin time, thromboplastin time
3.4.7	Clinical Chemisty	Yes Number of animals: all animals Time points: during week 4 Parameters: sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, sorbitol dehydrogenase, methaemoglobin, lipids, hormone (specify hormones), acid/base balance, cholinesterase inhibition.
3.4.8	Urinalysis	Yes Number of animals: all animals Time points: during week 4 Parameters: appearance, volume, osmolarity, specific gravity, pH, protein, glucose, blood
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes Organs: selected organs were weighed
3.5.2	Gross and histopathology	Yes A comprehensive list of tissues preserved. The tissues from the animals treated at 0 or 2.08mg/L, and gross lesions and respiratory tract tissues from all groups, were examined microscopically.
3.5.3	Other examinations	No
3.5.4	Statistics	Where appropriate, data were subjected to statistical analysis using one-way ANOVA. Levene's test was used to evaluate the homogeneity of variance. Where data were not heterogeneous Dunnett's test was employed. Clinical chemistry data were analysed using non-parametric tests, Kruskal-Wallis ANOVA and the Terpstra-Jonckheere test. Organ weight data was analysed using ANCOVA followed by Dunnett's test.
3.6	**Further remarks**	Blood and urine samples were collected after overnight deprivation of food and food/water, respectively. Necropsy was performed following overnight deprivation of food.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No effects, post-exposure clinical observations were predominantly localised thinning hair, attributable to the restraint procedure.
4.1.2	Mortality	No effects
4.2	**Body weight gain**	The body weight gains of all male treated groups were significantly ($p < 0.05$ or 0.01) lower than the control gain during week 1. Thereafter, weight gains were slightly lower than the controls, but not statistically significant ($p > 0.05$) at any exposure concentration. The group mean terminal body weights of the male treated groups were 5.2, 3.8 and 6.4% lower than the control value. Since the overall effect on male body weight gain was minimal and transient, and associated with a slight

Section A6.3.3 **Repeated dose toxicity**

Annex Point IIA6.3 **Inhalation**

Rat, 28-day

		decrease in food consumption, it is considered not to be an adverse effect. The weight gain of all female treated groups was comparable to the control values throughout the study. See Table A6.3.3-1	X1
4.3	**Food consumption and compound intake**	The mean weekly food consumption of all male treated groups in weeks 1 and 2, and in the high dose group in week 3, was 4.3 - 10.7% lower than the control consumption. However, the differences were not statistically ($p > 0.05$) significant. The food consumption of all female treated groups was unaffected by exposure to dinotefuran. See Table A6.3.3-1	
4.4	**Opthalmoscopic examination**	No effects	
4.5	**Blood analysis**		
4.5.1	Haematology	No effects	
4.5.2	Clinical chemistry	No effects	
4.5.3	Urinalysis	No effects	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No effects	
4.6.2	Gross and histopathology	No effects	
4.7	**Other**	There was a minimal, but statistically significant ($p < 0.05$) difference from the controls in the ratio of neutrophils/lymphocytes in males exposed to 2.08mg/L, but this was considered to reflect the biological variation inherent in small groups of animals. The group mean plasma ALT activity of the male group exposed to 2.08mg/L was significantly ($p < 0.01$) higher than the male control group by 38.7%, but since the absolute value was within the range of historical control values and in the absence of correlating histopathological alterations in the liver, the difference is considered to reflect normal biological variation. Histopathological alterations were infrequent, minor in nature and consistent with the normal pattern of findings in rats of the strain and age used. The nature and incidence of all microscopic findings were comparable in the treated and control groups.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Guidelines: OECD guideline 412 (1981), which is equivalent to 92/69/EEC (method B.8) No relevant deviations from test guidelines. Methods: Four groups of 10 male and 10 female rats were exposed for 6 hours/day for 29 or 30 days, by inhalation in nose-only chambers, to an atmosphere of dinotefuran as a dust in air at nominal concentrations of 0 (air only), 2.89, 16.02 and 61.24mg/L (equivalent to gravimetrically determined concentrations of 0, 0.22, 0.66 and 2.08mg/L).	
5.2	**Results and discussion**	There were no deaths or treatment-related clinical signs in any exposure group. The body weight gains of all male treated groups were reduced	

Section A6.3.3 **Repeated dose toxicity**

Annex Point IIA6.3 **Inhalation**

Rat, 28-day

		during week 1. Since the overall effect on male body weight gain was minimal and transient, and associated with a slight decrease in food consumption, it is considered not to be an adverse effect. The weight gain of all female treated groups was comparable to the control values throughout the study. The mean weekly food consumption of all male treated groups in weeks 1 and 2, and in the high dose group in week 3, was slightly lower than the control consumption. However, the differences were not statistically significant. The food consumption of all female treated groups was unaffected by exposure to dinotefuran. There were no treatment-related ophthalmological findings at the highest exposure concentration. There were no treatment-related effects on the hematological and plasma and urine clinical chemistry profiles in either sex at any exposure concentration. There were no treatment-related macroscopic findings, organ weight changes or histopathological alterations. No target organs were identified in either sex at the highest dose level employed.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	Not determined	X2
5.3.2	NO(A)EL	A no-observed-adverse-effect-level for respirable dinotefuran was established in both sexes as 2.08mg/L, the maximum technically achievable aerosol concentration with a MMAD ± GSD of 1.55 ± 2.96µm.	
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Table A6.3.3-1 Summary of body weight gain and food consumption

Study interval	Sex	Group mean body weight gain (g) at (mg/L):			
		0	0.22	0.66	2.08
Week 1	Male	26.7	17.1*	16.0**	14.3**
Week 2 - week 4		38.2	35.4	35.2	34.0
Overall (week 1 - week 4)		64.8	52.5	51.1*	48.3**
Week 1	Female	8.2	7.2	7.1	7.0
Week 2 - week 4		17.8	19.8	14.1	22.5
Overall (week 1 - week 4)		26.0	27.0	21.2	29.5
		Group mean food consumption (g/week):			
Week 1	Male	146.3	137.8	138.9	130.7
Week 2		155.1	148.4	147.0	140.0
Week 3		155.3	153.9	151.3	145.7
Week 4		134.7	137.3	138.9	135.9
Week 1	Female	109.5	110.1	109.0	109.7
Week 2		113.5	117.6	114.2	117.9
Week 3		117.2	122.8	119.2	120.2
Week 4		110.7	113.1	114.4	119.8

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *9 January 2013*

Materials and Methods *As described by Applicant*

Results and discussion *X1 Section 4.2 The significant reductions in bodyweight gain in all treated groups of males, accompanied by slightly reduced food consumption, are of sufficient magnitude to be regarded as a treatment-related adverse effects*

Conclusion *X2 Sections 5.3.1 &5.3.2 A NOAEC for males could not be identified because adverse effects on bodyweight gain and food consumption were present at the lowest concentration tested. A LOAEC of 0.22 mg/L is therefore identified for males. The NOAEL for females is 2.08 mg/L, the highest concentration tested.*

Reliability *As described by Applicant*

Acceptability *Acceptable*

Remarks *None*

COMMENTS FROM ... *(specify)*

Date

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

Section A6.4.1-1 **Repeated dose toxicity**

Annex Point IIA6.4 **Rat**

Oral, 13-week

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1997c, 13-week dietary toxicity study with MTI-446 in rats, [redacted], unpublished report no. [redacted] 6648-127, December 31, 1997. [redacted], 2000a, First amendment to report - 13-week dietary toxicity study with MTI-446 in rats, [redacted], unpublished report no. [redacted] 6648-127, April 5, 2000.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD guideline no. 408 (1981), which is equivalent to 88/302/EEC (method B.26) EPA-FIFRA, Subdivision F, § 82-1 JMAFF 59 NohSan no. 4200 (1985)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	22-00110
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	96.5% + 2.0% water, purity of dried material 99.1%
3.1.2.3	Stability	Expiration date: May 14, 2001
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Crl:CD® [SD]BR VAF/Plus ®
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	About 7 weeks old, weighing 235-284 g for males and 165-228 g for females
3.2.6	Number of animals per group	10 males and 10 females per group
3.2.7	Control animals	Yes

Section A6.4.1-1 Annex Point IIA6.4		Repeated dose toxicity Rat Oral, 13-week
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	7 days per week
3.3.3	Postexposure period	None
3.3.4	Oral	
3.3.4.1	Type	In food
3.3.4.2	Concentration	Nominal in food: 0, 500, 5000, 25000 and 50000 ppm in the diet Mean achieved dose levels: 0, 34, 336, 1623 and 3156 mg/kg bw/day in males 0, 38, 384, 1871 and 3616 mg/kg bw/day in females
3.3.4.3	Vehicle	No vehicle, added to basal diet
3.3.4.4	Concentration in vehicle	Not applicable
3.3.4.5	Total volume applied	Not applicable
3.3.4.6	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, weekly
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, weekly
3.4.3	Food consumption	Yes, weekly
3.4.4	Water consumption	Not examined
3.4.5	Ophthalmoscopic examination	Yes, pre-test and in week 14 on all animals
3.4.6	Haematology	Yes Number of animals: all animals Time points: pre-test and in week 14, following overnight food deprivation Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, clotting time, prothrombin time, thromboplastin time
3.4.7	Clinical Chemisty	Yes Number of animals: all animals Time points: pre-test and in week 14, following overnight food deprivation

Section A6.4.1-1 Annex Point IIA6.4		Repeated dose toxicity Rat Oral, 13-week	
		Parameters: sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, sorbitol dehydrogenase, methaemoglobin, lipids, hormone (specify hormones), acid/base balance, cholinesterase inhibition.	
3.4.8	Urinalysis	Yes Number of animals: all animals Time points: pre-test and in week 14, following overnight food deprivation Parameters: appearance, volume, osmolarity, specific gravity, pH, protein, glucose, blood	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes Organs: selected organs were weighed	
3.5.2	Gross and histopathology	Yes Samples of all major organs and tissues preserved. All preserved tissues from animals treated at 0 or 50000ppm were examined by light microscopy. Gross lesions, adrenal glands, kidneys, liver and lungs were also examined from all animals in the groups treated at 500, 5000 and 25000ppm	
3.5.3	Other examinations	None	
3.5.4	Statistics	Where appropriate, data were analysed statistically at the 5% level by one-way ANOVA on homogeneous or transformed data followed by Dunnett's multiple comparison t-test where ANOVA was significant. Levene's test was used to evaluate the homogeneity of variance.	
3.6	**Further remarks**	The animals were fed the diets *ad libitum* for at least 13 weeks, except when animals were fasted.	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	No effects	
4.1.2	Mortality	No effects	
4.2	**Body weight gain**	Males and females treated at 50000ppm lost weight during week 1, but subsequently gained weight. The animals of both sexes treated at 25000 or 50000ppm, and females treated at 5000ppm, showed statistically significant ($p < 0.05$) and dose-related reductions in overall body weight gain. At termination, the mean body weights of these groups were 7.2 to 24.1% lower than control values. See Table A6.4.1.1-1	
4.3	**Food consumption and compound intake**	The mean weekly food consumption of both sexes at 25000 or 50000ppm was significantly ($p < 0.05$) reduced for at least 11 weeks of the treatment period, and the overall mean food consumption was 11.5 to 24.5% lower than control values. Although the overall weight gain of females at 5000ppm was significantly lower than the control value and overall food consumption was 8.7% lower than the control value ($p < 0.05$ on 6 occasions), the observations are considered not to	X1

Section A6.4.1-1	Repeated dose toxicity	
Annex Point IIA6.4	Rat	
	Oral, 13-week	
	be adverse effects because the group mean body weight and food consumption remained within 10% of the control values throughout the study. See Table A6.4.1.1-1	
4.4 Ophtalmoscopic examination	There were no treatment-related ocular lesions at any dose level.	
4.5 Blood analysis		
4.5.1 Haematology	After 13 weeks of treatment effects were confined to the group treated at 50000ppm. Males at 50000ppm also showed slightly, but significantly ($p < 0.05$) lower blood glucose (Glu), total protein (Prot) and globulin (Glob) concentrations. These minor differences from the controls were not associated with overt histopathological alterations and are considered not to be of toxicological significance or adverse effects. See Table A6.4.1.1-2	
4.5.2 Clinical chemistry	After 13 weeks of treatment effects were confined to the group treated at 50000ppm. Activated partial thromboplastin times (APTT) were slightly shorter than control values and urea nitrogen concentrations were slightly elevated in both sexes, but were significantly ($p < 0.05$) different from the controls in males only. These minor differences from the controls were not associated with overt histopathological alterations and are considered not to be of toxicological significance or adverse effects. See Table A6.4.1.1-2	
4.5.3 Urinalysis	No effects	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	There were no effects on absolute organ weights or ratios that are considered to be a direct effect of treatment at any dose level. However, absolute heart, kidney, liver and spleen weights were significantly ($p < 0.05$) lower and their ratios relative to body weight and/or brain weight were significantly different from the controls in the groups treated at 25000 and 50000ppm. The absolute weights of the adrenals and pituitary were lower in females and the relative weights of brain and testes were higher in animals at 50000ppm. Since the body weights of these groups were significantly reduced at termination and because there were no correlating histopathological changes in these organs, the differences from control values are considered to be incidental to treatment or secondary to substantially reduced weight gain.	
4.6.2 Gross and histopathology	Treatment-related histopathological alterations were confined to increased cytoplasmic vacuolation of the adrenal cortex in both sexes treated at 25000 and 50000ppm and in males at 5000ppm. The vacuolation was apparent in both the zona glomerulosa and zona fasciculata of the males but was confined to the zona glomerulosa in the females. The severity of the lesion was graded as minimal or slight in all instances except for one female at 50000ppm that was graded moderate. Minimal to moderate adrenal cortical vacuolation is considered not to be an adverse finding in this study since there were no correlating clinical pathology findings indicating functional deficit. See Table A6.4.1.1-3	
4.7 Other	None	

Section A6.4.1-1 **Repeated dose toxicity**

Annex Point IIA6.4 **Rat**

Oral, 13-week

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Guidelines: OECD guideline no. 408 (1981), which is equivalent to 88/302/EEC (method B.26), EPA-FIFRA, Subdivision F, § 82-1, JMAFF 59 NohSan no. 4200 (1985). No relevant deviations from test guidelines. Method: In a 90-day study, the test substance was administered to groups of 20 Sprague-Dawley rats (10 males and 10 females) at constant concentrations of 0, 500, 5000, 25000 and 50000 ppm in the diet (mean achieved dose levels 0, 34, 336, 1623 and 3156 mg/kg bw/day in males and 0, 38, 384, 1871 and 3616 mg/kg bw/day in females).	
5.2	**Results and discussion**	There were no deaths and no treatment-related clinical signs, but a dose-related decrease in body weight gain, associated with reduced food consumption, occurred in both sexes at 25000 or 50000 ppm. Although females at 5000 ppm also showed slightly reduced food consumption and weight gain, the differences were considered not to be adverse because they remained within 10% of control values. Minor treatment-related effects on the haematological and clinical chemistry profiles occurred at 50000 ppm only, and comprised slightly lower APTT and slightly elevated blood urea nitrogen concentration. Other than secondary changes in organ weights and ratios due to body weight effects, there were no direct effects of treatment on organ weights. Treatment-related histopathological alterations occurred in both sexes at 25000 or 50000 ppm and in males at 5000 ppm, and comprised slightly increased cytoplasmic vacuolation of the adrenal cortex which was considered non-adverse since there were no correlating clinical pathology findings indicating functional deficit.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	Not determined	
5.3.2	NO(A)EL	25000 ppm (1623 and 1871 mg/kg/day in males and females, respectively), based on marked growth retardation and reduced food consumption at 50000 ppm.	X2
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Table A6.4.1.1-1 Summary of body weight gain and food consumption

Sex	Dose level (ppm)	Overall group mean weight gain (g)	Group mean terminal body weight (g)	Overall mean food consumption (g/wk)a
Male	0	315	572	208
	500	316	568	204
	5000	295	552	201
	25000	257*	515*	184
	50000	190*	446*	157
Female	0	141	345	160
	500	131	326	146
	5000	118*	320*	146
	25000	96*	291*	131
	50000	67*	262*	121

* $p < 0.05$; a statistical analysis not performed on overall group mean data

Table A6.4.1.1-2 Treatment related effects on hematology and serum chemistry – week 14

Sex	Dose level (ppm)	Group mean hematology and serum chemistry values:				
		APTT (sec)	UN (mg/dL)	Glu (mg/dL)	Prot (g/dL)	Glob (g/dL)
Male	0	14.4	14	102	7.9	3.1
	500	13.8	13	111	7.7	2.9
	5000	14.8	14	104	7.8	2.9
	25000	13.7	15	98	7.6	2.8
	50000	13.1*	17*	85*	7.5*	2.7*
Female	0	13.2	16	102	8.3	2.6
	500	12.6	16	107	8.3	2.7
	5000	12.8	15	105	8.2	2.5
	25000	12.6	16	98	8.1	2.6
	50000	12.2	19	104	8.0	2.7

Table A6.4.1.1-3 Treatment related histopathological alterations in the adrenal cortex

Sex	Dose level (ppm)	Incidence (%) of increased vacuolation in zona glomerulosa	Incidence (%) of increased vacuolation in zona fasciculata
Male	0	0	10
	500	0	0
	5000	30	20
	25000	20	30
	50000	40	50
Female	0	0	0
	500	0	0
	5000	0	0
	25000	60	0
	50000	100	0

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*9 January 2013*
Materials and Methods	*As described by Applicant*
Results and discussion	*As described by Applicant, except:* *X1 4.3 The reduction in bodyweight gain of females seen at 5000 ppm is considered to be an adverse effect of treatment by the RMS*
Conclusion	*X2 5.3.1 and 5.3.2 The NOAEL is 500 ppm (not 25000 ppm as concluded by the Applicant) based on the increased incidence of vacuolisation of the adrenal cortex at 5000 ppm in males and reduced bodyweight gain of females at 5000 ppm. Thus, the LOAEL is 5000 ppm*
Reliability	*As described by Applicant*
Acceptability	*Acceptable*
Remarks	*None*
	COMMENTS FROM ... *(specify)*
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.4.1-2 **Repeated dose toxicity**

Annex Point IIA6.4 **Mouse**

Oral, 13-week

Official use only

	1 REFERENCE	
1.1	**Reference**	[redacted], 1997d, 13-week dietary toxicity study with MTI-446 in mice, [redacted], unpublished report no. [redacted] 6648-126, December 31, 1997. [redacted], 2000b, First amendment to report: - 13-week dietary toxicity study with MTI-446 in mice, [redacted], unpublished report no. [redacted] 6648-126, April 5, 2000.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD guideline no. 408 (1981), which is equivalent to 88/302/EEC (method B.26) EPA-FIFRA, Subdivision F, § 82-1 JMAFF 59 NohSan no. 4200 (1985)
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	22-00110
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	96.5% + 2.0% water, purity of dried material 99.1%
3.1.2.3	Stability	Expiration date: May 14, 2001
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Crl:CD1® [ICR]BR VAF/Plus ®
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	About 7 weeks old, weighing 25.9 – 35.9 g for males and 18.8 – 32.2 g for females
3.2.6	Number of animals per group	10 males and 10 females per group See Table A6_04_1_02-1
3.2.7	Control animals	Yes

Section A6.4.1-2	Repeated dose toxicity	
Annex Point IIA6.4	Mouse	
	Oral, 13-week	
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	7 days per week
3.3.3	Postexposure period	None
3.3.4	**Oral**	
3.3.4.1	**Type**	In food
3.3.4.2	**Concentration**	Nominal in food: 0, 500, 5000, 25000 and 50000 ppm in the diet Mean achieved dose levels: 0, 81, 844, 4442 and 10635mg/kg bw/day in males 0, 102, 1064, 5414 and 11560mg/kg bw/day in females Food consumption per day: *ad libitum*
3.3.4.3	**Vehicle**	No vehicle, added to basal diet
3.3.4.4	**Concentration in vehicle**	Not applicable
3.3.4.5	**Total volume applied**	Not applicable
3.3.4.6	**Controls**	Plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, weekly
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, weekly
3.4.3	Food consumption	Yes, weekly
3.4.4	Water consumption	Not examined
3.4.5	Ophthalmoscopic examination	Yes, pre-test and in week 14 on all animals
3.4.6	Haematology	Yes Number of animals: 5 mice/sex/group Time points: in week 14 Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, clotting time, prothrombin time, thromboplastin time. Blood samples were withdrawn after a period of at least 4 hours food deprivation.
3.4.7	Clinical Chemisty	Yes Number of animals: 5 mice/sex/group

Section A6.4.1-2	Repeated dose toxicity	
Annex Point IIA6.4	Mouse	
	Oral, 13-week	

		Time points: In week 14 Parameters: sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, sorbitol dehydrogenase, methaemoglobin, lipids, hormone (specify hormones), acid/base balance, cholinesterase inhibition.
3.4.8	Urinalysis	Yes Number of animals: all animals Time points: in week 1 Parameters: appearance, volume, osmolality, specific gravity, pH, protein, glucose, blood
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes Organs: Selected organs were weighed.
3.5.2	Gross and histopathology	Yes Samples of all major organs and tissues preserved. All preserved tissues from animals treated at 0 or 50000ppm were examined by light microscopy. Gross lesions, kidneys, lungs and liver with gall bladder were also examined from all animals treated at 500, 5000 or 25000ppm.
3.5.3	Other examinations	None
3.5.4	Statistics	Where appropriate, data were analysed statistically at the 5% level by one-way analysis of variance on homogeneous or transformed data followed by Dunnett's t-test where ANOVA proved significant. Levene's test was used to evaluate the homogeneity of variance.
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No effects
4.1.2	Mortality	No effects
4.2	**Body weight gain**	Both sexes at 50000ppm lost weight during the first week of treatment and subsequently showed a treatment-related depression of body weight gain. The overall mean weight gains of both sexes were significantly ($p < 0.05$) lower than control values and at termination the group mean body weights were 15.4 and 21.9% lower than the controls in males and females, respectively. The overall weight gains of males at 25000ppm and females at 500, 5000 and 25000ppm were 15.9 to 31.4% lower than, but not significantly ($p > 0.05$) different from, the controls. Increased food spillage occurred during the first week in the groups treated at 25000 or 50000ppm and continued throughout the study at 50000ppm. Spillage is considered to indicate reduced diet palatability at concentrations ≥ 25000ppm and any apparent increase in food consumption is considered to represent spillage. No evidence of an effect on food consumption or food

Section A6.4.1-2	Repeated dose toxicity	
Annex Point IIA6.4	Mouse	
	Oral, 13-week	

		efficiency at dose levels up to 5000 ppm. See Table A6.4.1.2-2
4.3	**Food consumption and compound intake**	No effects. See Table A6.4.1.2-2
4.4	**Ophtalmoscopic examination**	No effects
4.5	**Blood analysis**	
4.5.1	Haematology	No effects, all group mean hematological values were similar to, and not significantly ($p > 0.05$) different from, the control values. See Table A6_04_1_02-3
4.5.2	Clinical chemistry	Treatment-related effects on serum chemistry after 13 weeks of treatment were confined to the group treated at 50000ppm. The serum albumin concentration of males was slightly, but significantly ($p < 0.05$) higher than the control value by 17.2%. See Table A6.4.1.2-3
4.5.3	Urinalysis	Urine pH was slightly lower in both sexes, but significantly different from the controls in the female group only. See Table A6.4.1.2-3
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No effects
4.6.2	Gross and histopathology	No effects
4.7	**Other**	There were no primary treatment-related effects on absolute and relative organ weights at any dose level. The absolute weights of the heart and liver in females at 50000ppm and of the kidneys in both sexes were significantly ($p < 0.05$) lower than control values. The differences are considered to be a consequence of growth retardation since the organ/body weight ratios were not affected. A significant ($p < 0.05$) increase in the brain/body weight ratios in both sexes at 50000ppm is also considered to reflect growth retardation.
	5	**APPLICANT'S SUMMARY AND CONCLUSION**
5.1	**Materials and methods**	Guidelines: OECD guideline no. 408 (1981), which is equivalent to 88/302/EEC (method B.26), EPA-FIFRA, Subdivision F, § 82-1, JMAFF 59 NohSan no. 4200 (1985) No relevant deviations from test guidelines. Method: Groups of 10 male and 10 female mice were treated orally for 13 weeks with dinotefuran, by admixture in the diet at constant nominal concentrations of 0, 500, 5000, 25000 and 50000ppm. Mean achieved dose levels were 0, 81, 844, 4442 and 10635mg/kg b.w./day (males) and 0, 102, 1064, 5414 and 11560mg/kg bw/day (females).
5.2	**Results and discussion**	There were no deaths and no treatment-related clinical signs at any dose level, but both sexes at 50000ppm lost weight during the first week of treatment and subsequently showed a treatment-related depression of body weight gain. The overall mean weight gains and body weights at termination of both sexes were reduced. The overall weight gains of males at 25000ppm and females at 500, 5000 and 25000ppm were 15.9 to 31.4% lower than, but not significantly

Section A6.4.1-2 **Repeated dose toxicity**

Annex Point IIA6.4 **Mouse**

Oral, 13-week

different from, the controls. Increased food spillage occurred during the first week in the groups treated at 25000 or 50000ppm and continued throughout the study at 50000ppm. Spillage is considered to indicate reduced diet palatability at concentrations ≥ 25000ppm and any apparent increase in food consumption is considered to represent spillage. There was no evidence of an effect on food consumption or food efficiency at dose levels up to 5000ppm.

There were no treatment-related ophthalmological and hematological effects at any dose level. Treatment-related effects on serum chemistry after 13 weeks of treatment were confined to males treated at 50000ppm that showed slightly raised serum albumin concentration. Urine pH was slightly lower in both sexes. These minor differences from the controls were not associated with overt histopathological alterations and are considered not to be adverse effects.

There were no primary treatment-related effects on absolute and relative organ weights at any dose level. The absolute weights of the heart and liver in females at 50000ppm and of the kidneys in both sexes were lower than control values. The differences are considered to be a consequence of growth retardation since the organ/body weight ratios were not affected. There were no treatment-related macroscopic findings at necropsy at any dose level. There were no treatment-related histopathological alterations and the nature, severity and incidence of microscopic findings were similar in all treated and control groups.

No target organs were identified in either sex at the highest dose level employed.

5.3 Conclusion

5.3.1	LO(A)EL	Not determined
5.3.2	NO(A)EL	25000ppm in both sexes, equivalent to dose levels of 4442mg/kg bw/day (males) and 5414mg/kg bw/day (females), based on the occurrence of reduced weight gain in both sexes at 50000ppm.
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Table A6.4.1.2-1: Animal assignment and treatment

Group number	Dose level of dinotefuran (ppm)	Number of animals	
		Male	Female
1	0	10	10
2	500	10	10
3	5000	10	10
4	25000	10	10
5	50000	10	10

Table A6.4.1.2-2: Summary of body weights, weight gains and good consumption

Interval	Males treated at (ppm):					Females treated at (ppm):				
	0	500	5000	25000	50000	0	500	5000	25000	50000
Group mean body weight (g)										
Week 1	31.0	31.6	30.2	30.1	31.1	26.0	26.1	25.3	26.1	25.6
Week 2	32.7	33.2	32.0	31.2	30.2	27.3	27.2	26.5	26.8	24.0
Week 3	34.4	35.0	33.7	32.9	30.4*	28.9	28.5	27.6	28.0	24.7*
Week 4	34.6	34.9	34.2	32.8	30.9*	29.1	28.5	27.8	28.1	25.1*
Week 8	37.9	38.1	37.0	36.1	33.2*	31.5	30.7	30.0	30.2	26.6*
Week 14	41.7	41.9	40.3	39.1	35.3*	36.5	34.1	32.5*	33.3	28.5*
Overall gain	10.7	10.3	10.1	9.0	4.2*	10.5	8.0	7.2	7.2	2.9*
Group mean food consumption (g/week)**										
Weeks 1 - 4	45.1	43.6	43.6	43.7	49.6	41.4	43.2	43.6	44.5	46.1
Weeks 5 - 8	45.6	44.4	45.1	45.8	51.4	45.0	46.1	45.1	45.9	42.3
Weeks 9 - 13	40.5	40.4	40.2	41.7	45.3	42.6	42.1	42.6	43.7	41.9

* $p < 0.05$
** food consumption of animals not showing substantial food spillage

Table A6.4.1.2-3: Treatment related serum and urine clinical chemistry findings – week 14

Sex	Dose level (ppm)	Mean serum albumin concentration ± SD (g/dL)	Mean urine pH (range)
Male	0	2.9 ± 0.14	7.3 (6.5 - 8.5)
	500	3.1 ± 0.31	7.5 (7.0 - 8.0)
	5000	3.2 ± 2.1	7.5 (7.0 - 8.0)
	25000	3.3 ± 0.18	6.9 (7.0 - 8.5)
	50000	3.4 ± 0.26*	6.3 (6.0 - 8.0)
Female	0	3.4 ± 0.09	7.2 (6.5 - 8.0)
	500	3.4 ± 0.25	7.2 (6.5 - 7.5)
	5000	3.4 ± 0.11	7.2 (6.5 - 8.0)
	25000	3.5 ± 0.38	6.8 (6.5 - 7.5)
	50000	3.6 ± 0.29	6.6* (6.0 - 7.0)

* $p < 0.05$

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*9 January 2013*
Materials and Methods	*As described by Applicant.*
Results and discussion	*As described by Applicant.*
Conclusion	*As described by Applicant.*
Reliability	*As described by Applicant.*
Acceptability	*Acceptable*
Remarks	*None*
	COMMENTS FROM ... *(specify)*
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.4.1-3 **Repeated dose toxicity**

Annex Point IIA6.4 **Dog**

Oral, 13-week

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1999a, 13-week dietary toxicity study with MTI-446 in dogs, [redacted], unpublished report no. [redacted] 6648-128, May 13, 1999. [redacted], 1999b, First amendment to report - 13-week dietary toxicity study with MTI-446 in dogs, [redacted], unpublished report no. [redacted] 6648-128, December 10, 1999.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD guideline no. 409 (1981), which is equivalent to 88/302/EEC EPA-FIFRA, Subdivision F, § 82-1 JMAFF 59 NohSan no. 4200 (1985)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	2200210
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	93.0% + 7.6% water, purity of dried material 98.9%
3.1.2.3	Stability	Expiration date: May 2002
3.2	**Test Animals**	
3.2.1	Species	Dog
3.2.2	Strain	Beagle
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	5-6 months old, weighing 7.7 – 8.8 kg for males and 6.3 – 8.8 kg for females
3.2.6	Number of animals per group	4 males and 4 females per group. See Table A6.4.1.3-1
3.2.7	Control animals	Yes

Section A6.4.1-3	Repeated dose toxicity
Annex Point IIA6.4	Dog
	Oral, 13-week

3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	7 days per week7
3.3.3	Postexposure period	None
3.3.4	Oral	
3.3.4.1	Type	In food
3.3.4.2	Concentration	Nominal in food: 0, 1600, 8000 and 40000/24000 ppm in the diet Mean achieved dose levels: 0, 58, 307 and 862mg/kg bw/day in males 0, 58, 323 and 950mg/kg bw/day in females
3.3.4.3	Vehicle	No vehicle, added to basal diet
3.3.4.4	Concentration in vehicle	Not applicable
3.3.4.5	Total volume applied	Not applicable
3.3.4.6	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, daily
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, weekly and at necropsy
3.4.3	Food consumption	Yes, daily for one week pre-dose and for the first 2 weeks of treatment and weekly thereafter.
3.4.4	Water consumption	Yes, 2 days/week throughout the treatment period
3.4.5	Ophthalmoscopic examination	Yes, pre-test and in week 14 on all animals
3.4.6	Haematology	Yes Number of animals: all animals Time points: pre-test, in weeks 5 and 14 after overnight food withdrawal Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, clotting time, prothrombin time, thromboplastin time
3.4.7	Clinical Chemisty	Yes Number of animals: all animals Time points: pre-test, in weeks 5 and 14 after overnight food

		withdrawal Parameters: sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, sorbitol dehydrogenase, methaemoglobin, lipids, hormone (specify hormones), acid/base balance, cholinesterase inhibition.
3.4.8	Urinalysis	Yes Number of animals: all animals Time points: pre-test, in weeks 5 and 14 after overnight food withdrawal Parameters: appearance, volume, osmolarity, specific gravity, pH, protein, glucose, blood
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes Organs: selected organs were weighed
3.5.2	Gross and histopathology	Yes Samples of all major organs and tissues preserved. All preserved tissues from all animals were examined by light microscopy.
3.5.3	Other examinations	None
3.5.4	Statistics	Where appropriate, data were analysed statistically at the 5% level by one-way ANOVA on homogeneous or transformed data followed by Dunnett's multiple comparison t-test where ANOVA proved significant. One-way ANCOVA was used to analyse body weights using initial body weight as covariate.
3.6	**Further remarks**	Due to a marked reduction in food consumption, the highest dietary concentration was progressively reduced from 40000ppm, through 30000ppm, to 24000ppm from day 12.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Treatment-related clinical signs were confined to animals treated at 30000/40000ppm. Discolored feces, few or no feces, thinness, slightly reduced activity and pale gums occurred in some animals. Two males and one female initially treated at 40000ppm showed black feces for one or 2 days, but the occurrence may be related to stress resulting from offering unpalatable diet, rather than a primary effect of dinotefuran. One male treated at 40000/30000ppm showing liquid/mucoid feces was diagnosed with enteritis on day 8 and was taken off dose for 4 days.
4.1.2	Mortality	No effects
4.2	**Body weight gain**	Body weights were significantly reduced in both sexes at 24000ppm from week 2 resulting in overall reductions in weight gain of 33.3 and 31.4% in males and females, respectively. However, a large proportion of the reduced weight gain was associated with the administration of 30000 - 40000ppm during the first 12 days of treatment, and subsequent treatment at 24000ppm resulted in only marginally lower weight gains. Females at 1600 and 8000ppm also showed significantly lower body weights during the last 8 weeks of treatment and lower

Section A6.4.1-3		Repeated dose toxicity	
Annex Point IIA6.4		Dog Oral, 13-week	
		overall weight gains (34.3 and 31.4%, respectively) but these are considered not to be adverse effects since there were no adverse clinical signs or pathological findings. See Table A6.4.1.3-2	X1
4.3	**Food consumption and compound intake**	Food consumption was markedly and significantly ($p < 0.05$) reduced in both sexes treated at 30000/40000ppm, indicating reduced diet palatability. Although food consumption increased after the reduction of the diet concentration to 24000ppm, reduced food consumption persisted in both sexes throughout the study. The food consumption of females treated at 1600 and 8000ppm was also low in comparison with the female control consumption. However, the differences in food consumption are considered not to be toxicologically relevant because they were not dose-related and the pre-dose food consumption at 1600ppm was 16.4% lower than the control consumption. See Table A6.4.1.3-3	
4.4	**Ophtalmoscopic examination**	No effects	
4.5	**Blood analysis**		
4.5.1	Haematology	No effects	
4.5.2	Clinical chemistry	No effect, males and females at 24000ppm showed slightly but significantly ($p < 0.05$) reduced serum ALT activity relative to both pre-dose values and the controls after 5 and 13 weeks treatment. See Table A6.4.1.3-4	
4.5.3	Urinalysis	No effects, urine pH of males at 24000ppm was marginally lower than control values in weeks 5 and 14.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No effects, All organ weights and ratios were unaffected by treatment at all dose levels and none at 24000ppm was significantly different from the controls ($p > 0.05$).	
4.6.2	Gross and histopathology	No effects, although hemorrhage in the mesenteric and/or mandibular lymph nodes occurred in 3 of the 4 males treated at 24000ppm, its occurrence is considered incidental to treatment with dinotefuran since hemorrhage was not evident in other organs and none of the macroscopic or clinical pathology observations suggested a hemorrhagic condition in these animals.	
4.7	**Other**	The highest diet concentration was reduced to 30000ppm on day 5 and further reduced to 24000ppm on day 12 due to very low food consumption. Markedly lower food consumption at ≥ 30000ppm is considered to reflect diet unpalatability. There was a concomitant decrease in water consumption during the first 11 days of treatment at concentrations of ≥ 30000/40000ppm. Subsequently, the water consumption of the males remained depressed, but female water consumption was comparable to, or higher than, the control females as from day 24. Water consumption of both sexes at 1600 or 8000 ppm was not affected by treatment.	

Section A6.4.1-3 **Repeated dose toxicity**

Annex Point IIA6.4 **Dog**

Oral, 13-week

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

OECD guideline no. 409 (1981) which is equivalent to 88/302/EEC, EPA-FIFRA, Subdivision F, § 82-1, JMAFF 59 NohSan no. 4200 (1985)

No relevant deviations from test guidelines.

Method:

Groups of 4 male and 4 female beagle dogs were treated orally for 13 weeks with dinotefuran incorporated into the diet at concentrations of 0, 1600, 8000 and 40000/24000ppm. Due to a marked reduction in food consumption, the highest dietary concentration was progressively reduced from 40000ppm, through 30000ppm, to 24000ppm from day 12. Mean achieved dose levels were 0, 58, 307 and 862mg/kg bw/day (males) and 0, 58, 323 and 950mg/kg bw/day (females).

5.2 Results and discussion

There were no deaths during the study. Treatment-related clinical signs were confined to a few animals treated at 30000/40000ppm that showed discoloured, mucoid, few or no feces, thinness, slightly reduced activity and pale gums. There were no other clinical signs after reduction of the diet concentration to 24000ppm. Markedly lower food consumption at ≥ 30000ppm was considered to reflect diet unpalatability, but consumption remained depressed at 24000 ppm. Low food consumption in females at 8000 or 1600 ppm was considered not toxicologically relevant. Body weights were significantly reduced in both sexes at 24000ppm from week 2, due mainly to treatment at 30000 - 40000ppm during the first 12 days of treatment, and subsequent treatment at 24000ppm resulted in only marginally lower weight gains. Females at 1600 and 8000ppm also showed significantly lower body weights during the last 8 weeks of treatment and lower overall weight gains (34.3 and 31.4%, respectively) but these are considered not to be adverse effects since there were no adverse clinical signs or pathological findings. There was a concomitant decrease in water consumption during the first 11 days of treatment at concentrations of ≥ 30000/40000ppm. Subsequently, the water consumption of the males remained depressed, but female water consumption recovered. Water consumption of both sexes at 1600 or 8000ppm was not affected by treatment.

There were no ocular lesions and no treatment-related hematological changes. Males and females at 24000ppm showed slightly reduced serum ALT activity after 5 and 13 weeks treatment. Urine pH of males at 24000ppm was marginally lower than control values in weeks 5 and 14. These findings are considered not be adverse effects and of no toxicological relevance in the absence of correlating histopathological alterations. There were no other treatment-related changes in the hematology, serum chemistry and urinalysis profiles at any dose level.

All organ weights and ratios were unaffected by treatment at all dose levels. There were no treatment-related macroscopic or microscopic histopathological alterations at any of the dose levels employed. Although hemorrhage in the mesenteric and/or mandibular lymph nodes occurred in 3 of the 4 males treated at 24000ppm, its occurrence is considered incidental to treatment since hemorrhage was not evident in other organs and none of the macroscopic or clinical pathology

Section A6.4.1-3 **Repeated dose toxicity**

Annex Point IIA6.4 **Dog**

Oral, 13-week

		observations suggested a hemorrhagic condition in these animals. No target organs were identified in either sex at the highest dose level employed. A no-observed-effect-level (NOEL) and NOAEL were established as 8000ppm in both sexes, equivalent to dose levels of 307mg/kg bw/day (males) and 323mg/kg bw/day (females), based on the occurrence of reduced food consumption, body weight gain and serum ALT activity in both sexes, and marginally reduced urine pH in males at 24000 - 40000ppm. The reviewer considers the established NOAEL to be a conservative value because reduced body weight gain was not apparent in either sex during the 11 weeks of treatment at 24000ppm.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	Not determined	
5.3.2	NO(A)EL	8000ppm in both sexes, equivalent to dose levels of 307mg/kg bw/day (males) and 323mg/kg bw/day (females), based on the occurrence of reduced food consumption, body weight gain and serum ALT activity in both sexes, and marginally reduced urine pH in males at 24000 - 40000ppm.	X2
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Table A6.4.1.3-1: Animal assignment and treatment

Group number	Dose level of dinotefuran (ppm)	Number of animals	
		Male	Female
1	0	4	4
2	1600	4	4
3	8000	4	4
4	24000*	4	4

* Group 4 animals were offered the diet containing 40000ppm on day 1 through 4; because animals did not readily consume the diet containing 40000ppm, the high dose was lowered to 30000ppm on day 5. The animals were offered the diet containing 30000ppm on day 5 through 11. On day 12, the dose level was again lowered to 24000ppm because the animals did not readily consume the diet containing 30000ppm.

Table A6.4.1.3-2: Selected group mean body weight data

Sex / dose level (ppm)	Mean body weight (kg) at week:					
	1	2	3	4	8	14
Males:						
0	8.1	8.9	9.5	9.9	10.8	11.7
1600	8.0	9.1	9.4	9.9	11.0	11.8
8000	8.2	8.9	9.2	9.6	10.6	11.5
24000	8.3	7.9*	8.6*	9.1*	10.0*	10.7*
Females:						
0	7.1	7.9	8.3	8.7	9.8	10.6
1600	7.0	7.7	8.0	8.2*	8.8*	9.3*
8000	7.2	7.8	8.2	8.4	9.0*	9.6*
24000	7.0	6.8*	7.3*	7.8*	8.6*	9.4*

* $p < 0.05$

Table A6.4.1.3-3: Representative group mean food and water consumption data

Sex	Dose level (ppm)	Mean food consumption (g/week) in week:				
		1	2	4	8	13
Male	0	2951	2919	2875	2958	2990
	1600	2913	2671	2645	2528	2722
	8000	2555	2603	2686	2708	2896
	24000	1394*	2381	2570	2391*	2411*
Female	0	2712	2863	2773	2722	2657
	1600	2406	2311*	2212*	2203*	2102*
	8000	2588	2557	2392*	2350	2631
	24000	869*	2240*	2295*	2207*	2402
		Mean water consumption (g/week) on day:				
		2	10	24	52	86
Male	0	915	1455	1423	1550	1505
	1600	780	958*	1090	980*	1095
	8000	743	1260	1295	1270	1313
	24000	138*	798*	1165	963*	1000*
Female	0	573	1030	945	977	980
	1600	503	903	790	658	833
	8000	390	818	735	683	747
	24000	38*	748	1035	940	1103

* $p < 0.05$

Table A6.4.1.3-4: Treatment related serum clinical chemistry findings

Sex dose level (ppm)	Mean serum ALT activity (IU/L) ± SD in:			Mean urine pH (range) in:		
	Week -1	Week 5	Week 14	Week -1	Week 5	Week 14
Males:						
0	25 ± 2.9	27 ± 3.3	32 ± 1.0	7.0 (7.0 - 7.0)	8.1 (7.5-8.5)	7.6 (7.0-8.5)
1600	30 ± 7.3	24 ± 0.5	27 ± 1.9	6.9 (6.5 - 7.5)	7.5 (6.5-8.5)	7.6 (7.5-8.0)
8000	28 ± 5.1	22 ± 6.8	27 ± 4.8	6.9 (6.5 - 7.0)	6.8 (6.0-7.5)	7.8 (7.0-8.5)
24000	29 ± 5.5	18* ± 1.6	19* ± 4.3	7.0 (6.5 - 7.5)	6.5 (6.0-7.0)	6.8 (6.0-7.5)
Females:						
0	32 ± 7.7	26 ± 3.5	29 ± 6.7	6.8 (6.5 - 7.5)	6.9 (6.5-7.0)	6.6 (6.5-7.0)
1600	28 ± 4.8	27 ± 3.8	26 ± 3.6	7.1 (6.0 - 7.5)	6.8 (6.5-7.0)	7.3 (6.5-8.0)
8000	26 ± 5.9	24 ± 5.4	22 ± 4.4	7.1 (7.0 - 7.5)	6.5 (6.0-7.0)	7.1 (7.0-7.5)
24000	25 ± 2.6	14* ± 1.5	20* ± 2.1	6.6 (6.5 - 7.0)	6.5 (6.0-7.0)	6.6 (6.0-7.0)

* $p < 0.05$

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*9 January 2013*
Materials and Methods	*As described by Applicant*
Results and discussion	*As described by Applicant, except:* *X1 Section 4.2 The RMS considers that the treatment related reduced bodyweight gains for females at 1600 and 8000 ppm should be regarded as adverse.*
Conclusion	*X2 Section 5.3.1 & 5.3.2: The RMS concludes that:* *(1) For males a study NOAEL of 8000 ppm and LOAEL of 24000 ppm are identified, based on the bodyweight and food consumption reductions in the high dose group. For females a NOAEL is not identified becasue bodyweight reductions occurred at all dose levels and therefore a LOAELis identified for femaless.* *(2) The reduced serum ALT activity in both sexes and marginally reduced urine pH in males in the highest dose group were incidental findings either because the direction of change is of unlikely clinical significance (ALT) or the differences were marginal and without correlates among the other parameters investigated (urinary pH).*
Reliability	*As described by Applicant*
Acceptability	*Acceptable*
Remarks	*None*
	COMMENTS FROM ... *(specify)*
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section IIIA 6.4.2 Annex Point IIA, VI.6.4	**Subchronic dermal toxicity test**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure** [X]	**Technically not feasible** [ ] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	Not required, as dinotefuran has a low dermal toxicity as indicated in a 28-day dermal toxicity study in rats. See Section A6.3.2.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*10 January 2013*	
Evaluation of applicant's justification	*The justification is valid*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section IIIA 6.4.3 Annex Point IIA, VI.6.4	**Subchronic inhalation toxicity test**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Not required, dinotefuran is not a volatile substance and has a vapour pressure of < 1.7 x 10-6 Pa at 30°C (see Section A3.2). Inhalation exposure is not significant as indicated in an acute inhalation toxicity study in the rat (see Section A6.1.3) and in a 28-day repeated inhalation toxicity study in the rat (see Section A6.3.3)	X1
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*10 January 2013*	
Evaluation of applicant's justification	*X1 Based of the fact that dinotefuran is not a volatile substance and, additionally, human exposure to dinotefuran is expected to be relatively low, a subchronic inhalation study is considered unnecessary.*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.5-1 **Repeated dose toxicity**

Annex Point IIA6.5 **Oral**

Rat, 104-week

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 2000c, 104-week dietary combined chronic toxicity and carcinogenicity study with MTI-446 in rats, [redacted], unpublished report no. [redacted]6648-131, April 5, 2000.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD guideline no. 453 (1981), which is equivalent to 88/302/EEC EPA-FIFRA Subdivision F, §83-2 (1985) JMAFF 59 NohSan 4200 (1985)
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	2200210
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	93.0% + 7.6% water, purity of dried material 98.9%
3.1.2.3	Stability	Expiration date: May 2002
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Crl:CD®(SD)BR VAF/Plus®
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	About 7 weeks old, weighing 173 - 271 g for males and 143 - 204 g for females
3.2.6	Number of animals per group	Main study: 60 males and 60 females per group Additional groups: 10 animals/sex/group were similarly treated for at least 26, 52 and 78 weeks Further groups: 10 animals/sex treated at 0 and 20000ppm were treated for 26 weeks and then maintained untreated for 6 weeks before necropsy

Section A6.5-1 Annex Point IIA6.5		Repeated dose toxicity Oral Rat, 104-week
		See Table A6.5.1-1
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	104 weeks
3.3.2	Frequency of exposure	7 days per week
3.3.3	Postexposure period	None
3.3.4	Oral	
3.3.4.1	Type	In food
3.3.4.2	Concentration	Nominal in food: 0, 60, 200, 2000 and 20000 ppm in the diet Mean achieved dose levels: 0, 3, 10, 100 and 991mg/kg bw/day in males 0, 4, 13, 127 and 1332mg/kg bw/day in females Food consumption per day: *ad libitum*
3.3.4.3	Vehicle	No vehicle, added to basal diet
3.3.4.4	Concentration in vehicle	Not applicable
3.3.4.5	Total volume applied	Not applicable
3.3.4.6	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, weekly
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, weekly for 13 weeks and every 4 weeks thereafter
3.4.3	Food consumption	Yes, weekly for 13 weeks and every 4 weeks thereafter
3.4.4	Water consumption	Not examined
3.4.5	Ophthalmoscopic examination	Yes, pre-test and after 26, 32, 78 and 104 weeks on all animals
3.4.6	Haematology	Yes Number of animals: 10 animals/sex/group Time points: after 26, 52, 78 and 104 weeks, and after 26 weeks of treatment at 0 and 20000ppm followed by 6 weeks treatment-free. Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, clotting time, prothrombin time, thromboplastin time
3.4.7	Clinical Chemisty	Yes

Section A6.5-1 Annex Point IIA6.5		Repeated dose toxicity Oral Rat, 104-week	
		Number of animals: 10 animals/sex/group Time points: after 26, 52, 78 and 104 weeks, and after 26 weeks of treatment at 0 and 20000ppm followed by 6 weeks treatment-free. Parameters: sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, sorbitol dehydrogenase, methaemoglobin, lipids, hormone (specify hormones), acid/base balance, cholinesterase inhibition.	
3.4.8	Urinalysis	Yes Number of animals: 10 animals/sex/group Time points: after 26, 52, 78 and 104 weeks, and after 26 weeks of treatment at 0 and 20000ppm followed by 6 weeks treatment-free. Parameters: appearance, volume, osmolality, specific gravity, pH, protein, glucose, blood	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes Organs: selected organs were weighed from 10 animals/sex/group	
3.5.2	Gross and histopathology	Yes After 26, 52 and 78 weeks of treatment 10 animals/sex/group were killed. The additional 10 animals/sex/group treated at 0 and 20000ppm for 26 weeks were killed after a 6-week treatment-free period. All other survivors were killed after at least 104 weeks treatment. All decedents and scheduled kill animals were subjected to necropsy and *post mortem* examination of major organs and tissues. Samples of major organs and tissues were preserved from all animals. Samples of liver, kidney and adrenal were retained from 2 animals/sex/group at each scheduled sacrifice for possible electron microscopic examination. All preserved tissues from the animals treated at 0 or 20000ppm and from decedents in all groups were examined by light microscopy. Gross lesions, endocrine glands, tissue masses, kidneys, liver, lungs and reproductive organs of both sexes were also examined from all intermediate dose group animals.	
3.5.3	Other examinations	No	
3.5.4	Statistics	In life and organ weight data were statistically analysed by one-way ANOVA followed by Dunnett's multiple comparison t-test, where appropriate. The incidences of non-neoplastic pathological findings were analysed using the Fisher-Irwin exact test and Cochran-Armitage trend test. Survival data was analysed by life table techniques and Kaplan-Meier. Tumour data were analysed using interval-based prevalence or exact permutation tests. Fatal and palpable tumour data were analysed by Cox-Tarone binary regression and log-rank tests. Neoplastic lesions were selected for statistical analysis if the incidence in one or more treated groups differed from the controls by at least two occurrences.	
3.6	**Further remarks**	None	

Section A6.5-1 **Repeated dose toxicity**

Annex Point IIA6.5 **Oral**

Rat, 104-week

		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	No effects, including the incidences of palpable tissue masses.	
4.1.2	Mortality	Survival of both sexes to 104 weeks was unaffected by treatment at all dose levels. Control male survival in week 104 was 47% compared to 25 - 38% in the intermediate dose groups and 53% in the high dose dinotefuran group. Control female survival after 104 weeks was 33% compared to 30 - 43% in the dinotefuran treated groups.	
4.2	**Body weight gain**	The body weight gains of both sexes were reduced by treatment at 20000ppm from week 2 but the effect was more severe in the females with overall (week 1 - 104) body weight gains reduced by 5.5 and 44.1% in males and females, respectively. The effect in males is considered not to be adverse. Body weight gain was unaffected by treatment at lower dose levels. See Table A6.5.1-2	X1
4.3	**Food consumption and compound intake**	The mean weekly food consumption of animals treated at 20000ppm was reduced by up to 10.0% during the first 77 weeks of treatment, but water intake was unaffected by treatment at all dose levels. Food consumption was not affected by treatment at dose levels up to 2000ppm. Table A6.5.1-2	
4.4	**Ophtalmoscopic examination**	No effects	
4.5	**Blood analysis**		
4.5.1	Haematology	No effects	
4.5.2	Clinical chemistry	No effects	
4.5.3	Urinalysis	No effects	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	There were no treatment-related organ weight changes in either sex at any dose level after 26, 52, 78 and 104 weeks treatment other than in females treated at 20000ppm in which liver weight at week 79 was reduced as a consequence of growth retardation.	
4.6.2	Gross and histopathology	The mean number of uterine masses was higher than the controls in the groups treated at 2000 and 20000ppm. There were 3, 1, 5 and 8 masses in the groups treated at 60, 200, 2000 and 20000ppm, respectively, compared with 2 masses in the control group. The masses were commonly endometrial stromal polyps, but the incidence of the lesion and other uterine neoplasms was not significantly different from the controls ($p > 0.05$). There were no other notable differences in the incidence of macroscopic lesions between the treated and control groups. There were no treatment-related effects on the nature and incidence of adverse non-neoplastic histopathlogical findings at any dose level. However, males treated at 20000ppm showed higher incidences of the renal changes pelvic mineralisation, lymphohistiocytic infiltrate, tubular epithelial basophilia and thickening of the basement membrane. None of the renal changes is considered to be an adverse effect since they are either common findings in rats or can be	

correlated with the lower incidence of chronic progressive nephropathy in males at 20000ppm. Similarly, increased incidences of thymic lymphocyte depletion and prostatic chronic active inflammation in males at 20000ppm are considered not to be adverse effects since they occur commonly in the rat.

There were no treatment-related effects at any dose level on the nature and incidence of tumors. However, pooled data from all animals showed differences between the control and 20000ppm group in the incidence of four tumor types. The incidence of thyroid C-cell adenomas was significantly higher ($p < 0.05$) than the controls in males treated at 20000ppm, but was within the historical control range of 1.7 - 24%. Since this neoplasm is a common finding in the rat and because the total number of thyroid neoplasms (adenomas + carcinomas) was not significantly higher than the controls ($p > 0.05$), the statistical finding is considered not biologically relevant. Benign testicular interstitial cell tumors occurred at an incidence of 5.6% in animals treated at 20000ppm compared to a control incidence of 2.0%. Since the difference from the controls was not statistically significant ($p > 0.05$) and the incidence was within the historical control range of 1.3 - 6.7%, the higher incidence is considered not to be treatment-related. Benign endometrial stromal polyps occurred at a higher incidence in the uterus of animals treated at 20000ppm than in the controls, but the incidence in the uterus alone was not statistically significant ($p > 0.05$). The combined incidence of this lesion in uterus, cervix and vagina at 20000ppm (7.0%) was significantly higher than the control incidence of 2.0% ($p < 0.05$), but remained within the historical control range of 1.0 - 14%. Therefore, the increased incidence of this common neoplasm is considered unrelated to the administration of dinotefuran. The incidence of mammary gland carcinomas was higher in the group treated at 20000ppm (22%) than in the controls (13%), but remained within the historical control range of 10.0 - 26.7%. The difference was not statistically significant ($p > 0.05$) and is considered unrelated to the administration of dinotefuran. With the exception of significantly lower incidences of pituitary adenomas in both sexes ($p < 0.05$) and adrenal pheochromocytomas in males ($p < 0.01$), the incidences of all other tumors in the group treated at 20000ppm were not significantly different from the controls. See Table A6.5.1-3 and Table A6.5.1-4

4.7 Other

None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

OECD guideline no. 453 (1981), which is equivalent to 88/302/EEC, EPA-FIFRA Subdivision F, §83-2 (1985), JMAFF 59 NohSan 4200 (1985)

No relevant deviations from test guidelines.

Method:

Five groups of 60 male and 60 female SD rats were treated orally for at least 104 weeks with dinotefuran, by admixture in the diet at constant nominal concentrations of 0, 60, 200, 2000 and 20000ppm. Additional groups of 10 animals/sex/group were similarly treated for at least 26, 52 and 78 weeks, and further groups of 10 animals/sex treated at 0 and 20000ppm were treated for 26 weeks and then

Section A6.5-1	Repeated dose toxicity	
Annex Point IIA6.5	Oral Rat, 104-week	
	maintained untreated for 6 weeks before necropsy. Mean achieved dose levels were 0, 3, 10, 100 and 991mg/kg bw/day (males) and 0, 4, 13, 127 and 1332mg/kg bw/day (females).	
5.2 Results and discussion	There were no treatment-related deaths, adverse clinical signs or ophthalmological findings at any dose level. The body weight gains of both sexes were reduced by treatment at 20000ppm from week 2, but since male body weights remained within 10% of control values, the effect in males was considered not to be adverse. The effect in females was more marked. The mean weekly food consumption of animals treated at 20000ppm was reduced during the first 77 weeks of treatment, but water intake was unaffected by treatment at all dose levels. Body weight gain and food consumption were not affected by treatment at dose levels up to 2000ppm.	X2
	There were no treatment-related effects on hematology, serum clinical chemistry and urinalysis parameters at any dose level or sampling interval. There were no treatment-related effects on the incidence of macroscopic findings at any necropsy interval. However, the mean number of uterine masses was higher than the controls in the groups treated at 2000 and 20000ppm. There were no primary treatment-related organ weight changes at any sacrifice interval. There were no treatment-related effects on the nature and incidence of adverse non-neoplastic histopathlogical findings at any dose level.	
	There were no treatment-related effects at any dose level on the nature and incidence of tumors. Although pooled data from all animals treated at 20000 ppm showed slightly higher incidences of thyroid C-cell adenomas, benign testicular interstitial cell tumors, benign endometrial stromal polyps and mammary gland carcinomas, all were either not statistically significant and/or remained within the historical control ranges and were therefore considered unrelated to treatment.	
5.3 Conclusion		
5.3.1 LO(A)EL	Not determined	X3
5.3.2 NO(A)EL	For all effects was established as 20000 and 2000ppm, equivalent to dose levels of 991mg/kg bw/day (males) and 127mg/kg bw/day (females), based on growth retardation in females	
5.3.3 Reliability	1	
5.3.4 Deficiencies	No	

Table A6.5.1-1: Animal assignment and treatment

Group number	Dose level of dinotefuran (ppm)	Number of animals killed after:										Total number	
		Recovery*		26 weeks		52 weeks		78 weeks		104 weeks			
		M	F	M	F	M	F	M	F	M	F	M	F
1	0	10	10	10	10	10	10	10	10	60	60	100	100
2	60	0	0	10	10	10	10	10	10	60	60	90	90
3	200	0	0	10	10	10	10	10	10	60	60	90	90
4	2000	0	0	10	10	10	10	10	10	60	60	90	90
5	20000	10	10	10	10	10	10	10	10	60	60	100	100

M; male; F; female
*Aanimals were treated for 26 weeks and then untrerated for 6 weeks.

Table A6.5.1-2: Treatment related effects on body weight, body weight gain and food consumption

Week of study	Group mean body weight (g) of:									
	Males treated at (ppm):					Females treated at (ppm):				
	0	60	200	2000	20000	0	60	200	2000	20000
1	228	232	229	231	228	178	177	174	175	176
26	685	694	692	702	638*	344	352	362*	350	314*
50	794	802	815	822	722*	411	418	439*	418	347*
78	836	841	865	867	777*	482	490	529*	497	377*
105	758	792	808	800	729	553	608	565	545	417*
Weight gain (wks 1 - 104)	530	560	579	569	501	375	431	391	370	241*
Interval (weeks)	Group mean food consumption (g/week) of:									
	Males treated at (ppm):					Females treated at (ppm):				
	0	60	200	2000	20000	0	60	200	2000	20000
1 - 25	201.3	197.7	199.1	198.1	182.4[a]	141.9	210.8	139.6	138.0	128.9[a]
29 - 49	202.8	201.3	203.5	209.5	186.7[a]	152.7	151.5	156.2	153.7	143.3[a]
53 - 77	205.9	202.7	207.1	209.4	193.7[a]	164.4	166.3	165.6	168.3	148.0[a]
81 - 101	192.7	199.8	196.0	195.2	186.0	162.7	169.2	160.3	167.5	150.5

* $p < 0.05$;
[a] $p < 0.05$ for most weekly values

Table A6.5.1-3: Treatment related, non-adverse, non-neoplastic histopathological findings

Finding	Incidence in males treated at (ppm):				
	0	60	200	2000	20000
No. examined (kidneys)	100	90	89	89	100
- lymphohistiocytic infiltrate	42	51*	39	49*	65**
- tubular epithelial basophilia	42	47	37	51*	57*
- thickening of basement membrane	30	35	32	44**	44*
- pelvic mineralisation	5	5	4	7	27**
- chronic progressive nephropathy	35	26	36	24	14**
No. examined (thymus)	96	39	38	40	99
- lymphocytic depletion	5	3	3	3	13*
No. examined (prostate)	100	90	89	89	100
- chronic active inflammation	23	46**	53**	51**	36*
	Incidence in females treated at (ppm):				
	0	60	200	2000	20000
No. examined (kidneys)	100	90	89	90	100
- lymphohistiocytic infiltrate	43	43	35	41	40
- tubular epithelial basophilia	48	42	34	40	26**
- thickening of basement membrane	23	24	19	19	15
- pelvic mineralisation	42	42	42	44	47
- chronic progressive nephropathy	5	8	12*	8	0*
No. examined (thymus)	100	42	44	41	98
- lymphocytic depletion	9	4	2	2	11

* $p < 0.05$;
** $p < 0.01$

Table A6.5.1-4: Incidences of neoplastic findings at 20000 ppm higher than controls

Finding	Incidence in males treated at (ppm):				
	0	60	200	2000	20000
No. examined (thyroid)	99	89	90	88	100
- C-cell adenoma (b)	8	12	10	12	17*
- C-cell carcinoma (m)	1	0	0	0	0
- total (adenoma + carcinoma)	9	12	10	12	17
No. examined (testes)	100	89	90	89	99
- benign interstitial cell tumor	2	1	3	1	5
	Incidence in females treated at (ppm):				
	0	60	200	2000	20000
No. examined (thyroid)	100	90	90	89	100
- C-cell adenoma (b)	12	11	12	5	13
- C-cell carcinoma (m)	0	0	1	1	1
- total (adenoma + carcinoma)	12	11	13	6	14
No. examined (uterus)	100	90	90	90	100
- benign endometrial stromal polyps	1	0	3	3	6
No. examined (cervix)	100	40	43	40	100
- benign endometrial stromal polyps	1	1	1	1	1
No. examined (vagina)	100	89	90	90	100
- benign endometrial stromal polyps	0	0	1	1	0
Total (uterus, vagina and cervix)	2	1	5	5	7*
No. examined (mammary gland)	100	90	89	89	100
- adenoma (b)	11	11	7	9	9
- carcinoma (m)	13	15	17	18	22
- total (adenoma + carcinoma)	24	26	24	27	31

* $p < 0.05$;
b benign;
m malignant

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*15 January 2013*
Materials and Methods	*As described by Applicant*
Results and discussion	*As described by Applicant, except:* *X1, section 4.2: the bodyweight gain for females at 20000 ppm for weeks 1-104 were reduced by 36%, not 44.1% as stated by Applicant.* *X2, section 5.2: the RMS considers that the treatment related reduction in bodyweight gain in males at 20000 ppm should be considered to be an adverse effect; the Applicant regards this change as non-adverse.*
Conclusion	*As described by Applicant, except:* *X3, section 5.3.1 & 5.3.2: based on the reduced bodyweight gain and reduced food consumption at the highest dose level, the LOAEL is 20000 ppm and the NOAEL is 2000 ppm.*
Reliability	*As described by Applicant*
Acceptability	*Acceptable*
Remarks	*See A6.7-1 for RMS evaluation of neoplastic aspects of this study.*
	COMMENTS FROM ... *(specify)*
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.5-2 Repeated dose toxicity

Annex Point IIA6.5 **Dog**

Oral, 52-week

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1999c, 52-week dietary chronic toxicity study with MTI-446 in dogs, [redacted], unpublished report no. 6648-129, December 10, 1999. [redacted], 2005, Historical control data for 52-week dog studies, [redacted], unpublished [redacted] No. 45639720, May 17, 2005.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD guideline no. 452 (1981), which is equivalent to 88/302/EEC EPA-FIFRA 83-1 (1982), JMAFF 59 NohSan 4200 (1985)
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	2200210
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	93.0% + 7.6% water, purity of dried material 98.9%
3.1.2.3	Stability	Expiration date: May 2002
3.2	**Test Animals**	
3.2.1	Species	Dog
3.2.2	Strain	Beagle
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	5-5.5 months old, weighing 8.0 – 9.8 kg for males and 7.1 – 8.9 kg for females
3.2.6	Number of animals per group	4 males and 4 females per group See Table A6.5.2-1
3.2.7	Control animals	Yes