Section A6.5-2		Repeated dose toxicity
Annex Point IIA6.5		Dog Oral, 52-week
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	52 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, 640, 3200 and 16000 ppm in the diet Mean achieved dose levels: 0, 20, 111 and 559mg/kg bw/day in males 0, 22, 108 and 512mg/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 for 16 weeks and at 4-week intervals thereafter
3.4.3	Food consumption	Yes, weekly for 16 weeks and at 4-week intervals thereafter
3.4.4	Water consumption	Yes, weekly for 16 weeks and at 4-week intervals thereafter
3.4.5	Ophthalmoscopic examination	Yes, pretest and during week 52 on all animals
3.4.6	Haematology	Yes Number of animals: all animals Time points: pretest and in weeks 14, 27 and 53 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: pretest and in weeks 14, 27 and 53 Parameters: sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, alanine

Section A6.5-2 **Repeated dose toxicity**

Annex Point IIA6.5 **Dog**

Oral, 52-week

		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: pretest and in weeks 14, 27 and 53 Parameters: appearance, volume, osmolality, specific gravity, pH, protein, glucose, blood
3.5	**Sacrifice and 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 All animals Samples of organs and tissues were preserved and prepared for histological evaluation and then examined microscopically from all animals.
3.5.3	Other examinations	None
3.5.4	Statistics	Variance homogeneity was analysed by Levene's test. One-way ANOVA was performed and, if significant, Dunnett's t-test was performed to compare treated groups with the control group.
3.6	**Further remarks**	The animals were assigned to treatment groups based on the results of a 13-week dietary study (see Section A6.4.1-3).
		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**	There was a treatment-related decrease in the overall body weight gains of both sexes at 16000ppm and of females at 3200ppm. Body weight gains were 30.0 - 37.1% lower than the controls and statistically significant ($p < 0.05$) in the female groups. The body weight gains of males at 3200ppm and both sexes at 640ppm were unaffected by treatment. See Table A6.5.2-2
4.3	**Food consumption and compound intake**	Females treated at 3200 and 16000ppm showed 8.0 and 12.3% decreases, respectively, in food consumption, but consumption was unaffected by treatment in the other groups. See Table A6.5.2-2
4.4	**Ophtalmoscopic examination**	No effects
4.5	**Blood analysis**	
4.5.1	Haematology	No effects, slightly higher serum albumin and potassium ion concentrations at week 53 in females.

Section A6.5-2		Repeated dose toxicity
Annex Point IIA6.5		Dog Oral, 52-week
4.5.2	Clinical chemistry	No effects
4.5.3	Urinalysis	No effects, statistically significant differences between the 16000ppm group and the controls were higher urine pH at week 27.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No effects, An apparent, treatment-related effect on the group mean thymus weight of all male treated groups was considered not to be an effect of treatment on comparison with laboratory historical control data (HCD) which showed only one low dose and one high dose animal with values outside the HCD range.
4.6.2	Gross and histopathology	No effects
4.7	**Other**	Water consumption was unaffected by treatment at all dose levels
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Guidelines: OECD guideline no. 452 (1981), which is equivalent to 88/302/EEC, EPA-FIFRA 83-1 (1982), JMAFF 59 NohSan 4200 (1985) No relevant deviations from test guidelines. Methods: Groups of 4 male and 4 female beagle dogs were treated orally, in diet, for 52 weeks with dinotefuran at concentrations of 0, 640, 3200 and 16000ppm. Mean achieved dose levels were 0, 20, 111 and 559mg/kg bw/day (males) and 0, 22, 108 and 512mg/kg bw/day (females).
5.2	**Results and discussion**	There were no deaths, treatment-related clinical signs or ocular defects at any dose level. There was a treatment-related decrease in the overall body weight gains of both sexes at 16000ppm and of females at 3200ppm. The body weight gains of males at 3200ppm and both sexes at 640ppm were unaffected by treatment. Females treated at 3200 and 16000ppm showed minor decreases in food consumption, but consumption was unaffected by treatment in the other groups. Water consumption was unaffected by treatment at all dose levels. There were no treatment-related effects at any dose level on the hematological, serum clinical chemistry and urinalysis profiles. There were no treatment-related effects at any dose level on the incidence of macroscopic findings at necropsy or on organ weights and ratios. An apparent, treatment-related effect on the group mean thymus weight of all male dogs treated for 52 weeks was considered not to be an effect of treatment on comparison with laboratory historical control data which showed only one low dose and one high dose animal with values outside the HCD range. There were no treatment-related histopathological alterations at any dose level. No target organs were identified in either sex at the highest dose level employed. The no-observed-effect-level (NOEL) for all effects was established as 3200ppm diet (males) and 640ppm (females), equivalent to a dose level of 111mg/kg bw/day (males) and 22mg/kg bw/day (females), based on the occurrence of growth retardation in males at 16000ppm and growth retardation and reduced food consumption in

Section A6.5-2 **Repeated dose toxicity**

Annex Point IIA6.5 **Dog**

Oral, 52-week

		females at 3200 and 16000ppm.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	Not determined	
5.3.2	NO(A)EL	16000ppm in both sexes, equivalent to dose levels of 559mg/kg bw/day (males) and 512mg/kg bw/day (females), based on the absence of correlative clinical and pathological alterations associated with the observed growth retardation.	X1
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Table A6.5.2-1: Animal assignment and treatment

Group number	Dose level of dinotefuran (ppm)	Number of animals	
		Male	Female
1	0	4	4
2	640	4	4
3	3200	4	4
4	16000	4	4

Table A6.5.2-2: Treatment related effects on body weight gain and food consumption

Treatment (ppm)	Group mean body weight (kg) in week:					Overall weight gain (kg)	Mean food intake (g/day)
	1	14	28	40	52		
Males:							
0	8.7	11.4	11.6	11.7	11.7	3.0	349
640	8.7	11.4	11.7	11.9	11.6	2.9	330
3200	8.9	11.0	11.5	12.1	11.9	3.0	368
16000	8.5	11.0	10.7	11.1	10.6	2.1	363
Females:							
0	8.0	10.8	11.3	12.0	11.5	3.5	350
640	7.9	10.2	10.6	11.1	11.4	3.5	348
3200	7.9	9.9*	10.3	10.4*	10.1*	2.2*	322
16000	7.9	9.7*	10.2	10.2*	10.2*	2.3*	307

* $p < 0.05$

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10 January 2013
Materials and Methods	As described by Applicant
Results and discussion	As described by Applicant
Conclusion	X1Section 5.3.1.and 5.3.2 The RMS concludes that NOAELs of 3200 ppm for males and 640 ppm for females are identified, based on the bodyweight changes in both sexes and food consumption reductions in females. Thus, LOAELs are 16000 ppm for males and 3200 ppm for females.
Reliability	As described by Applicant
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ... *(specify)*
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.1-1 Genotoxicity *in vitro*
Annex Point IIA6.6.1 Gene mutation in bacteria

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1996, MTI-446: Microbial reverse mutation assay, [redacted], unpublished report no. [redacted] 3133, October 3, 1996.
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 proposal for updated guideline 471-472 (1994), which is equivalent to 92/69/EEC (method B.14) US-EPA OPPTS 870.5100/870.5265 JMAFF 59 NohSan no. 4200 (1985) JMHW, Part 1 (1990); Japan Ministry of Labor, Notification nos. 76 and 77(1988)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes, in addition to the required 4 *S. typhimurium* strains, one strain of *E. coli* was also included since this is a requirement for Japanese authorities. The deviation does not affect the validity of the study.

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 (Not specified in report)
3.1.2.2	Purity	96.5% + 2% water, purity of dried material 99.1% (Not specified in report)
3.1.2.3	Stability	Expiration date: May 14, 2001 (Not specified in report)
3.2	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	*S. typhimurium*: TA 1535, TA 1537, TA 98, TA 100 *E. coli*: WP2 uvr A
3.2.2	Deficiencies / Proficiencies	*S. typhimurium*: histidine-auxotrophic strains *E. coli*: tryptophan-auxotrophic strain
3.2.3	Metabolic activation system	S9 mix S9 liver fraction was obtained from [redacted]. (source and inducing agent not specified).

Section A6.6.1-1 **Genotoxicity *in vitro***

Annex Point IIA6.6.1 **Gene mutation in bacteria**

3.2.4	Positive control	Without S9: 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2), 9-Aminoacridine and Sodium azide With S9: 2-Aminoanthracene
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Pre-incubation: 0 (solvent control), 313, 625, 1250, 2500 and 5000µg/plate Main assay: 0 (solvent control), 1.2, 4.9, 20, 78, 313, 1250 and 5000 µg/plate dinotefuran and the relevant positive controls both with and without metabolic activation. See Table A6.6.1.1-1
3.3.2	Way of application	dissolved in medium
3.3.3	Pre-incubation time	20 minutes at 37°C
3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Evaluated for colonies by an automatic counter and for precipitation and growth inhibition of the background lawn by microscopy.

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	Dose range-finding study: No, normal background growth occurred in all strains without metabolic activation. None of the strains showed an appreciable increase in the reversion frequency at any of the dose levels tested. Main study: No appreciable increase in the reversion frequencies occurred in any strain tested in the dose range 313 - 5000µg/plate. See Table A6.6.1-2 and Table A6.6.1-3.
4.1.2	with metabolic activation	Dose range-finding study: No, normal background growth occurred in all strains with metabolic activation. None of the strains showed an appreciable increase in the reversion frequency at any of the dose levels tested. Main study: No appreciable increase in the reversion frequencies occurred in any strain tested in the dose range 313 - 5000µg/plate. See Table A6.6.1.1-2 and Table A6.6.1.1-3.

Section A6.6.1-1 Genotoxicity *in vitro*
Annex Point IIA6.6.1 Gene mutation in bacteria

4.2	**Cytotoxicity**	Dose range-finding study: No appreciable cytotoxicity was observed at up to 5000µg/plate, the highest concentration evaluated. Main study: Dinotefuran did not influence the growth of any strain tested at dose levels of up to 5000µg/plate.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Guidelines: OECD proposal for updated guideline 471-472 (1994), which is equivalent to 92/69/EEC (method B.14), US-EPA OPPTS 870.5100/870.5265, JMAFF 59 NohSan no. 4200 (1985), JMHW, Part 1 (1990); Japan Ministry of Labor, Notification nos. 76 and 77(1988) No relevant deviations from test guidelines. Method: Dinotefuran in dimethylsulfoxide (DMSO) solvent was tested in 4 histidine-auxotrophic strains (TA98, TA100, TA1535 and TA1537) of *Salmonella typhimurium* and on the tryptophan-auxotrophic strain WP2uvrA of *Escherichia coli*, by pre-incubation, at concentrations of 0 (solvent control), 313, 625, 1250, 2500 and 5000µg/plate. Dose levels for the main assay were determined from the results of a dose range-finding study in all 5 strains in which a solvent control and dose levels of 1.2, 4.9, 20, 78, 313, 1250 and 5000 µg/plate dinotefuran and the relevant positive controls (3 plates/dose), both with and without metabolic activation were pre-incubated for 20 minutes. Following incubation for 48 hours, signs of precipitation or growth inhibition were recorded and revertant colonies were counted.
5.2	**Results and discussion**	No appreciable cytotoxicity was observed in all strains, with and without metabolic activation at up to 5000µg/plate, the highest concentration evaluated. None of the strains showed an appreciable increase in the reversion frequency at any of the dose levels tested. In the main assay, dinotefuran did not influence the growth of any strain tested at dose levels of up to 5000µg/plate. No appreciable increase in the reversion frequencies occurred in any strain tested in the dose range 313 - 5000µg/plate. In contrast, the positive control substances produced marked increases in the number of revertant colonies in all strains tested. Under the conditions of this study, dinotefuran and/or metabolites does not induce gene mutations in the strains of *S. typhimurium* and *E. coli* used in the study at doses up to 5000µg/plate.
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

A6.6.1.1-1: Strain specific positive control substances and dose levels employed

Strain	Without S9		With S9	
	Positive control	Dose (µg/plate)	Positive control	Dose (µg/plate)
S. typhimurium TA100	AF-2	0.01	2-AA	1.0
S. typhimurium TA98	AF-2	0.01	2-AA	2.0
S. typhimurium TA1535	NaN3	0.5	2-AA	2.0
S. typhimurium TA1537	9-AA	80.0	2-AA	1.5
E. coli WP2uvrA	AF-2	0.01	2-AA	10.0

AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide; NaN3: sodium azide; 9-AA: 9-Aminoacridine;
2-AA: -Aminoanthracene

Table A6.6.1.1-2: Summary of the incidences of revertant colonies for the dose range-finding assay

Treatment	Dose (µg/plate)	Mean (n = 3) ± SD revertant colonies/plate for strain:				
		TA100	TA1535	WP2uvrA	TA98	TA1537
		Without S9				
DMSO	0	119	7	14	11	3
Dinotefuran	1.2	138	4	17	9	4
	4.9	128	5	13	13	3
	20	129	9	17	10	4
	78	129	6	16	10	2
	313	134	7	13	8	4
	1250	122	6	15	5	4
	5000	124	6	17	9	3
AF-2	0.01	829	-	-	-	-
NaN3	0.5	-	180	-	-	-
AF-2	0.01	-	-	104	-	-
AF-2	0.1	-	-	-	475	-
9-AA	80	-	-	-	-	376
		With S9				
DMSO	0	121	8	15	17	9
Dinotefuran	1.2	106	10	18	22	9
	4.9	119	10	15	19	8
	20	121	10	14	17	8
	78	120	10	12	19	5
	313	116	7	18	13	7
	1250	118	10	13	14	6
	5000	112	10	11	14	8
2-AA	1	1006	-	-	-	-
	2	-	208	-	-	-
	10	-	-	845	-	-
	0.5	-	-	-	286	-
	2	-	-	-	-	71

Table A6.6.1.1-3: Summary of the incidence of revertant colonies for the main assay

Treatment	Dose (µg/plate)	Mean (n = 3) ± SD revertant colonies/plate for strain:				
		TA100	TA1535	WP2uvrA	TA98	TA1537
		Without S9				
DMSO	0	101	5	25	12	3
Dinotefuran	313	111	7	26	12	3
	625	104	7	18	7	3
	1250	121	9	22	11	2
	2500	103	6	19	9	5
	5000	103	9	18	16	5
AF-2	0.01	727	-	-	-	-
NaN3	0.5	-	244	-	-	-
AF-2	0.01	-	-	106	-	-
AF-2	0.1	-	-	-	460	-
9-AA	80	-	-	-	-	578
		With S9				
DMSO	0	104	9	24	18	12
Dinotefuran	313	102	8	24	21	8
	625	107	10	25	16	8
	1250	111	9	27	26	10
	2500	112	7	28	25	13
	5000	106	7	25	19	13
2-AA	1	665	-	-	-	-
	2	-	210	-	-	-
	10	-	-	799	-	-
	0.5	-	-	-	209	-
	2	-	-	-	-	81

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*10 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 ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.1-2 **Genotoxicity *in vitro***
Annex Point IIA6.6.1 **Gene mutation in bacteria**
DNA repair assay

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1999, A DNA repair assay of *Bacillus subtilis* on MTI-446, [redacted], unpublished report no. 4731, October 2, 1996.
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**	No applicable EU guideline JMAFF 59 NohSan no. 4200 (1985)
2.2	**GLP**	Yes
2.3	**Deviations**	No applicable EU guideline.

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	Solid
3.1.2.2	Purity	96.5% + 2% water, purity of dried material 99.1%
3.1.2.3	Stability	Expiration date: May 14, 2001 (Not specified in report)
3.2	**Study Type**	Unscheduled DNA synthesis in mammalian cells in vitro
3.2.1	Organism/cell type	*Bacillus subtilis:* M45 Rec- and H17 Rec+
3.2.2	Deficiencies / Proficiencies	Not applicable
3.2.3	Metabolic activation system	S9 mix
3.2.4	Positive and negative control	

Control	± S9	Substance	Dose (µg/disc)
Negative	- S9	Kanamycin sulfate (KM)	10
Negative	+S9	Streptomycin sulfate (SM)	100
Positive	- S9	Mitomycin C (MMC)	0.01
Positive	+S9	Trp-P-1	3.0

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	0, 62.5, 250, 1000, 4000 and 16000µg/disc dinotefuran with and without metabolic activation.

Section A6.6.1-2 Genotoxicity *in vitro*
Annex Point IIA6.6.1 Gene mutation in bacteria
DNA repair assay

3.3.2	Way of application	An 8mm paper disc (2 discs/dose) was treated with 20 µL solvent or test substance solution and placed on the spore-innoculated agar without metabolic activation. With metabolic activation, an 8mm paper disc (2 discs/dose level) was treated with 20 µL coenzyme solution and 20 µL solvent or test substance solution and placed on the spore-innoculated agar containing S9 mix.
3.3.3	Pre-incubation time	24 hours
3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Evaluation criteria	Results are judged positive when the difference in diameter of growth inhibition zones between strains H17 and M45 is more than 5mm, and negative if less than 2.5mm. Where differences in inhibition zone diameter are between 2.5 and 5.0mm the results are evaluated by consideration of dose-response and reproducibility.

4 RESULTS AND DISCUSSION

4.1	**Preliminary study**	
4.1.1	Without and without metabolic activation	No growth inhibition occurred at any dose level in either strain with or without metabolic activation (Table A6.6.1.2-1). Based on these results, dinotefuran in DMSO solvent was tested in H17 and M45 (approx. 2 x 107cells/mL) at concentrations of 0 (DMSO only), 1000, 2000, 4000, 8000 and 16000µg/disc with and without S9 metabolic activation.
4.2	**Main study**	
4.2.1	With and without metabolic activation	DMSO solvent alone and all doses of DINOTEFURAN employed, up to and including the highest dose, 16000µg/disc, produced no growth inhibition of either strain of B. subtilis either with or without S9 metabolic activation (Table A6.6.1.2-2). The negative control substances, KM without S9 and SM with S9 produced differences in the inhibition zone diameters of 1.7 and 1.1mm, respectively, whereas the positive control substances, MMC without S9 and Trp-P-1 with S9, produced differences of 7.2 and 6.0mm, respectively.

Section A6.6.1-2 **Genotoxicity *in vitro***
Annex Point IIA6.6.1 **Gene mutation in bacteria**
DNA repair assay

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

JMAFF 59 NohSan no. 4200 (1985)

Deviations: No relevant EU guideline.

Method:

A DNA repair assay (rec-assay) was performed with dinotefuran using *Bacillus subtilis* strains M45 Rec- and H17 Rec+. A series of 5 dose levels with a dose increment of 4, up to the limits of solubility, were tested both with and without metabolic activation. The dose levels selected were 0, 62.5, 250, 1000, 4000 and 16000µg/disc, with and without S9, on the basis of results from a range-finding experiment.
Solvent (DMSO), negative (kanamycin or streptomycin) and positive controls (mitomycin C or Trp-P-1) were tested concurrently.

5.2 Results and discussion

In the main assay, DMSO solvent alone and all doses of dinotefuran employed, up to and including the highest dose, 16000µg/disc, produced no growth inhibition of either strain of *B. subtilis* with or without S9 metabolic activation. The negative control substances without and with S9 produced differences in the inhibition zone diameters of 1.7 and 1.1mm, respectively, whereas the positive control substances without and with S9 produced differences of 7.2 and 6.0mm, respectively.

It was concluded that under the conditions of the study, dinotefuran and/or metabolites does not exhibit DNA-damaging activity in *B. subtilis* under the conditions of the study at doses up to 16000µg/disc.

5.3 Conclusion

5.3.1 Reliability: 1

5.3.2 Deficiencies: No applicable EU guideline

Table A6.6.1.2-1: Summary of growth inhibition for the dose range-finding study

Compound	Dose level	± S9	Growth inhibition zone (mm):		
	(µg/disc)		M45 (Rec-)	H17 (Rec+)	Difference (M45 – H17)
DMSO	0	-	0	0	0
Dinotefuran	1000	-	0	0	0
	2000	-	0	0	0
	4000	-	0	0	0
	8000	-	0	0	0
	16000	-	0	0	0
KM	10	-	10.0	8.4	1.6
MMC	0.01	-	6.9	0	6.9
DMSO	0	+	0	0	0
Dinotefuran	1000	+	0	0	0
	2000	+	0	0	0
	4000	+	0	0	0
	8000	+	0	0	0
	16000	+	0	0	0
SM	100	+	3.3	2.7	0.6
Trp-P-1	3	+	5.6	0	5.6

Table A6.6.1.2-2: Summary of growth inhibition for the main assay

Compound	Dose level	± S9	Growth inhibition zone (mm):		
	(µg/disc)		M45 (Rec-)	H17 (Rec+)	Difference (M45 – H17)
DMSO	0	-	0	0	0
Dinotefuran	1000	-	0	0	0
	2000	-	0	0	0
	4000	-	0	0	0
	8000	-	0	0	0
	16000	-	0	0	0
KM	10	-	10.5	8.8	1.7
MMC	0.01	-	7.2	0	7.2
DMSO	0	+	0	0	0
Dinotefuran	1000	+	0	0	0
	2000	+	0	0	0
	4000	+	0	0	0
	8000	+	0	0	0
	16000	+	0	0	0
SM	100	+	4.0	2.9	1.1
Trp-P-1	3	+	6.0	0	6.0

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*10 January 2013*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	*This is a non-standard test not required under 98/8/EC. The study has not been evaluated by the RMS*
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.2 Genotoxicity in vitro
Annex Point IIA6.6.2 Cytogenicity in mammalian cells
Mycoplasma-free Chinese hamster lung

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1996, MTI-446: In vitro mammalian cytogenetics test, [redacted], unpublished report no. [redacted]0076, October 15, 1996.
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. 473 (1983) 92/69/EEC (method B.10) US-EPA OPPTS 870.5375 JMAFF 59 NohSan no. 4200 (1985) JMHW, Part 1 (1990); Japan Ministry of Labor, Appendix 1, Notification nos. 143 (1987)
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 (Not specified in report)
3.1.2.2	Purity	96.5% + 2% water, purity of dried material 99.1% (Not specified in report)
3.1.2.3	Stability	Expiration date: May 14, 2001 (Not specified in report)
3.2	**Study Type**	In Vitro mammalian chromosome aberration test
3.2.1	Organism/cell type	Mammalian cell lines: Chinese hamster lung (CHL/IU)
3.2.2	Deficiencies / Proficiencies	Mycoplasma-free
3.2.3	Metabolic activation system	S9 mix Derived from male Sprague-Dawley rat liver pre-treated with phenobarbital and 5,6-benzoflavone.
3.2.4	Positive control	Mitomycin C (without S9) Cyclophosphamide (with S9)

Section A6.6.2 **Genotoxicity in vitro**

Annex Point IIA6.6.2 **Cytogenicity in mammalian cells**

Mycoplasma-free Chinese hamster lung

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	2000 µg/mL
3.3.2	Way of application	Dinotefuran was dissolved in saline. For preparation of the original solution and serial dilution of dinotefuran, the solution in 11-fold of each final concentration was prepared. In the cell growth inhibition test, 101.6 mg of dinotefuran dissolved in 4.6 mL of saline (final concentration is 2000 mg/mL which was equal to 10 mM) both with and without S9. In the main study, 146.3 mg of of dinotefuran dissolved in 6.65 mL of saline without S9 and 86.8 mg of dinotefuran dissolved in 3.9 mL of saline with S9 (final concentration is 2000 mg/mL which was equal to 10 mM). Mitomycin C (MMC - 0.03µg/mL) was the positive control material for 24 and 48 hour exposures without activation and cyclophosphamide (CP - 12µg/mL) for 6 hour exposures with and without activation.
3.3.3	Pre-incubation time	None
3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Two hours prior to harvesting, the cultures were treated with 0.2µg/mL colcemide to arrest cells in metaphase. The cell suspensions were trypsinised, centrifuged and re-suspended in KCl for 15 minutes to permit swelling. The cells were fixed, spread on to glass slides, air-dried and stained with Giemsa. A cytotoxicity test was performed on one slide/group by trypan blue dye exclusion using automated cell counting and calculation of the percentage viable cells relative to the solvent control. Whenever possible, two hundred well spread metaphase figures from two cultures (100 metaphases per replicate culture) in each group, including the positive controls, were scored. The slides were examined blind for specific and non-specific structural chromatid and chromosome aberrations and numerical aberrations. Cells with more than one aberration were scored as a single aberrant cell and polyploidy was defined as more than tetraploidy and including endoreduplication.

Section A6.6.2 **Genotoxicity in vitro**
Annex Point IIA6.6.2 **Cytogenicity in mammalian cells**
Mycoplasma-free Chinese hamster lung

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No Structural or numerical aberrations were not induced after 6 hours exposure to dinotefuran either in the absence of metabolic activation. Cell survival was unaffected at all dose levels after 6 hours exposure. MMC in the absence of metabolic activation produced marked increases in the incidences of structural aberrations, notably chromatid breaks and exchanges. See Table A6.6.2-1 and Table A6.6.2-2.
4.1.2	with metabolic activation	No Structural or numerical aberrations were not induced after 6 hours exposure to dinotefuran either in the presence of metabolic activation. Cell survival was unaffected at all dose levels after 6 hours exposure. Cyclophosphamide in the presence of metabolic activation produced marked increases in the incidences of structural aberrations, notably chromatid breaks and exchanges. See Table A6.6.2-1 and Table A6.6.2-2.
4.2	**Cytotoxicity**	No In the main study, structural or numerical aberrations were not induced by dinotefuran either after 24 or 48 hours exposure to dose levels up to 2000 µg/mL. However, slight growth inhibition occurred at 2000 µg/mL, 28% after 24 hours and 38% after 48 hours exposure. See Table A6.6.2-1 and Table A6.6.2-2.

Section A6.6.2 **Genotoxicity in vitro**
Annex Point IIA6.6.2 **Cytogenicity in mammalian cells**
Mycoplasma-free Chinese hamster lung

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

OECD guideline no. 473 (1983), 92/69/EEC (method B.10), US-EPA OPPTS 870.5375, JMAFF 59 NohSan no. 4200 (1985), JMHW, Part 1 (1990); Japan Ministry of Labor, Appendix 1, Notification nos. 143 (1987)

No relevant deviations from test guidelines.

Methods:
Dinotefuran was assessed for clastogenic activity in a chromosome aberration test performed in cultured CHL/IU cells. Dose levels for the main assay were determined following a preliminary cytotoxicity test and the high dose levels selected were 2000 µg/mL (10mM). A series of 3 dose levels were used for each of the 4 treatment regimens, 6-hour exposure without S9, 6-hour exposure with S9 and 24- or 48-hour exposures without S9. All dose levels were evaluated for clastogenicity by evaluating 200 metaphase cells/dose level. Cell survival was determined at harvest.

5.2 Results and discussion

In the main study, structural or numerical aberrations were not induced by dinotefuran either after 24 or 48 hours exposure to dose levels up to 2000µg/mL. However, slight growth inhibition occurred at 2000µg/mL, 28% after 24 hours and 38% after 48 hours exposure. Similarly, structural or numerical aberrations were not induced after 6 hours exposure to dinotefuran either in the presence or absence of metabolic activation. Cell survival was unaffected at all dose levels after 6 hours exposure.

It was concluded that under the conditions of this study dinotefuran and/or metabolites does not induce structural or numerical chromosomal aberrations in Chinese hamster lung cells at dose levels up to and including 2000µg/mL (equivalent to 10 mM).

5.3 Conclusion

5.3.1 Reliability 1

5.3.2 Deficiencies No

Table A6.6.2-1: Cell growth rate (%) relative to solvent control

Dose level (µg/mL)	Preliminary study exposed for:		Main study exposed for:			
	48hr (-S9)	6hr (+S9)	24hr (-S9)	48hr (-S9)	6hr (+S9)	6hr (-S9)
0	=100	=100	=100	=100	=100	=100
7.81	99	100	-	-	-	-
15.6	88	93	-	-	-	-
31.3	96	102	-	-	-	-
62.5	93	88	-	-	-	-
125	92	85	-	-	-	-
250	92	85	-	-	-	-
500	88	90	85	93	100	103
1000	82	88	84	95	99	104
2000	65	100	72	62	104	106
MMC 0.03	-	-	70	74	-	-
CP 12.0	-	-	-	-	86	99

Table A6.6.2-2: Summary of results from the main assay

Compound	Dose (µg/mL)	Cell survival (%)	No. cells	No. cells with structural aberrations:						Total less gaps (%)	Polyploidy (%)
				gap	ctb	cte	csb	cse	other		
		6-hour exposure, without S9									
Saline	0	100	200	0.5	0	0	0	0	0	0.5	0.5
Dinotefuran	500	103	200	0	0	0.5	0	0	0	0.5	1.0
	1000	104	200	0.5	0	0	0	0	0	0.5	0
	2000	106	200	0	0.5	0.5	0	0	0	1.0	1.0
CP	12.0	99	200	1.0	0	0	0.5	0	0	1.5	0.5
		6-hour exposure with S9									
Saline	0	100	200	0	0.5	0	0	0	0	0.5	1.0
Dinotefuran	500	100	200	0	0.5	0	0	1.0	0	1.5	1.0
	1000	99	200	0	0.5	0	0	0	0	0.5	2.5
	2000	104	200	0	0	0	0	1.0	0	1.0	1.0
CP	12.0	86	200	0	15.5	43.5	0	0.5	0	51.0	1.0
		24-hour exposure, without S9									
Saline	0	100	200	0.5	0.5	0	0	1.5	0	2.5	0
Dinotefuran	500	85	200	0	2.5	2.0	0	0	0	4.5	0
	1000	84	200	0.5	1.0	1.0	0	0	0	2.5	0.5
	2000	72	200	0.5	2.0	0.5	0.5	0	0	3.5	0
MMC	0.03	70	200	2.0	42.5	38.5	1.0	0.5	0	65.5	0.5
		48-hour exposure, without S9									
Saline	0	100	200	1.0	1.5	0	0.5	0	0	3.0	2.5
Dinotefuran	500	93	200	0	1.0	0.5	0	0.5	0	2.0	0.5
	1000	95	200	1.0	0	0	0	1.0	0	2.0	0.5
	2000	62	200	0	1.0	0	0	0	0	1.0	1.5
MMC	0.03	74	200	2.0	36.0	66.5	0	0.5	0	76.0	0.5

ctb-chromatid break;
cte-chromatid exchange;
csb-chromosome break;
cse-chromosome exchange including dicentric and ring chromosomes;
other-otheraberrations including fragmentation;
gap - includes both chromatid and chromosome gaps

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*
Conclusion	*As described by Applicant*
Reliability	*As described by Applicant*
Acceptability	*Acceptable*
Remarks	*The highest concentration of MTI-446 tested, 2000 µg/mL, did not induce significant cytotoxicity. However, this concentration is equal to 0.01M which is the limit concentration for substances of low toxicity in this type of assay.*
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.3 Genotoxicity in vitro
Annex Point IIA6.6.3 Gene mutation in mammalian cells
Mouse lymphoma cell line

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1 REFERENCE

1.1	**Reference**	[redacted], 2002, MTI-446 technical material: mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the microtitre fluctuation technique, [redacted], unpublished report no. 719/15-D6173, February 8, 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 no. 476 (1997) US-EPA OPPTS 870.5300 (1998) UKEMS Guideline (1990)
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	**Study Type**	In vitro mammalian cell gene mutation test
3.2.1	Organism/cell type	Mammalian cell lines: Mouse lymphoma L5178Y cells
3.2.2	Deficiencies / Proficiencies	Mycoplasma-free *tk*$^{+/-}$
3.2.3	Metabolic activation system	S9 mix Derived from the liver post-mitochondrial fraction of male Sprague-Dawley rats induced with Aroclor 1254.
3.2.4	Positive control	4-nitroquinoline-1-oxide (without S9) benz(a)pyrene (with S9)
3.3	**Administration / Exposure; Application of test substance**	

Section A6.6.3	**Genotoxicity in vitro**	
Annex Point IIA6.6.3	**Gene mutation in mammalian cells**	
	Mouse lymphoma cell line	
3.3.1	Concentrations	Experiment 1 and 2: 0 (saline), 400, 800, 1200, 1600 and 2022 µg/mL Both with and without metabolic activation. See Table A6.6.3-1.
3.3.2	Way of application	Dinotefuran was dissolved in saline, under subdued lighting conditions, immediately prior to assay to give the required concentration. Stock solutions were filter-sterilised and further dilutions were made using saline. The dinotefuran solutions were protected from light and used within 2 hours of preparation. No change in osmolality occurred at the highest concentration tested.
3.3.3	Pre-incubation time	None
3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Cultures for mutation assessment were adjusted to 10^4 cells/mL, 3µg/mL TFT was added, plated on to four 96-well plates/concentration and incubated for 12 days.
	4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	Experiment 1: No: relative survival at 2022µg/mL was 102.68 without activation. Experiment 2: No: relative survival at 2022µg/mL was 76.16 without metabolic activation. See Table A6.6.3-3
4.1.2	with metabolic activation	Experiment 1: No: relative survival at 2022µg/mL was 118.06% with metabolic activation. Experiment 2: No: relative survival at 2022µg/mL was 100.28% with metabolic activation. See Table A6.6.3-3

Section A6.6.3	**Genotoxicity in vitro**
Annex Point IIA6.6.3	**Gene mutation in mammalian cells**
	Mouse lymphoma cell line

4.2	**Cytotoxicity**	No: no statistically significant increases in mutation frequency occurred at any dose level in the absence or presence of metabolic activation in either independent experiment. The proportion of small colony mutants for the solvent controls without and with metabolic activation ranged from 38 to 41% in experiment 1 and from 52 to 55% in experiment 2. Marked increases in the numbers of both small and large colony mutants occurred in response to both positive control materials. Analysis of dinotefuran formulations demonstrated achieved concentrations within 100 ± 10% of nominal concentrations. The assay acceptance criteria were met and the study is considered valid. See Table A6.6.3-2.	

Section A6.6.3 **Genotoxicity in vitro**
Annex Point IIA6.6.3 **Gene mutation in mammalian cells**
Mouse lymphoma cell line

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

OECD guideline no. 476 (1997), US-EPA OPPTS 870.5300 (1998), UKEMS Guideline (1990)

No relevant deviations from test guidelines.

Method:

A gene mutation assay of dinotefuran dissolved in saline was performed on a mouse lymphoma cell line, L5178Y $tk^{+/-}$, at the thymidine kinase locus. The highest dose levels for the main assay, equivalent to 10mM, were determined on the basis of two preliminary cytotoxicity tests. One culture/dose level were exposed for 3 hours at 37°C to a series of 6 dose levels in the ranges 62.5 - 2022 µg/mL with and without S9 metabolic activation in first preliminary test, and 9 dose levels in the ranges .7.81 - 2022 µg/mL in second preliminary study. In the preliminary cytotoxicity tests, cells survived the 3-hour exposure to dinotefuran at all concentrations with and without metabolic activation, and for 24 hours without metabolic activation. Accordingly, 5 concentrations in the range 400 - 2022µg/mL were selected for both independent experiments to determine viability and 5-TFT resistance 2 days after treatment.

In experiment 1, duplicate cultures were exposed for 3 hours to dinotefuran in saline at final concentrations of 0 (saline), 400, 800, 1200, 1600 and 2022µg/mL, both with and without metabolic activation. In experiment 2, the same final concentrations were assayed without metabolic activation for a 24-hour exposure period, and with metabolic activation for a 3-hour exposure period. Concurrent positive controls with and without S9 were also tested. Cultures for mutation assessment were adjusted to 10^4 cells/mL, 3µg/mL TFT was added, plated on to four 96-well plates/concentration and incubated for 12 days. Wells containing clones were identified by eye and counted. Wells containing large and small colonies were enumerated for the negative and positive controls.

5.2 Results and discussion

In both experiments relative survival at 2022µg/mL was at least 76.16 % with and without metabolic activation. No statistically significant increases in mutation frequency occurred at any dose level in the absence or presence of metabolic activation in either independent experiment. Marked increases in the numbers of both small and large colony mutants occurred in response to both positive control materials.

It was concluded that dinotefuran technical and/or metabolites does not induce mutation at the tk locus of L5178Y mouse lymphoma cells at concentrations up to 10mM, and is considered not mutagenic in this test system.

5.3 Conclusion

5.3.1 Reliability 1

Section A6.6.3	**Genotoxicity in vitro**
Annex Point IIA6.6.3	**Gene mutation in mammalian cells**
	Mouse lymphoma cell line

5.3.2	Deficiencies	Yes No evaluation criteria specified in the report. However, the reviewer considers the results to be clearly negative since there were no statistically significant differences in mutation frequencies between the solvent control and treated groups, and the few slightly higher than control mutation frequencies that occurred in the treated groups showed no dose-response relationship.

Table A6.6.3-1: Dose levels in two preliminary studies and main assay

Study	Dose levels of dinotefuran used (µg/mL)								
Cytotoxicity ± S9, 3h	62.5	125	250	500	1000	2022			
Cytotoxicity - S9, 24h	7.81	15.63	31.25	62.5	125	250	500	1000	2022
Experiment 1 in main assay ± S9, 3h	400	800	1200	1600	2022				
Experiment 2 in main assay - S9, 24h; +S9, 3h	400	800	1200	1600	2022				

Table A6.6.3-2: Summary of relative survival in the cytotoxicity range-finding study

Compound	Dose level (µg/mL)	Relative survival (%):		
		3-hour exposure (-S9)	3-hour exposure (+S9)	24-hour exposure (-S9)
DMSO	0	= 100	= 100	= 100
Dinotefuran	7.81	-	-	150.17
	15.63	-	-	122.63
	31.25	-	-	116.22
	62.5	47.60	58.55	101.05
	125	103.20	72.40	111.20
	250	97.81	82.08	109.29
	500	93.43	94.94	133.42
	1000	132.27	66.25	97.63
	2022	87.00	70.00	109.58

- not assayed

Table A6.6.3-3: Relative survival and mutant frequency – main experiments

Concn. (µg/mL)	Experiment 1					
	3-hr exposure (-S9)			3-hr exposure (+S9)		
	%RS[a]	RTG[b]	MF[c]	%RS[a]	RTG[b]	MF[c]
0	= 100	1.00	67.92	= 100	1.00	81.65
400	100.42	0.87	70.05	103.94	1.11	80.01
800	98.63	1.06	70.70	116.66	1.18	54.15
1200	112.29	1.12	60.56	105.90	1.12	64.32
1600	103.08	0.99	85.24	105.69	1.14	68.82
2022	102.68	0.91	81.84	118.06	1.03	61.05
NQO						
0.05	102.70	0.85	149.24	-	-	-
0.01	94.13	0.54	337.64	-	-	-
0.02	-	-	-	-	-	-
0.04	-	-	-	-	-	-
BP						
2.0	-	-	-	91.27	0.76	365.23
3.0	-	-	-	81.81	0.69	525.10
	Experiment 2					
	24-hr exposure (-S9)			3-hr exposure (+S9)		
	%RS[a]	RTG[b]	MF[c]	%RS[a]	RTG[b]	MF[c]
0	= 100	1.00	72.69	= 100		101.87
400	96.10	1.04	61.05	105.89		78.81
800	88.13	0.99	62.01	112.71		82.82
1200	83.95	1.09	85.11	116.11		129.05
1600	107.55	1.01	56.23	101.87		105.00
2022	76.16	0.94	82.33	100.28		107.68
NQO						
0.05	-	-	-	-	-	-
0.01	-	-	-	-	-	-
0.02	61.06	0.84	194.58	-	-	-
0.04	55.07	0.92	196.02	-	-	-
BP						
2.0	-	-	-	102.04	0.58	423.87
3.0	-	-	-	62.54	0.41	606.07

[a] % relative survival adjusted by post-treatment cell count;

[b] relative total growth;

[c] 5-TFT resistant mutants/10^6 viable cells 2 days after treatment;

- not assayed

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*17 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	*The highest concentration of MTI-446 tested, 2022 µg/mL, did not induce significant cytotoxicity. However, this concentration is equal to 0.01M which is the limit concentration for substances of low toxicity in this type of assay.*
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.4 Genotoxicity *in vivo*
Annex Point IIA6.6.4 Micronucleus test, mouse

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 1995, Micronucleus test of EXP-316 with mice, [redacted], unpublished report no. 2498, March 15, 1995.	X1
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 JMAFF 59 NohSan no. 4200 (1985) 92/69/EEC (method B.12)	
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	OFU-1207	
3.1.2	Specification		
3.1.2.1	Description	Crystal	
3.1.2.2	Purity	>99%	
3.1.2.3	Stability	Not specified in report	
3.1.2.4	Maximum tolerable dose	1080mg/kg bw/day	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	BDF1 (C57BL/6 x DBA/2)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Weighing 24.8 – 27.1g	
3.2.6	Number of animals per group	6 males per group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Number of applications	2 Daily dosing for 2 days is considered appropriate for dinotefuran because elimination from rodent plasma and tissues is rapid (rat $T_{1/2}$	

Section A6.6.4 **Genotoxicity *in vivo***

Annex Point IIA6.6.4 **Micronucleus test, mouse**

		values for elimination after single oral doses of 50 and 1000mg/kg are 3.6 and 13.8 hours, respectively [[redacted], 2000], unpublished report no. [redacted] 6648-136	
3.3.2	Interval between applications	24 h	
3.3.3	Postexposure period	24 h after final treatment	
		Oral	
3.3.4	Type	Gavage	
3.3.5	Concentration	0 (vehicle), 270, 540 and 1080 mg/kg/day on 2 consecutive days, 24 hours apart (total doses: 540, 1080 or 2160 mg/kg). A similar group of male mice received a single intraperitoneal injection of 2 mg/kg MMC, 24 hours before necropsy, as a positive control group	
3.3.6	Vehicle	0.5% aqueous carboxymethyl cellulose solution	
3.3.7	Concentration in vehicle	Not applicable	
3.3.8	Total volume applied	10 mL/kg	
3.3.9	Controls	Vehicle Sngle intraperitoneal injection of 2 mg/kg Mitomycin C(MMC), 24 hours before necropsy, as a positive control group	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes, after treatment and before sacrifice.	
3.4.2	Body weight	Yes, first day of treatment and on the day of sacrifice.	
3.4.3	Tissue	Bone marrow Number of animals: all animals Number of cells: 2000 Time points: 24 h after final treatment Type of cells: Micronucleated polychromatic erythrocytes in bone marrow Parameters: Polychromatic/normochromatic erythrocytes ratio	X2
3.5	**Further remarks**	The dose levels for the main study were determined from the results of a dose range-finding study in which 5 groups of 6 male mice were treated once daily, by gavage, for 2 days with dinotefuran at dose levels of 648, 1080, 1800, 3000 or 5000 mg/kg. Where survival permitted, 2 mice/group were killed 24, 48 and 72 hours after the final dose. Bone marrow smears were prepared from all survivors, stained and examined for micronucleated polychromatic erythrocytes. All mice treated at 3000 or 5000mg/kg bw/day and 4 of the 6 mice treated at 1800mg/kg bw/day died during the dose range-finding study and the mean incidences of MNPCE were similar between treated groups (0.18, 0.12 and 0.15% at 648, 1080 and 1800mg/kg bw/day, respectively) and between sampling intervals (0.20, 0.06 and 0.20% at	

Section A6.6.4 **Genotoxicity *in vivo***

Annex Point IIA6.6.4 **Micronucleus test, mouse**

		24, 48 and 72 hours, respectively). The incidences of MNPCE was assessed by the stochastic method of Kastenbaum and Bowman (1970)[1] and the PCE/NCE ratio by Dunnett's t-test, at the 95 and 99% significance levels.
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	No effects
4.2	**Body weight**	No effects
4.3	**Haematology / Tissue examination**	The frequency of occurrence of MNPCE in the groups treated with dinotefuran ranged from 0.15 to 0.22% compared with a solvent control incidence of 0.18%. The ratio of PCE/total erythrocytes in the groups treated with dinotefuran ranged from 50.0 to 55.2% compared with a solvent control ratio of 51.6%. The MNPCE frequencies and PCE/total erythrocyte ratios were not significantly different ($p > 0.05$) from the control values at any dose level. See Table A6.6.4-1. In contrast, mitomycin C produced a statistically significant ($p < 0.01$) increase in the MNPCE frequency (7.03%) and a statistically significant ($p < 0.01$) decrease in the PCE/total erythrocyte ratio (29.8%) compared with the solvent control group. Since the incidences of MNPCE and the ratios of PCE/total erythrocytes in both the vehicle control and positive control groups were within the laboratory historical control ranges, the test is considered to be valid.
4.4	**Genotoxicity**	No
4.5	**Other**	None

[1] Kastenbaum, M.A. and Bowman, K.O. (1970): Tables for determining the statistical significance of mutation frequencies, Mut. Res., 9, 527 - 549

Section A6.6.4 **Genotoxicity *in vivo***
Annex Point IIA6.6.4 **Micronucleus test, mouse**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

JMAFF 59 NohSan no. 4200 (1985), 92/69/EEC (method B.12)

No relevant deviations from test guidelines.

Method:

Dose levels for the main study were based on the results of a dose range-finding study. All mice treated at 3000 or 5000mg/kg bw/day and 4 of the 6 mice treated at 1800mg/kg bw/day died during the dose range-finding study and the mean incidences of MNPCE were similar between treated groups and between sampling. Therefore, a high dose level of 1080mg/kg bw/day and a sampling interval of 24 hours were selected for the main study.

Four groups of 6 male mice were treated orally, by gavage, with 10 mL/kg dinotefuran suspension at dose levels of 0 (vehicle), 270, 540 or 1080 mg/kg/day on 2 consecutive days, 24 hours apart (total doses 540, 1080 and 2160 mg/kg). A positive control group received a single intraperitoneal injection of 2 mg/kg MMC. Body weights were recorded on the first day of treatment and on the day of sacrifice. General signs of toxicity were recorded after treatment and before sacrifice. All mice were sacrificed 24 hours after the final treatment. Femoral bone marrow samples were aspirated into fetal calf serum, smears were prepared, air-dried, fixed and stained with giemsa, coded and scored by light microscopy. The incidence of micronucleated polychromatic erythrocytes (MNPCE) in 1000 polychromatic erythrocytes (PCE) and the ratio of PCE to total erythrocytes (NCE) in 1000 erythrocytes were determined for each slide.

5.2 Results and discussion

There were no deaths during the treatment period and no signs of toxicity including body weight change were evident at any dose level. The MNPCE frequencies and PCE/total erythrocyte ratios of all dinotefuran-treated groups were comparable to and not significantly different from control values.

In contrast, mitomycin C produced a statistically significant increase in the MNPCE frequency and a statistically significant decrease in the PCE/total erythrocyte ratio.

Since the incidences of MNPCE and the ratios of PCE/total erythrocytes in both the vehicle control and positive control groups were within the laboratory historical control ranges, the test was considered to be valid.

Dinotefuran at dose levels approaching the maximum tolerated dose does not induce the formation of micronuclei and does not inhibit spindle formation in the bone marrow of mice.

5.3 Conclusion

5.3.1 Reliability 1

5.3.2 Deficiencies Yes

6 male mice/group were employed rather than 5 mice/sex/group as specified in 92/69/EEC. However, since there is no sex-related difference in the acute oral toxicity of dinotefuran to mice (see Glaza 1997b, unpublished report no. CHW 6648-119), the use of a single sex is considered not to have affected the validity of the study. X3

Table A6.6.4-1: Summary of MNPCE incidences from the main study

Compound	Dose level (mg/kg)	No. of animals examined	MNPCE (%) (mean ± SD)	Range of MNPCE/2000PCE	PCE (%) (mean ± SD)
Vehicle	0	6	0.18 ± 0.15	0 - 4	51.6 ± 3.7
EXP-316 (Dinotefuran)	270	6	0.20 ± 0.14	0 - 4	55.2 ± 4.2
	540	6	0.15 ± 0.05	1 - 2	50.0 ± 4.2
	1080	6	0.22 ± 0.13	0 - 4	52.8 ± 5.1
MMC	2	6	7.03 ± 0.88**	60 - 85	29.8 ± 4.7**

** $p < 0.01$

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15 January 2013
Materials and Methods	As described by Applicant, except X1 Section 1.1 EXP-316 is confirmed as a code name for dinotefuran. X2 Section 3.4.3: 1000 polychromatic erythrocytes/animal were assessed for the presence of micronuclei, not 2000 polychromatic erythrocytes/animal as inferred in this section.
Results and discussion	As described by Applicant
Conclusion	As described by Applicant, except X3 Section 5.3.2 Deficiencies: The following deficiency is identified by the RMS- only 1000 polychromatic erythrocytes/animal were assessed for the presence of micronuclei, not 2000 polychromatic erythrocytes/animal as specified in OECD Test Guideline 474. This deficiency will reduce the power of the study to detect genotoxicity.
Reliability	The reliability of the study is reduced because of the above deficiency.
Acceptability	Although a significant deficiency is identified, this study is considered acceptable as providing supporting information because the substance tested negative in the in vitro genotoxicity assays.
Remarks	None
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.7-1 **Carcinogenicity**
Annex Point IIA6.7 **Rat**
Oral, 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	60 males and 60 females per group. See Table A6.7.1-1

Section A6.7-1 Annex Point IIA6.7		Carcinogenicity Rat Oral, 104-week
3.2.6.1	at interim sacrifice	Additional groups of 10 animals/sex/group were similarly treated for at least 26, 52 and 78 weeks. Further groups of 10 animals/sex treated at 0 and 20000ppm were treated for 26 weeks and then maintained untreated for 6 weeks before necropsy.
3.2.6.2	at terminal sacrifice	All surviving animals
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	Rats 104 weeks
3.3.2	Interim sacrifice(s)	After 26, 52 and 78 weeks
3.3.3	Final sacrifice	After 104 weeks
3.3.4	Freqency of exposure	7 days/week
3.3.5	Postexposure period	None
		Oral
3.3.6	Type	In food
3.3.7	Concentration	Nominal in food: 0, 60, 200, 2000 and 20000 ppm Mean achieved dose levels: 0, 3, 10, 100 and 991mg/kg/day in males 0, 4, 13, 127 and 1332mg/kg/day in females
3.3.8	Vehicle	No vehicle, admixture to the diet
3.3.9	Concentration in vehicle	Not applicable
3.3.10	Total volume applied	Not applicable
3.3.11	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Body weight	Yes
3.4.2	Food consumption	Yes
3.4.3	Water consumption	No
3.4.4	Clinical signs	Yes
3.4.5	Macroscopic investigations	Tumours
3.4.6	Ophthalmoscopic examination	Yes

Section A6.7-1
Annex Point IIA6.7

Carcinogenicity
Rat
Oral, 104-week

3.4.7	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:	Red blood cell count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, white blood cell count, blood cell morphology, differential blood cell count.
			None
3.4.8	Clinical Chemistry		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:	Glucose, urea nitrogen, creatinine, total protein, albumin, globulin calculated, albumin/globulin ratio, total bilirubin, cholesterol, triglycerides, aspartate aminotransferase, alaine aminotransferase, alkaline phosphatase, gamma glutamyltransferase, creatine kinase, calcium, inorganic phosphorus, sodium, potassium, chloride, prothrombin time, activated partial thromboplastin time.
		Other	None
3.4.9	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:	Specific gravity, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, urine volume, red blood cell hight-power field, white blood cells per high-power field, epithelial cells per high-power field, bacteria per high-power field, casts per low power field, crystals per low-power field, urine appearance.
		Other	None
3.4.10	Pathology	Yes	
3.4.10.1	Organ Weights	Yes	
		from:	10 animals/sex/group

Section A6.7-1 **Carcinogenicity**
Annex Point IIA6.7 **Rat**
Oral, 104-week

		Organs:	Aderenal, brain, epidiymis, heart, kidney, liver, ovary, pituitary, prostate, spleen, seminal vesicle, testis, thymus, thyroid with parathyroid, uterus with cervix. Gross lesions, endocrine glands, tissue masses, kidneys, liver, lungs and reproductive organs of both sexes were also examined from all intermediate dose group animals.
		Other	None
3.4.11	Histopathology	Yes	
		from:	0 or 20000 ppm dose groups
		from:	all surviving animals and decedents in all groups
		Organs:	Macroscopic lesions, adrenals, tissue masses, lungs, liver, kidneys, pituitary, parathyroid, thyroid, testes, seminal vesicle, prostate, epididymides, female mammary, ovary, uterus and vagina.
		Other	None
3.4.12	Other examinations	None	
3.5	**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	

4 RESULTS AND DISCUSSION

4.1	**Body weight**	The body weight gains of both sexes were reduced by treatment at 20000ppm from week 2 (Table A6.7.1-2) 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.
4.2	**Food consumption**	The mean weekly food consumption of animals treated at 20000ppm was reduced by up to 10.0% during the first 77 weeks of treatment (Table A6.7.1-2). Food consumption was not affected by treatment at dose levels up to 2000ppm.
4.3	**Water consumption**	No effect: water intake was unaffected by treatment at all dose levels.
4.4	**Clinical signs**	No effects: there were no treatment-related clinical signs of toxicity including the incidences of palpable tissue masses.

Section A6.7-1 Annex Point IIA6.7		Carcinogenicity Rat Oral, 104-week
4.5	**Macroscopic investigations**	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 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.
4.6	**Ophthalmoscopic examination**	No effects: there were no treatment-related ophthalmological findings at any dose level at any of the examination intervals.
4.7	**Haematology**	No effects
4.8	**Clinical Chemistry**	No effects
4.9	**Urinalysis**	No effects
4.10	**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.
4.11	**Histopathology**	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 (Table A6.7.1-3). 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.
4.12	**Other examinations**	None

Section A6.7-1	Carcinogenicity	
Annex Point IIA6.7	Rat	
	Oral, 104-week	
4.13 Time to tumours	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 (Table A6.7.1-4). 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	
4.14 Other	None	

Section A6.7-1 **Carcinogenicity**

Annex Point IIA6.7 **Rat**

Oral, 104-week

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.

Methods:

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 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.

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.

The no-observed-effect-level (NOEL) for carcinogenicity was established as >20000ppm, equivalent to dose levels of 991mg/kg bw/day (males) and 1332mg/kg bw/day (females), based on no excess treatment-related tumors in any tissue at the highest dose level employed.

The no-observed-adverse-effect-level (NOAEL) 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.

Section A6.7-1 **Carcinogenicity**

Annex Point IIA6.7 **Rat**

Oral, 104-week

5.3 Conclusion

5.3.1 Reliability 1

5.3.2 Deficiencies No

Table A6.7.1-1 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 A6.7.1-2 Treatment related effects on bodyweight, bodyweight 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 A6.7.1-3 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 A6.7.1-4 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*
Conclusion	*As described by Applicant i.e. there was no evidence of carcinogenic activity and therefore the NOAEL for neoplastic effects is 20000 ppm, the highest dose level investigated*
Reliability	*As described by Applicant*
Acceptability	*Acceptable*
Remarks	*See A6.5-1 for RMS evaluation of non-neoplastic aspects of this study.*
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.7-2 **Carcinogenicity**
Annex Point IIA6.7 **Mouse**
Oral, 78-week

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 2000d, 78-week dietary carcinogenicity study with MTI-446 in mice, [redacted], unpublished report no. 6648-130, January 25, 2000. [redacted], 2000e, First amendment to report - 78-week dietary carcinogenicity study with MTI-446 in mice, [redacted], unpublished report no. 6648-130, 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 Method complies with or exceeds 87/302/EEC (1987) OECD guideline no. 451 (1981) EPA-FIFRA, Subdivision F, § 83-2 (1985) 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	Mouse
3.2.2	Strain	Crl:CD-1®(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 27.1 – 36.7 g for males and 20.0 – 29.5 g for females
3.2.6	Number of animals per group	60 males and 60 females per group See Table A6.7.2-1.

Section A6.7-2 Annex Point IIA6.7		Carcinogenicity Mouse Oral, 78-week	
3.2.6.1	at interim sacrifice	An additional 10 animals/group/sex killed after 52-weeks	
3.2.6.2	at terminal sacrifice	All animals	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	Mice 78 weeks	
3.3.2	Interim sacrifice(s)	After 52 weeks	
3.3.3	Final sacrifice	After 104 weeks	X1
3.3.4	Freqency of exposure	7 days/week	
3.3.5	Postexposure period	None	
		Oral	
3.3.6	Type	In food	
3.3.7	Concentration	Nominal in food: 0, 25, 250, 2500, and 25000ppm Mean achieved dose levels 0, 3, 34, 345 and 3694mg/kg bw/day in males 0, 4, 45, 441 and 4728mg/kg bw/day in females	
3.3.8	Vehicle	No vehicle, admixture to diet	
3.3.9	Concentration in vehicle	Not applicable	
3.3.10	Total volume applied	Not applicable	
3.3.11	Controls	Plain diet	
3.4	**Examinations**		
3.4.1	Body weight	Yes	
3.4.2	Food consumption	Yes	
3.4.3	Water consumption	Yes	
3.4.4	Clinical signs	Yes	
3.4.5	Macroscopic investigations	Tumours	
3.4.6	Ophthalmoscopic examination	No	
3.4.7	Haematology	Yes	

Section A6.7-2
Annex Point IIA6.7

Carcinogenicity
Mouse
Oral, 78-week

		Number of animals:	Interim sacrifice: 10 animals/sex/group Terminal sacrifice: all survivours
		Time points:	In week 53 and week 79
		Parameters:	Red blood cell count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, white blood cell count, blood cell morphology, differential blood cell count.
		Other:	None
3.4.8	Clinical Chemistry	No	
		Number of animals:	Not applicable
		Time points:	Not applicable
		Parameters:	Not applicable
		Other	None
3.4.9	Urinalysis	No	
		Number of animals:	Not applicable
		Time points:	Not applicable
		Parameters:	Not applicable
		Other	None
3.4.10	Pathology	Yes	
3.4.10.1	Organ Weights	Yes	
		from:	10 animals/sex/group at interim sacrifice and at terminal sacrifice. No organ weights were recorded in decedents
		Organs:	Adrenal, brain, epididymis, heart, kidney, liver with gall bladder (drained), ovary, pituitary, prostate, spleen, seminal vesicle, testis, thymus, thyroid with parathyroid, uterus with cervix.
		Other:	None
3.4.11	Histopathology	Yes/No	
		from:	All tissues from decedents and animals treated at 0 or 25000ppm, and gross lesions, lungs, liver and kidneys from all animals were examined

Section A6.7-2 **Carcinogenicity**
Annex Point IIA6.7 **Mouse**
Oral, 78-week

		Organs:	Adrenal, aorta, brain, cecum, cervix, colon, duodenum, epidiymis, esophagus, eye, femur with bone marrow (articular surface of the distal end), gallbladder, Harderian gland, heart, ileum, jejunum, kidney, lesions, liver, lun with mainstream bronchi, lymph node (mandibular and mesenteric), mammary gland (females only), masses and associated tissues, muscle (thigh), optic nerve, ovary, pancreas, pituitary, prostate rectum, salivary gland (mandibular), sciatic nerve, seminal vesicle, skin, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus, vagina.
			Other
3.4.12	Other examinations	None	
3.5	**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	
		4 RESULTS AND DISCUSSION	
4.1	**Body weight**	Overall body weight gains of both sexes were significantly ($p < 0.05$) reduced by treatment at 25000ppm (Table A6.7.2-2) but were unaffected by treatment at lower dose levels. At 25000ppm, overall body weight gains were reduced by 17.9 and 25.2% in males and females, respectively, and group mean body weights at week 78 were 95.3 and 90.9% of control values in males and females, respectively.	
4.2	**Food consumption**	Food consumption wase unaffected by treatment at all dose levels.	
4.3	**Water consumption**	Water consumption was unaffected by treatment at all dose levels.	
4.4	**Clinical signs**	There were no treatment-related clinical signs of toxicity including the incidences of palpable tissue masses.	
4.5	**Macroscopic investigations**	There were no treatment-related macroscopic pathology findings in either sex at any dose level after 52 and 78 weeks of treatment	
4.6	**Ophthalmoscopic examination**	Not performed	

Section A6.7-2	**Carcinogenicity**	
Annex Point IIA6.7	**Mouse**	
	Oral, 78-week	
4.7	**Haematology**	Treatment-related hematological effects were confined to slightly lower platelet counts in both sexes after 78 weeks treatment at 25000ppm (Table A6.7.2-2). The group mean counts were 19.9 and 17.4% lower than control values in males and females, respectively, but were statistically significant ($p < 0.05$) in males only. The effect was not apparent at lower dose levels after 78 weeks or in any group after 52 weeks of treatment.
4.8	**Clinical Chemistry**	Not performed
4.9	**Urinalysis**	Not performed
4.10	**Organ Weights**	There were no treatment-related organ weight changes in either sex at any dose level after 52 and 78 weeks of treatment
4.11	**Histopathology**	The nature and incidence of non-neoplastic histopathological findings were similar in decedent animals and those surviving to termination and there were no treatment-related differences between the control and treated groups.
4.12	**Other examinations**	None
4.13	**Time to tumours**	The nature and incidence of neoplastic changes (Table A6.7.2-3) were not influenced by treatment with dinotefuran at any dose level. In male animals, adenoma / carcinoma of the lung and liver and haemangioma / haemangiosarcoma in multiple organs met the criteria for statistical analysis. In females, adenoma / carcinoma of the lung, ovarian granulosa / theca cell tumour and haemangioma / haemangiosarcoma, endometrial stromal polyp and sarcoma, and leiomyoma / leiomyosarcoma in multiple organs met the criteria for statistical analysis. There were no statistically significant trends or group differences in the incidences of any common tumours ($p > 0.01$) or rare tumours ($p > 0.05$) and all differences between the groups are considered to be normal biological variation.
4.14	**Other**	None

Section A6.7-2 **Carcinogenicity**

Annex Point IIA6.7 **Mouse**

Oral, 78-week

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

Method complies with or exceeds 87/302/EEC (1987)
OECD guideline no. 451 (1981)
EPA-FIFRA, Subdivision F, § 83-2 (1985)

JMAFF 59 NohSan no. 4200 (1985)

No relevant deviations from test guidelines.

Methods:

Five groups of 60 male and 60 female albino mice were administered orally for at least 78 weeks, by admixture in the diet, at constant nominal concentrations of 0, 25, 250, 2500, and 25000ppm. Mean achieved dose levels were 0, 3, 34, 345 and 3694mg/kg bw/day (males) and 0, 4, 45, 441 and 4728mg/kg bw/day (females). An additional 10 animals/sex/group, similarly treated, were killed after at least 52 weeks treatment for interim evaluation.

5.2 Results and discussion

There were no treatment-related deaths or clinical signs at any dose level. Overall body weight gains of both sexes were reduced by treatment at 25000ppm but were unaffected by treatment at lower dose levels. Food and water consumption were unaffected by treatment at all dose levels. Treatment-related hematological effects were confined to slightly lower platelet counts in both sexes after 78 weeks treatment at 25000ppm. The effect was not apparent at lower dose levels after 78 weeks or in any group after 52 weeks of treatment.

There were no treatment-related macroscopic pathology findings or organ weight changes in either sex at any dose level after 52 and 78 weeks of treatment. The nature and incidence of non-neoplastic histopathological findings were similar in decedent animals and those surviving to termination and there were no treatment-related differences between the control and treated groups. The nature and incidence of neoplastic changes were not influenced by treatment with dinotefuran at any dose level. There were no statistically significant trends or group differences in the incidences of any common or rare tumours and all differences between the groups were considered to be normal biological variation.

Dietary administration of dinotefuran at concentrations up to 25000ppm, equivalent to dose levels of 3694mg/kg bw/day (males) and 4728mg/kg bw/day (females), for 78 weeks is not tumorigenic in mice.

A no-observed-effect-level (NOEL) for all effects was established as 2500ppm, equivalent to dose levels of 345mg/kg bw/day (males) and 441mg/kg bw/day (females), based on reduced body weight gain and lower circulating platelet counts at 25000ppm.

5.3 Conclusion

5.3.1 Reliability 1

5.3.2 Deficiencies No

Table A6.7.2-1 Animal assignment and treatment

Group number	Dose level of dinotefuran (mg/kg/day)	Number of animals	
		Male	Female
1	0	70	70
2	25	70	70
3	250	70	70
4	2500	70	70
5	25000	70	70

Table A6.7.2-2 Treatment related effects in life

Parameter	Males treated at (ppm):					Females treated at (ppm):				
	0	25	250	2500	25000	0	25	250	2500	25000
Mean BW gain (g)[a]	13.4	14.6	13.5	12.6	11.0*	13.9	14.8	13.4	13.6	10.4*
Plt (103/µl)[b] - week 53	1125	1138	1241	1329	1147	1028	1139	988	1063	1035
Plt (103/µl)[b] - week 79	1405	1309	1545	1245	1125*	1038	1011	1021	966	857

[a] Group mean body weight gain weeks 1 - 78;
[b] Group mean platelet count;
* $p < 0.05$

Table A6.7.2-3 Incidence of neoplastic lesions achieving the criteria for statistical analysis

Organ and lesion	Males treated at (ppm):					Females treated at (ppm):				
	0	25	250	2500	25000	0	25	250	2500	25000
No. examined	70	70	70	70	70	70	70	70	70	70
Lung:										
adenoma (b)	4	5	6	3	6	5	4	5	5	3
carcinoma (m)	3	0	0	1	0	0	0	0	1	0
total (b + m)	7	5	6	4	6	5	4	5	6	3
Liver:										
adenoma (b)	9	13	8	6	5	-	-	-	-	-
carcinoma (m)	4	3	1	1	2	-	-	-	-	-
total (b + m)	13	16	9	7	7	-	-	-	-	-
Multiple organs:										
haemangioma (b)	0	NE	NE	NE	1	1	NE	NE	NE	3
haemangiosarcoma	2	NE	NE	NE	0	1	NE	NE	NE	1
(m)	2	-	-	-	1	2	-	-	-	4
total (b + m)										
Multiple organs:										
stromal polyp (b)	-	-	-	-	-	3	NE	NE	NE	1
stromal sarcoma (m)	-	-	-	-	-	2	NE	NE	NE	3
total (b + m)	-	-	-	-	-	5	-	-	-	4
Multiple organs:										
leiomyoma (b)	-	-	-	-	-	1	NE	NE	NE	3
leiomyosarcoma (m)	-	-	-	-	-	1	NE	NE	NE	2
total (b + m)	-	-	-	-	-	2	-	-	-	5
Ovary:										
granulosa/theca cell										
tumour (b)	-	-	-	-	-	2	NE	NE	NE	1
granulosa/theca cell										
tumour (m)	-	-	-	-	-	1	NE	NE	NE	0
total (b + m)	-	-	-	-	-	3	-	-	-	1

NE = not evaluated;
b = benign;
m = malignant

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *17 January 2013*

Materials and Methods *As described by Applicant, except:*
X1 section 3.3.3 The final sacrifice took place in Week 79

Results and discussion *As described by Applicant, but see remarks below*

Conclusion *As described by Applicant*

Reliability *As described by Applicant*

Acceptability *Acceptable*

Remarks *Though not discussed either by Applicant or in the study report, spleen weights were lower in males from 25 ppm and females from 250 ppm at the 79 week kill only (as shown in table below). These differences were considered to be chance findings and unrelated to treatment by the RMS because (1) clear dose response relationships were not present, (2) statistical significance was not achieved, (3) similar changes were not present at 53 weeks and (4) treatment-related histopathological changes were not reported in this organ.*

Table: Spleen weights at 79 week kill

Sex	Males					Females				
Dose (ppm)	**0**	**25**	**250**	**2500**	**25000**	**0**	**25**	**250**	**2500**	**25000**
Spleen wt. (g)	0.287	0.168	0.129	0.200	0.150	0.317	0.305	0.196	0.179	0.146
As % bwt	0.69	0.37	0.31	0.47	0.37	0.87	0.80	0.55	0.50	0.45

COMMENTS FROM ...

Date

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

Section A6.8.1-1 Teratogenicity Study
Annex Point IIA6.8.1 Oral, rat

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1998a, A dose finding teratogenicity study of MTI-446 given orally to rats, [redacted], unpublished report no. H-97162, January 16, 1998. [redacted], 1998b, Teratogenicity study of MTI-446 given orally to rats, [redacted], unpublished report no. H-97163, June 8, 1998.
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. 414 (1981), which is equivalent to 88/302/EEC EPA FIFRA, Subdivision F, §83-3 (1984) 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	92.9% + 6.9% water, purity of dried material 99.1%
3.1.2.3	Stability	Not specified in report
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Crj:CD(SD) IGS (SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Females
3.2.5	Age/weight at study initiation	10-12 weeks old, weighing 212.22-269.37 g for females
3.2.6	Number of animals per group	24 mated females per group See Table A6.8.1.1-1
3.2.7	Control animals	Yes
3.2.8	Mating period	12 – 13 days
3.3	**Administration/ Exposure**	Oral

Section A6.8.1-1 **Teratogenicity Study**

Annex Point IIA6.8.1 **Oral, rat**

3.3.1	Duration of exposure	Rat, day 6-15, post-mating
3.3.2	Post-exposure period	5 days
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	Nominal dose levels of 0 (vehicle only), 100, 300 or 1000 mg/kg/day
3.3.5	Vehicle	0.5% aqueous carboxymethyl cellulose
3.3.6	Total volume applied	10 mL/kg
3.3.7	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Yes, on days 0 and 3 of gestation and then daily from day 5 of gestation until sacrifice.
3.4.2	Food consumption	Yes, on days 0 and 3 of gestation and then daily from day 6 of gestation until sacrifice.
3.4.3	Clinical signs	Yes, at least once daily on non-treatment days and at least twice daily during the treatment period.
3.4.4	Examination of uterine content	Yes, the uterine tract and ovaries were removed and pregnancy was confirmed. If implantations were not visible macroscopically, the uterus was immersed in ammonium sulphate to aid visualisation. Maternal organs of the cranial, thoracic and abdominal cavities, and ovaries (including corpora lutea count) and uteri (implantation site count) were examined macroscopically. The uterine contents were classified as live fetuses, embryo/fetal deaths, placental remnants, early or late resorptions, or macerated fetuses.
3.4.5	Examination of foetuses	
3.4.5.1	General	Fetuses were sexed, examined for external malformations, and weighed.
3.4.5.2	Skelet	Yes, approximately half of the foetuses from each litter were subjected to skeletal evaluation using a dual staining technique for cartilage and bone and examined for skeletal malformations and variations including counting the number of ossification centers in vertebrae, metacarpals, metatarsals, proximal and medial phalanges.
3.4.5.3	Soft tissue	Yes, approximately half of the foetuses were examined for soft-tissue malformations and variations by fixation in Bouin's solution and subsequent micro-dissection of the cranial and abdominal cavities by Wilson's method [1] and of the thoracic cavity by the method of Nishimura[2].

[1] Wilson, J. G. (1965): Methods for administering agents and detecting malformations in experimental animals, in Teratology: Principles and Techniques, Eds. Wilson J. G. and Warkany, J., Univ. Chicago Press, 262-277.

[2] Nishimura, K. (1974): Microdissection method for examination of mouse and rat fetuses for visceral malformations, Cong. Anom., 14, 23-40.