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

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

3.5	**Statistics**	Where appropriate, data were analysed for homogeneity of variance using Bartlett's test followed by one-way ANOVA for homogeneous data. If significant, Dunnett's test was performed. Non-homogeneous data and percentage/dam data were analysed using the Kruskal-Wallis H-test followed by Dunnett's test if significant.
		4 RESULTS AND DISCUSSION
4.1	**Maternal toxic Effects**	There were no deaths during the study. With the exception of a single animal treated at 1000mg/kg bw/day that showed transient hypoactivity on days 8 to 10 of gestation, there were no clinical signs of toxicity at any doe level. The body weight gain from day 6 to 11 of the group treated at 1000mg/kg bw/day was significantly reduced by 21% (Table A6.8.1.1-2). Thereafter, weight gain was not significantly different from the controls and on day 20 of gestation the group mean body weights of all treated groups were not significantly different from control values. The mean food consumption of the group treated at 1000mg/kg bw/day was significantly reduced by 10.5 to 13.0% on days 6, 7 and 9 of gestation. The mean water consumption of this group was significantly increased by 19.8 to 23.8% on days 10 to 12 of gestation. On other occasions during the treatment period the food and water consumption at 1000mg/kg bw/day were comparable to control values. There were no treatment-related effects on food and water consumption in the groups treated at 100 or 300mg/kg bw/day.
4.2	**Teratogenic / embryotoxic effects**	There were no treatment-related macroscopic findings in the maternal animals at any dose level. Litter parameters as assessed by pregnancy incidence, numbers of corpora lutea, implantations and live fetuses, post-implantation loss, external anomalies, fetal weights and sex ratio, were unaffected by treatment at all dose levels (Table A6.8.1.1-3). Although pre-implantation loss in the group treated at 1000mg/kg bw/day was high (24.0%) in relation to the control group (9.2%), it was not significantly different from the control value and is considered incidental to treatment with dinotefuran since implantation was complete at the initiation of treatment. The mean number of implantations in the 1000mg/kg bw/day group was slightly lower than, but not significantly different from, the control group as a consequence of higher pre-implantation loss. There were no external fetal abnormalities in any of the treated or control groups. There were no treatment-related or statistically significant differences between treated and control groups on the incidence and nature of skeletal and visceral abnormalities and variations. No skeletal abnormalities occurred in any group and the incidences of visceral abnormalities, thymic remnant, microphthalmia, ectopic ovary, pyeloectasia, ureteroectasia and left umbilical artery, were similar in all groups (Table A6.8.1.1-4). Delayed ossification, as assessed by the number of vertebral and phalangeal ossification centers, was not apparent at any dose level
4.3	**Other effects**	None

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

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

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

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)

No relevant deviations from test guidelines

Method:
Four groups of 24 mated female rats were treated orally, by gavage from day 6 to day 15 of gestation, at dose levels of 0, 100, 300 or 1000 mg/kg/day at 10 mL/kg in 0.5% aqueous carboxymethyl cellulose. The animals were killed on Day 20 after mating for reproductive assessment and fetal examination.

5.2 Results and discussion

There were no premature deaths during the study. With the exception of a single animal treated at 1000 mg/kg bw/day that showed transient hypoactivity on days 8 to 10 of gestation, there were no clinical signs of toxicity at any dose level.

The body weight gain from day 6 to 11 of the group treated at 1000mg/kg bw/day was significantly reduced by 21%. Thereafter, weight gain was not significantly different from the controls. The mean food consumption of the group treated at 1000 mg/kg bw/day was reduced up to day 9 of gestation. The mean water consumption of this group was increased on days 10 to 12 of gestation. There were no treatment-related effects on food and water consumption in the groups treated at 100 or 300mg/kg bw/day.

There were no treatment-related macroscopic findings in the maternal animals at any dose level. Litter parameters were unaffected by treatment at all dose levels. The mean number of implantations in the 1000mg/kg bw/day group was slightly reduced as a consequence of a non-treatment-related higher pre-implantation loss.

There were no external fetal abnormalities in any of the treated or control groups. There were no treatment-related or statistically significant differences between treated and control groups in the incidence and nature of skeletal and visceral abnormalities and variations. Delayed ossification, as assessed by the number of vertebral and phalangeal ossification centers, was not apparent at any dose level.

5.3 Conclusion

5.3.1 LO(A)EL maternal toxic effects

Not specified in report

5.3.2 NO(A)EL maternal toxic effects

A no-observed-effect-level (NOEL) for maternal animals was established as 300mg/kg bw/day, based on the occurrence of a transient decrease in weight gain and food consumption and increased water consumption in maternal rats at 1000mg/kg bw/day.

5.3.3 LO(A)EL embryotoxic / teratogenic effects

Not specified in report

Section A6.8.1-1		Teratogenicity Study	
Annex Point IIA6.8.1		Oral, rat	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	A no-observed-effect-level (NOEL) for embryonic development was established as 1000mg/kg bw/day, based on the absence of effects on embryonic development and no excess incidences of skeletal and visceral abnormalities and variations at this dose level.	
5.3.5	Reliability	1	
5.3.6	Deficiencies	No	

Table A6.8.1.1-1: Animal assignment and treatment

Group number	Dose level of dinotefuran (mg/kg/day)	Number of females
1	0 (vehicle)	24
2	100	24
3	300	24
4	1000	24

Table A6.8.1.1-2: Group mean body weights and weight gains of pregnant animals

Treatment group (mg/kg bw/day)	Mean body weight on day 6 (g)	Mean body weight gain (g) on days:			Mean body weight on day 20 (g)
		6 -11	6 -15	6 -20	
0	280	21.9	41.2	107	386
100	281	22.0	40.3	110	390
300	281	22.2	43.0	109	390
1000	276	17.3*	37.3	97	373

* $p < 0.05$

Table A6.8.1.1-3: Group mean caesarean data

Parameter	0 mg/kg	100mg/kg	300mg/kg	1000mg/kg
No. pregnant / no. mated	22 / 24	22 / 24	20 / 24	20 / 24
No. corpora lutea ± SD (mean/dam)	15.6 ± 2.0	16.2 ± 1.6	15.3 ± 2.7	15.7 ± 2.1
No. implantations ± SD (mean/dam)	14.2 ± 3.0	14.8 ± 1.6	14.1 ± 3.5	12.2 ± 5.3
Pre-implantation loss (%)	9.2	8.3	10.1	24.0
Total embryofetal loss (%)	5.1	5.1	3.4	3.6
- Implant remnant (%)	0.0	0.0	0.0	0.0
- Retained placenta (%)	4.3	3.7	3.4	2.7
- Early death (%)	0.0	1.1	0.0	0.9
- Late death (%)	0.8	0.0	0.0	0.0
- Macerated fetuses (%)	0.0	0.3[b]	0.0	0.0
No. live fetuses ± SD (mean/dam)	13.5 ± 3.1	14.0 ± 2.1	13.6 ± 3.4	11.8 ± 5.3
Sex ratio (% males)[a]	53.0	55.7	50.4	42.8
Mean body weight ± SD (g) - males	3.73 ± 0.29	3.72 ± 0.19	3.83 ± 0.23	3.71 ± 0.25
Mean body weight ± SD (g) - females	3.55 ± 0.23	3.51 ± 0.22	3.65 ± 0.23	3.47 ± 0.35
Live fetuses with external abnormality (%)	0.0	0.0	0.0	0.0

[a] re-calculated by reviewer;

[b] conjoined twin macerated fetuses

Table A6.8.1.1-4: Group mean skeletal and visceral examination data

Parameter	0 mg/kg	100mg/kg	300mg/kg	1000mg/kg
No. litters examined	22	22	19	20
No. fetuses examined (skeletal)	143	150	132	114
Total no. abnormal fetuses (skeletal):	0	0	0	0
Skeletal variations (mean %) ± SD:				
Total variations	18.4 ± 17.5	7.6 ± 12.4	10.5 ± 14.9	12.1 ± 23.3
- cervical rib	1.2 ± 4.0	0.0 ± 0.0	0.7 ± 2.9	0.6 ± 2.5
- 14th rib	13.6 ± 16.6	7.6 ± 12.4	9.1 ± 14.6	11.5 ± 23.5
- shortened 13th rib	3.6 ± 11.2	0.0 ± 0.0	0.8 ± 3.3	0.0 ± 0.0
Mean no. ossification centres ± SD:				
- caudal centra	2.7 ± 1.0	2.6 ± 0.7	2.8 ± 0.8	2.7 ± 0.8
- caudal arches	0.8 ± 0.5	0.8 ± 0.4	0.9 ± 0.4	0.8 ± 0.3
- forelimb phalanges	2.7 ± 1.0	2.8 ± 0.5	2.8 ± 0.6	2.5 ± 1.1
- hindlimb phalanges	2.5 ± 1.2	2.4 ± 1.1	2.5 ± 1.2	1.9 ± 1.4
No. fetuses examined (visceral)	155	159	140	122
No. abnormal fetuses (visceral):				
Total abnormal fetuses (mean %) ± SD	5.0 ± 9.0	3.5 ± 11.1	8.4 ± 22.9	5.1 ± 11.9
- thymic remnant	3.1 ± 7.3	3.5 ± 11.1	2.7 ± 7.2	4.6 ± 11.9
- microphthalmia	0.6 ± 2.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
- ectopic ovary	0.8 ± 3.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
- pyeloectasia	0.0 ± 0.0	0.0 ± 0.0	5.7 ± 22.4	0.0 ± 0.0
- ureteroectasia	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 22.4	0.0 ± 0.0
- left umbilical artery	1.3 ± 4.3	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 2.5

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*22 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 A6.8.1-2 Teratogenicity Study
Annex Point IIA6.8.1 Oral, rabbit

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1998c, A single and 2-week repeated dose preliminary study of MTI-446 given orally to rabbits, [redacted], unpublished report no. H-97164, January 16, 1998. [redacted], 1998d, A dose finding teratogenicity study of MTI-446 given orally to rabbits, [redacted], unpublished report no. H-97165, June 8, 1998. [redacted], 1998e, Teratogenicity study of MTI-446 given orally to rabbits, [redacted], unpublished report no. H-97166, December 3, 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	Rabbit
3.2.2	Strain	New Zealand White
3.2.3	Source	[redacted]
3.2.4	Sex	Females
3.2.5	Age/weight at study initiation	5 – 6 months old, weighing 2.7-3.6 kg
3.2.6	Number of animals per group	22 mated females per group See Table A6.1.1.2-1

3.2.7	Control animals	Yes
3.2.8	Mating period	Not specified in the report
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	Rabbit, day 6-18, post mating
3.3.2	Post-exposure period	10 days
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	Nominal concentration 0, 52, 125 or 300 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, recorded on day 0 (not reported), daily from day 6 to day 19, days 21, 23, 25 and 27 of gestation and on the day of necropsy.
3.4.2	Food consumption	Yes, food and water consumption were recorded daily from day 6 of gestation until necropsy.
3.4.3	Clinical signs	Yes, recorded 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 2% potassium hydroxide 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. Gross lesions were preserved for subsequent histological examination. The uterine contents were classified as live or dead fetuses, placental remnants, early or late resorptions, or macerated fetuses.
3.4.5	Examination of foetuses	
3.4.5.1	General	Live fetuses were weighed and examined for external and oral cavity abnormalities. The thoracic and abdominal organs were examined macroscopically and sexes recorded by examination of the internal reproductive organs.
3.4.5.2	Skelet	Yes, the carcasses of all fetuses were subjected to skeletal evaluation using a dual staining technique for cartilage and bone and examined for skeletal malformations and variations including enumeration of ossification centers in vertebrae, metacarpals, metatarsals, and right limb proximal and medial phalanges.
3.4.5.3	Soft tissue	Yes, The heads of approximately one half of the fetuses from each litter and the thoracic viscera from all fetuses were examined for soft-tissue malformations and variations by fixation in Bouin's solution and subsequent micro-dissection of the cranial 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.

3.5 Statistics

Where appropriate, data were analysed for homogeneity of variance using Bartlett's test followed by one-way ANOVA or Dunnett's test and the Kruskal-Wallace H-test. Percentages were analysed using the Kruskal-Wallace H-test and Dunnett's test.

4 RESULTS AND DISCUSSION

4.1 Maternal toxic Effects

There were no treatment-related deaths or abortions during the study, but a control animal died on day 7 of gestation due to mal-dosing. One animal to be treated at 300mg/kg bw/day was excluded from the study on day 6 due to body weight loss prior to the start of treatment. Clinical signs of toxicity were confined to the group treated at 300mg/kg bw/day. This group showed hypoactivity, prone position, panting, flushing of the nose and ears and tremors from the start of treatment until day 14 of gestation. The mean body weight gain of the group treated at 300mg/kg bw/day was significantly ($p < 0.05$ or $p < 0.01$) reduced by 50% during the treatment period (Table A6.8.1.2-2). Subsequently, the group gained weight at a greater rate than the controls and at termination body weights were similar to the control group. The mean body weight gain of the group treated at 125mg/kg bw/day was slightly but significantly ($p < 0.05$) reduced on day 8 of gestation only. The mean weight gain of the group treated at 52mg/kg bw/day was unaffected by treatment with dinotefuran. The mean food consumption during treatment of the group treated at 300mg/kg bw/day was reduced by 22.7% and water consumption was significantly ($p < 0.05$) reduced by up to 34.2% on days 14 to 16 of gestation. The food and water consumption of the groups treated at 125 or 52mg/kg bw/day were unaffected by treatment with dinotefuran.

4.2 Teratogenic / embryotoxic effects

Treatment-related macroscopic findings in maternal animals occurred at 125 and 300mg/kg bw/day. Pale brown discoloration of the liver and gray/white plaque formation in the fundic region of the stomach occurred in most animals treated at 300mg/kg bw/day and in a smaller proportion of animals treated at 125mg/kg bw/day (Table A6.8.1.2-3). One animal at 300mg/kg b.w./day also showed liver enlargement. Histological examination of representative maternal liver and stomach lesions revealed no correlative histopathological alterations. No macroscopic changes occurred at 52mg/kg bw/day.

Litter parameters as assessed by pregnancy incidence, numbers of corpora lutea, implantations and live fetuses, post-implantation loss, external anomalies, fetal weights and sex ratio, were unaffected by treatment at all dose levels (Table A6.8.1.2-4).

There were no treatment-related effects on the incidence and nature of skeletal and visceral abnormalities and variants at any dose level (Table A6.8.1.2-6). The incidences of skeletal and visceral abnormalities and skeletal variants were not significantly different ($p > 0.05$) from the control group. Although one litter from a dam treated at 300mg/kg bw/day had 3 fetuses with hydrocephalus, this abnormality occurs spontaneously in rabbits of the strain and source employed. Delayed ossification, measured by the number of vertebral and phalangeal ossification centers, was not apparent at any dose level.

4.3 Other effects

None

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

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

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)

No relevant deviations from test guidelines

Method:

Four groups of 22 mated female NZW rabbits were treated orally, by gavage, from day 6 to day 18 of gestation, at dose levels of 0, 52, 125 or 300 mg/kg/day at 10 mL/kg in 0.5% aqueous carboxymethyl cellulose. The animals were killed on Day 28 for reproductive assessment and fetal examination.

5.2 Results and discussion

There were no treatment-related deaths or abortions during the study. Clinical signs of toxicity were confined to the group treated at 300mg/kg bw/day that showed hypoactivity, prone position, panting, flushing of the nose and ears and tremors from the start of treatment until day 14 of gestation. The mean body weight gain of the group treated at 300mg/kg bw/day was markedly reduced during the treatment period. Subsequently, the group gained weight at a greater rate than the controls and at termination body weights were similar to the control group. The mean body weight gain of the group treated at 125mg/kg bw/day was slightly but significantly reduced on day 8 of gestation only. The mean food consumption during treatment of the group treated at 300mg/kg bw/day was reduced and water consumption was reduced on days 14 to 16 of gestation. The food and water consumption of the groups treated at 125 or 52mg/kg bw/day were unaffected by treatment with dinotefuran.

Treatment-related macroscopic findings in maternal animals occurred at 125 and 300 mg/kg bw/day. Pale brown discoloration of the liver and gray/white plaque formation in the fundic region of the stomach occurred in most animals treated at 300 mg/kg bw/day and in some animals at 125 mg/kg bw/day. One animal at 300 mg/kg b.w./day also showed liver enlargement. There were no correlative histopathological findings.

Litter parameters were unaffected by treatment at all dose levels. There were no treatment-related effects on the incidence and nature of skeletal and visceral abnormalities and variants at any dose level. Delayed ossification, measured by the number of vertebral and phalangeal ossification centers, was not apparent at any dose level.

5.3 Conclusion

5.3.1 LO(A)EL maternal toxic effects

Not specified in report

5.3.2 NO(A)EL maternal toxic effects

A no-observed-effect-level (NOEL) for pregnant rabbits was established as 52mg/kg bw/day, based on the occurrence of a minimal reduction in weight gain and macroscopic necropsy findings at 125mg/kg bw/day, and reduced weight gain, food and water consumption and macroscopic necropsy findings in maternal animals treated at 300mg/kg bw/day.

5.3.3 LO(A)EL embryotoxic / teratogenic effects

Not specified in report

5.3.4	NO(A)EL embryotoxic / teratogenic effects	A no-observed-effect-level (NOEL) for embryonic development was established as 300mg/kg bw/day, based on no effects on embryonic development and no excess incidences of skeletal and visceral abnormalities and variants at this dose level.
5.3.5	Reliability	1
5.3.6	Deficiencies	Yes, a dual staining technique for cartilage and bone was employed as specified in OECD revised draft guideline no. 414(1996). The deviation does not affect the validity or integrity of the study.

Table A6.08.1.2-1: Animal assignment and treatment

Group number	Dose level ofdinotefuran (mg/kg/day)	Number of females
1	0 (vehicle)	22
2	52	22
3	125	22
4	300	22

Table A6.8.1.2-2: Group mean body weights and weight gains of pregnant rabbits

Treatment group (mg/kg bw/day)	Mean body weight (kg) on day:				Mean weight gain (kg):	
	6	8	19	28	Day 6-18	Day 19-28
0	3.31	3.33	3.53	3.71	0.22	0.18
52	3.27	3.28	3.51	3.67	0.24	0.16
125	3.42	3.39	3.61	3.78	0.19	0.17
300	3.37	3.33	3.48	3.73	0.11	0.25
	Mean food consumption (g/animal/day) on day:					
	6	8	18	28	Day 6-18	Day 19-28
0	155	154	149	114	154	124
52	154	161	159	115	158	123
125	159	138	137	123	147	123
300	152	118*	143	136	119	142

* $p < 0.05$

Table A6.8.1.2-3: Nature and incidence of treatment related macroscopic findings in maternal animals

Macroscopic finding	Incidence of macroscopic findings at necropsy at (mg/kg bw/day):			
	0	52	125	300
Number examined*	22	22	22	22
Pale brown discoloration of liver	0	0	8	20
Liver enlargement	0	0	0	1
Gray-white discoloration of gastric mucosa	0	0	0	1
Gray-white plaque in fundus of stomach	0	0	15	20
Thickening of gastric mucosa	0	0	0	2

* including non-pregnant animals

Table A6.8.1.2-4: Group mean caesarean data

Parameter	Group value at (mg/kg bw/day):			
	0	52	125	300
No. pregnant / no. mated	20 / 22	19 / 22	21 / 22	19 / 22
Mean no. corpora lutea ± SD	8.6 ± 1.9	8.5 ± 2.1	9.0 ± 2.3	8.8 ± 1.7
Mean no. implantations ± SD	7.9 ± 2.0	8.3 ± 2.3	8.7 ± 2.7	8.4 ± 1.6
Pre-implantation loss (%)	7.7	3.2	5.0	4.4
Total dead fetuses (%)	5.1	2.1	6.9	6.9
- Implant remnant (%)	0	0	0	0
- Retained placenta (%)	4.1	2.1	2.9	1.8
- Early death (%)	0	0	1.3	0.5
- Late death (%)	0.5	0	2.1	3.6
- Macerated fetuses (%)	0.5	0	0.6	1.0
Mean no. live fetuses ± SD	7.6 ± 2.2	8.1 ± 2.2	8.1 ± 2.8	7.7 ± 1.6
Sex ratio (M / F x 100)	59.2	43.9	52.0	51.6
Mean body weight ± SD (g) - males	42.7 ± 5.9	40.6 ± 6.2	39.7 ± 7.1	40.4 ± 5.3
Mean body weight ± SD (g) - females	42.1 ± 6.3	39.9 ± 6.2	38.7 ± 6.6	39.9 ± 6.0
No. (%) fetuses with external abnormality	0 (0.0)	1 (0.9)	2 (2.1)	1 (0.7)
- omphalocele	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)
- gastroschisis	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
- cleft palate + hydrocephalus	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
- club foot + arthrogryposis (fore limbs)	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)

Table A6.8.1.2-5: Group mean skeletal and visceral examination data

Parameter	Group value at (mg/kg bw/day):			
	0	52	125	300
No. litters	20	19	21	19
No. fetuses examined (skeletal)	151	153	170	147
No. abnormal fetuses (%) mean ± SD:	0.9 ± 2.8	0.0	0.6 ± 2.7	0.0
- separation of sternebrae	0.9 ± 2.8	0.0	0.0	0.0
- flexion of caudal sternebrae	0.0	0.0	0.6 ± 2.7	0.0
Skeletal variants (%) mean ± SD:				
Total variants	58.9 ± 24.8	70.2 ± 30.5	60.7 ± 27.0	67.6 ± 21.0
- lumbar rib	49.4 ± 25.5	55.7 ± 33.2	60.7 ± 27.0	55.4 ± 23.1
- sacralisation	0.0	2.3 ± 6.2	0.6 ± 2.7	1.9 ± 6.1
- 8th lumbar	42.5 ± 25.3	66.3 ± 28.1	57.3 ± 33.4	58.3 ± 25.3
- asymmetrical sternebrae	0.0	0.0	0.0	0.6 ± 2.5
Ossification centers (%) mean ± SD:				
- caudal centra	16.1 ± 0.5	16.0 ± 0.3	16.0 ± 0.4	16.1 ± 0.5
- caudal arches	8.1 ± 0.3	8.1 ± 0.3	8.0 ± 0.3	8.0 ± 0.4
- forelimb phalanges	13.9 ± 0.2	14.0 ± 0.1	13.9 ± 0.2	14.0 ± 0.0
- hindlimb phalanges	12.0 ± 0.2	12.0 ± 0.2	12.0 ± 0.1	12.0 ± 0.0
No. fetuses examined (visceral)	151	153	170	138
No. abnormal fetuses:				
Total (%) mean ± SD	1.6 ± 5.1	0.7 ± 2.9	2.9 ± 8.2	3.0 ± 9.5
hydrocephalus	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 9.1
dilatation of lateral ventricle	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 2.9	0.0 ± 0.0
dilatation of 3rd ventricle	0.6 ± 2.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
supernumerary coronary ostium	1.0 ± 4.5	0.0 ± 0.0	0.5 ± 2.3	0.8 ± 3.5
ventricular septal defect	0.0 ± 0.0	0.7 ± 2.9	0.0 ± 0.0	0.0 ± 0.0
patent ductus arteriosus	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 3.5
persistent truncus arteriosus	0.0 ± 0.0	0.7 ± 2.9	0.0 ± 0.0	0.0 ± 0.0
hydronephrosis	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 7.6	0.0 ± 0.0
ureteroectasia	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 7.6	0.0 ± 0.0

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21 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 A6.8.1-3 Teratogenicity Study
Annex Point IIA6.8.1 Oral, rabbit

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 2013, Dinotefuran: prenatal developmental toxicity study in rabbits, [redacted], unpublished report no. [redacted]12005, January 28, 2013. [redacted], 2012, Dinotefuran: preliminary prenatal developmental toxicity study in rabbits, [redacted], unpublished report no. [redacted]12004, October 03, 2012.
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 (main study) OECD guideline no. 414 (2001) , which is equivalent to method B.31 (Annex 2F, Corrigendum to Directive 2004/73/EC) US-EPA OPPTS 870.3700 (August 1998) Notification no. 12-Nousan-8147 of the APB, MAFF (November 2000) and its latest amendment no. 22-Shouan-10015 (April 2011).
2.2	**GLP**	Yes (main study)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	K1223134
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.6%
3.1.2.3	Stability	CoA Expiry date: April 2017
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	Kbl:JW
3.2.3	Source	[redacted]
3.2.4	Sex	Females
3.2.5	Age/weight at study initiation	20 weeks old, weighing 3.27 – 4.25 kg
3.2.6	Number of animals per group	25 mated females per group See Table 1
3.2.7	Control animals	Yes

3.2.8	Mating period	11 days
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	Gestation days 6-27, day of mating = day 0.
3.3.2	Post-exposure period	1 day
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	Nominal concentrations: 0, 12, 35, 100 mg/mL Nominal dose levels: 0, 60, 175 or 500 mg/kg/day
3.3.5	Vehicle	0.5% aqueous carboxymethyl cellulose
3.3.6	Total volume applied	5 mL/kg
3.3.7	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Recorded on gestation day 0 and daily from day 6 to day 28 (day of necropsy).
3.4.2	Food consumption	Measured throughout gestation until necropsy for the following intervals: days 0 – 3, 3 – 6, 6 – 9, 9 – 12, 12 – 15, 15 – 18, 18 – 21, 21 – 24, 24 – 27, and 28.
3.4.3	Clinical signs	Animals examined at least once daily on non-treatment days and at least twice daily, before and after treatment, during the treatment period.
3.4.4	Examination of uterine content	The uterine tract and ovaries were removed and pregnancy was confirmed. If implantations were not visible macroscopically, the uterus was stained with 10% ammonium sulphide to aid visualization of very early resorptions. All major maternal organs and tissues were examined macroscopically, including ovaries (with corpora lutea count) and uteri (implantation site count). Gravid uterine weight was recorded. The uterine contents were removed and embryos, fetuses and placentae examined and classified as live or dead fetuses, placental remnants, early or late resorptions, or macerated fetuses. Fetuses were labeled to uniquely identify location in uterus.
3.4.5	Examination of foetuses	
3.4.5.1	General	All live fetuses were weighed and examined for external and oral cavity abnormalities including all orifices. Placentae were weighed, and fixed in 10% neutral buffered formalin. The sexes were recorded by examination of the internal reproductive organs.
3.4.5.2	Skeletal	The carcasses of all fetuses were subjected to skeletal evaluation after staining with alizarin red S and examined for skeletal malformations and variations including enumeration of ossification centers in vertebrae, sternebrae, and all limb phalanges.
3.4.5.3	Soft tissue	Approximately one half of the fetuses/litter had the head severed at the atlas and preserved for free-hand razor sectioning (Wilson's technique[1]). In the other fetuses, an incision was made in the skull to observe the ventricles. All fetuses were necropsied and examined with tissues and organs in situ. The thoracic and abdominal organs were removed, preserved and then examined for soft-tissue abnormalities

and variations. The heart was examined for abnormalities by Nishimura's microdissection method[2].

3.5 Statistics

Where appropriate, data were analysed for homogeneity of variance using Bartlett's test followed by one-way ANOVA, followed by Dunnett's test if significant or the Kruskal-Wallis test when variances were not homogeneous, for differences among groups. Steel's test was used to detect differences between treated and control groups.

4 RESULTS AND DISCUSSION

4.1 Maternal toxic Effects

There was one treatment-related death and an increased incidence of premature delivery or abortion (3/24 = 12.5%) at 500 mg/kg/day. Premature delivery or abortion was considered to be related to markedly reduced food consumption from the onset of treatment. Two dams at 175 mg/kg/day and one control dam also showed premature delivery or abortion during the study, but the incidence at 175 mg/kg/day (2/25 = 8.0%) remained within the historical control range of 0 – 8.3%. One dam at 60 mg/kg/day died on day 25 following premature delivery, but was considered not to be test substance related because at necropsy there was evidence of gavage dosing error.

All dams at 500 mg/kg/day showed tachypnea on days 6 and 7, and increased incidences of small amount or no feces and reduced urine output.

Body weight gain at 500 mg/kg/day was significantly reduced throughout the treatment period, resulting in slight overall group mean body weight loss from day 6 to day 28. (Table 2). Body weight gain was not affected at lower dose levels. Food consumption at 500 mg/kg/day was lower than control consumption throughout the dosing period, and was significantly lower on gestation days 6–24 (up to 47% lower on gestation days 12-15). Food consumption was not affected at lower dose levels (Table 2).

There were no maternal, treatment-related necropsy findings at any dose level. All necropsy findings in treated animals occurred in isolation or at comparable incidence to the controls.

There was no effect on pregnancy incidence, 22, 23, 23 and 20 pregnant animals survived to scheduled necropsy at 0, 60, 175 and 500 mg/kg/day, respectively. Live litters were obtained from all animals with the exception of one animal at 175 mg/kg/day with one resorbed implantation site and one at 500 mg/kg/day with two resorbed implantation sites.

The NOAEL for maternal effects was 175 mg/kg/day.

4.2 Teratogenic / embryotoxic effects

Litter parameters as assessed by gravid uterine weight, numbers of corpora lutea, implantations and live fetuses, post-implantation loss, external abnormalities, fetal and placental weights and sex ratio, were not significantly different from the controls and were unaffected by treatment at all dose levels (Table 3). Although post-implantation losses at 175 and 500 mg/kg/day (11.3 and 14.2%) were higher than the control incidence of 6.1%, the differences were due almost entirely to a single incidence of 100% post-implantation loss in each of the two groups. The mean number of live foetuses was unaffected by treatment at all dose levels.

There were no treatment-related effects on the incidence and nature of fetal external abnormalities, skeletal and visceral abnormalities and variants at any dose level (Table 4). The incidences of skeletal and visceral abnormalities and skeletal variants were not significantly different ($p > 0.05$) from the control group. Delayed ossification was not apparent at any dose level, as assessed by the number of vertebral,

		sternebral and phalangeal ossification centres Table 5). The NOAEL for embryofetal effects was 500mg/kg bw/day, the highest dose level tested.	
4.3	**Other effects**	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Guidelines: OECD guideline no. 414 (1981) , equivalent to method B.31 (Annex 2F, Corrigendum to Directive 2004/73/EC) US-EPA OPPTS 870.3700 (August 1998) Notification no. 12-Nousan-8147 of the APB, MAFF (November 2000) and its latest amendment no. 22-Shouan-10015 (April 2011). No relevant deviations from test guidelines Method: Four groups of 25 mated Kbl:JW female rabbits were treated orally, by gavage, from day 6 to day 27 of gestation, at dose levels of 0, 60, 175 or 500 mg/kg bw/day at 5 mL/kg in 0.5% aqueous carboxymethyl cellulose. The animals were killed on Day 28 for reproductive assessment and fetal examination.	
5.2	**Results and discussion**	There was one treatment-related death and an increased incidence of premature delivery/abortion at 500 mg/kg/day, the latter considered to be related to markedly reduced food consumption from the onset of treatment. All dams at 500 mg/kg/day showed tachypnea on days 6 and 7, and increased incidences of small amount or no feces and reduced urine output. Tachypnea did not occur at lower dose levels. Body weight gain and food consumption at 500 mg/kg/day were significantly reduced throughout the treatment period, resulting in slight overall group mean body weight loss from day 6 to day 28. These effects did not occur at lower dose levels. There were no maternal, treatment-related necropsy findings at any dose level. There was no effect on pregnancy incidence or the number of live litters at termination at any dose level. Litter parameters were unaffected by treatment at all dose levels. There were no effects on the incidence and nature of fetal external abnormalities, skeletal and visceral abnormalities and variants at any dose level. Delayed ossification was not apparent at any dose level.	
5.3	**Conclusion**		
5.3.1	LO(A)EL maternal toxic effects	500 mg/kg bw/day, based on the occurrence of one death, tachypnea, reduced food consumption, body weight loss and increased incidence of premature delivery/abortion.	
5.3.2	NO(A)EL maternal toxic effects	A no-observed-effect-level (NOEL) for pregnant rabbits was established as 175 mg/kg bw/day.	
5.3.3	LO(A)EL embryotoxic / teratogenic effects	Not applicable. No embryotoxicity or teratogenicity at the highest dose level employed.	X1
5.3.4	NO(A)EL embryotoxic / teratogenic effects	A no-observed-effect-level (NOEL) for embryonic development was established as 500mg/kg bw/day, based on no effects on embryonic development and no excess incidences of skeletal and visceral abnormalities and variants at this dose level.	X2
5.3.5	Reliability	1	
5.3.6	Deficiencies	None.	

Table 1: Animal assignment and treatment

Group number	Dose level of dinotefuran (mg/kg/day)	Number of females
1	0 (vehicle)	25
2	60	25
3	175	25
4	500	25

Table 2: Group mean body weights and weight gains of pregnant rabbits

Treatment group (mg/kg bw/day)	Mean body weight (kg) on day:					Adjusted body weight (kg)
	6	9	15	24	28	
0	3.86	3.84	3.91	3.96	4.02	3.62
60	3.91	3.91	3.98	4.03	4.08	3.64
175	3.85	3.83	3.84	3.91	3.98	3.56
500	3.84	3.77	3.73*	3.69**	3.83	3.45
	Mean food consumption (g/animal/day) on days:					
	6 – 9	9 – 12	12 – 15	15 – 18	18 – 21	24 – 27
0	156	142	136	136	136	105
60	165	152	131	143	143	101
175	155	136	108	127	127	96.2
500	120**	98.7**	63.9**	87.7**	87.7**	82.1

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

Table 3: Summary of group caesarean data

Parameter	Group value at (mg/kg bw/day):			
	0	60	175	500
No. pregnant / no. mated	22 / 25	23 / 25	23 / 25	20 / 25
Mean no. corpora lutea ± SD	10.0 ± 1.8	9.9 ± 2.1	10.0 ± 2.4	10.0 ± 2.5
Mean no. implantations ± SD	7.7 ± 2.7	8.6 ± 2.8	8.6 ± 2.8	8.2 ± 2.9
Pre-implantation loss index[c] (%) ± SD	22.0 ± 24.8	14.5 ± 21.5	14.9 ± 19.1	17.9 ± 20.6
Dead/resorbed embryos – means/litter				
- early stage[a]	0.3	0.5	0.7	0.5
- late stage[b]	0.1	0.2	0.0	0.5
- total	0.4	0.7	0.7	0.9
- incidence (%)	6.1	8.9	11.3	14.2
Mean no. live fetuses ± SD	7.4 ± 2.8	7.9 ± 2.8	7.9 ± 2.7	7.3 ± 2.9
Sex ratio (% M)	50.1	56.2	46.9	52.2
Mean body weight ± SD (g) - males	38.9 ± 6.1	39.8 ± 6.4	38.3 ± 3.8	36.8 ± 6.6
Mean body weight ± SD (g) - females	37.7 ± 3.8	38.8 ± 4.0	36.5 ± 4.4	35.7 ± 5.8
Mean placental weight (g)	5.46	5.79	5.10	5.30
No. litters examined	22	23	22	19
No. foetuses examined	162	181	182	145
No. foetuses with external abnormality	1	0	0	0
- omphalocele	1	0	0	0

[a] includes implantation sites and placental remnants
[b] includes macerated and dead term foetuses
[c] [(no. corpora lutea – no. implantations)/no. corpora lutea] x 100

Table 4: Group mean skeletal and visceral examination data

Parameter	Group value at (mg/kg bw/day):			
	0	60	175	500
SKELETAL:				
Heads:				
Litters examined	22	23	22	19
Fetuses examined	81	92	89	74
No. fetuses with skeletal abnormality	0	0	0	0
Bodies:				
Litters examined	22	23	22	19
Fetuses examined	162	181	182	145
No. (%) fetuses with skeletal abnormality:	1 (0.51)	0	5 (2.65)	0
- lumbar hemivertebra	0	0	1 (0.41)	0
- bifurcation of rib cartilage	0	0	4 (2.24)	0
- supernumerary sternebrae	0	0	4 (2.24)	0
- fusion of sternebrae	1 (0.51)	0	0	0
Heads:				
Litters examined	22	23	22	19
Fetuses examined	81	92	89	74
No. fetuses with skeletal variation	0	0	0	0
Bodies:				
Litters examined	22	23	22	19
Fetuses examined	162	181	182	145
No. (%) fetuses with skeletal variation:	72 (45.74)	62 (33.98)	73 (38.61)	49 (34.21)
- 8 lumbar vertebrae	2 (0.91)	0	6 (2.79)	2 (1.32)
- Lumbarisation of sacral vertebra	0	4 (1.59)	2 (0.92)	1 (0.66)
- Cervical rib	0	1 (0.48)	2 (1.02)	1 (0.53)
- Lumbar rib	46 (28.68)	36 (21.30)	36 (18.89)	25 (16.36)
- 13th ribs	4 (3.64)	6 (3.42)	4 (2.35)	5 (3.50)
- Shortening of 12th rib	1 (1.14)	0	0	0
- Absence of 12th rib	1 (1.14)	0	0	0
- Unossified sternebra(e)	16 (9.35)	14 (6.11)	24 (12.72)	16 (12.50)
- Incompletely ossified sternebra	1 (1.14)	0	3 (1.53)	0
- Bipartite ossification of sternebra	4 (2.47)	2 (2.66)	0	2 (1.32)
- Supernumerary ossification site in sternum	7 (3.35)	6 (3.43)	4 (1.84)	2 (1.58)
VISCERAL:				
Heads fixed in Bouins:				
Litters examined	22	22	22	19
Fetuses examined	81	89	93	71
No. fetuses with visceral abnormality	0	0	0	0
Fresh heads:				
Litters examined	22	23	22	19
Fetuses examined	81	92	89	74
No. fetuses with visceral abnormality	0	0	0	0
Bodies:				
Litters examined	22	23	22	19
Fetuses examined	162	181	182	145
No. foetuses with visceral abnormality	0	2	1	0
- persistent ductus arteriosus	0	0	1	0
- narrowed aorta	0	1	0	0
- ventricular septal defect	0	1	1	0
- abnormal lung lobation	0	1	0	0
- abnormal liver lobation	0	1	0	0
No. foetuses with visceral variations:				
Litters examined	22	23	22	19
Fetuses examined	162	181	182	145
No. fetuses with visceral variation	0	0	0	0

Table 5: Group mean fetal skeletal development data

Parameter	Mean no. ossification centres[a] at (mg/kg bw/day):			
	0	60	175	500
Litters examinedFetuses examined	22 162	23 181	22 182	19 145
Sacrovertebral body	19.22	19.17	19.18	19.06
Sternebra	5.91	5.94	5.87	5.88
Forelimb phalanges – proximal (L / R)	4.97 / 4.97	5.00 / 5.00	5.00 / 4.99	5.00 / 5.00
Forelimb phalanges – medial (L / R)	3.94 / 3.91	3.99 / 3.98	3.95 / 3.95	3.96 / 3.95
Forelimb phalanges – distal (L / R)	5.00 / 5.00	5.00 / 5.00	5.00 / 5.00	5.00 / 5.00
Hindlimb phalanges – proximal (L / R)	4.00 / 4.00	4.00 / 4.00	4.00 / 4.00	4.00 / 4.00
Hindlimb phalanges – medial (L / R)	3.99 / 3.97	4.00 / 4.00	4.00 / 4.00	4.00 / 4.00
Hindlimb phalanges – distal (L / R)	4.00 / 4.00	4.00 / 4.00	4.00 / 4.00	4.00 / 4.00

[a] The litter is the unit evaluated

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	10 October 2013
Materials and Methods	As described by Applicant
Results and discussion	As described by Applicant
Conclusion	X1 & X2 The LOAEL and NOAEL for embryotoxic/teratogenic effects are 500 and 175 mg/kg/day, respectively, based on 3/24 abortions at 500 mg/kg/day. In the opinion of the RMS, abortions should be regarded as an adverse effect on development, although we agree that these abortions are likely to be a secondary consequence of the prolonged and severe reduction in maternal food consumption observed in this group. The RMS agrees that there were no treatment-related effects on the other developmental parameters, including post-implantation loss, litter size, foetal weights and sex ratios, and incidence of foetal malformations and variants.
Reliability	As described by Applicant
Acceptability	Acceptable
Remarks	Table 2 contains typographical errors; mean food consumption on days 15-18 at 60, 175 and 500 mg/kg/day should be 147, 111 and 71** g/animal/day, respectively.

COMMENTS FROM ...

Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.8.2-1
Annex Point IIA6.8.2

Multigeneration Reproduction Toxicity Study
Rat
Oral

Official use only

1 REFERENCE

1.1 Reference

[redacted], 2001, MTI-446 technical preliminary two generation study in the Han Wistar rat, [redacted], unpublished report no. 774990, August 8, 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 No applicable EU guideline (dose range-finding study)

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: 5400810

3.1.2 Specification

3.1.2.1 Description: Solid

3.1.2.2 Purity: 98.9%

3.1.2.3 Stability: Expiration date: 14 March 2005

3.2 Test Animals Non-entry field

3.2.1 Species: Rat

3.2.2 Strain: Hanlbm: WIST (SPF)

3.2.3 Source: [redacted]

3.2.4 Sex: Males and females

3.2.5 Age/weight at study initiation: 11 – 12 weeks old, weighing 279-345 g for males and 182-209 g for females

3.2.6 Number of animals per group: 6/sex/group

3.2.7 Mating: The P generation animals were randomly grouped using a computer-generated random algorithm:

Group number	Dose level of dinotefuran (ppm)	Number of P generation animals	
		Male	Female
1	0	6	6
2	10000	6	6
3	20000	6	6

3.2.8 Duration of mating: 2 weeks

3.2.9 Deviations from standard protocol: None

3.2.10 Control animals: Yes

3.3 Administration/ Oral

Section A6.8.2-1 Multigeneration Reproduction Toxicity Study
Annex Point IIA6.8.2 **Rat**
Oral

Exposure

3.3.1 Animal assignment to dosage groups

Group number	Dose level of dinotefuran (ppm)	Number of P generation animals	
		Male	Female
1	0	6	6
2	10000	6	6
3	20000	6	6

3.3.2 Duration of exposure before mating: 2 weeks

3.3.3 Duration of exposure in general P, F1, F2 males, females: From beginning of the study until sacrifice of parent and F1generation

Oral

3.3.4 Type: In food

3.3.5 Concentration

Sex / generation	Study period	10000ppm (≡ mg/kg bw/day)	20000ppm (≡ mg/kg bw/day)
Male / P	Pre-mating	700	1340
	Post-mating	637	1254
Female / P	Pre-mating	779	1507
	Gestation	749	1541
	Lactation	1348 - 2145	2436 - 3192
Male / F1	From weaning	745 - 1693	1585 - 3380
Female / F1	From weaning	770 - 1720	1796 - 3534

3.3.6 Vehicle: The calculated amount of dinotefuran was mixed with diet in a Buehler Mixer typr DDMA-0.5 and then pelleted. Water was added to each feed preparation (a volume/weight ratio of approximately 2:10) and the pellets were dried with warm air for approximately 48 hours.

3.3.7 Concentration in vehicle: Not applicable

3.3.8 Total volume applied: Not applicable

3.3.9 Controls: Plain diet

3.4 Examinations

3.4.1 Clinical signs

Parental animals:
At least twice daily
F1 offspring:
Daily

3.4.2 Body weight

Parental animals:
Body weights were recorded weekly except during pairing. After mating, females were weighed on days 0, 7, 14 and 21 of gestation and days 1, 4, 7, 14 and 21 *post partum*.
F1 offspring:
Pups were weighed on days 0/1, 4, 7, 14 and 21 of lactation. F1 animals selected for 2 weeks further treatment were weighed on days 24, 28 and 35 *post partum*

3.4.3 Food/water consumption

Parental animals:
Food consumption was measured weekly throughout the study until day 14 *post partum*, except during mating.

Section A6.8.2-1 **Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2 **Rat**
Oral

		F1 offspring F1 animals selected for 2 weeks further treatment, food consumption was recorded at 4 or 6 day intervals.
3.4.4	Oestrus cycle	During the prepairing period, males and females were housed separately one to a cage. Cages of males were interspersed amongst those holding females to promote the development of regular oestrus cycles.
3.4.5	Sperm parameters	Motility, morphology, cauda epididymal sperm number, organ weights (testis and epididymis).
3.4.6	Offspring	number and sex of pups, stillbirths, live births, presence of gross anomalies, weight gain, physical or behavioural abnormalities.
3.4.7	Organ weights P and F1	Organ weights (testis and epididymis) P males.
3.4.8	Histopathology P and F1	Ovarian histopathology comprising quantitative primordial follicle counts in 10 levels/ovary and a comparison with secondary/tertiary follicles was performed on all females treated at 0 or 20000ppm.
3.4.9	Histopathology F1 not selected for mating, F2	Not performed
3.5	**Further remarks**	Statistics: Univariate ANOVA was used to analyse the significance of inter-group differences. Normally distributed variables were subjected to the Dunnett many-to-one t-test based on a pooled variance and the Steel many-to-one rank test was used for non-normally distributed variables. Fisher's exact test was applied if the data could be dichotomised without loss of information.
		4 RESULTS AND DISCUSSION
4.1	**Effects**	Non-entry field
4.1.1	Parent males	There were no deaths in the P generation at any dose level. Males treated at 20000ppm also showed reduced body weight gain, and significantly ($p < 0.05$) reduced food consumption during the first week of treatment. There were no treatment-related effects at either dose level in P generation males on sperm motility (% progressively motile sperm), sperm morphology and sperm counts (homogenisation-resistant spermatids and caudal epididymal sperm counts).
4.1.2	Parent females	There were no deaths in the P generation at any dose level and treatment-related clinical signs were confined to soft faeces in females at 20000ppm during lactation. There was a treatment- and dose-related decrease in food consumption and body weight gain throughout the treatment period in females treated at 10000 and 20000ppm (Table A6.8.2.1-1). Thus, the group mean body weights were significantly ($p < 0.05$ or 0.01) lower than the control group at the end of the pre-pairing, gestation and lactation periods. Males treated at 20000ppm also showed reduced body weight gain, and significantly ($p < 0.05$) reduced food consumption during the first week of treatment. Treatment-related gross findings at necropsy in the P generation were confined to an increased incidence of small thymus in females at 20000ppm. The incidences were 2/6, 3/6 and 6/6 in the groups treated at 0, 10000 and 20000ppm, respectively. There were no treatment-related gross findings at necropsy in the surplus F1 generation pups culled on day 4 or 21 post partum or in animals selected for 2 weeks treatment after weaning, although one male animal at 20000ppm died in the first week after selection. There was no treatment-related effect

Section A6.8.2-1 **Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2 **Rat**
Oral

		on the number and distribution of ovarian follicle types in females treated at 20000ppm (Table A6.8.2.1-2). Although the chi-square test revealed a significant ($p < 0.001$) difference between the control and treated groups in the distribution of follicle types, the difference was due to a slightly higher number of primordial follicles in the treated group which is considered to be within the normal range of variation. Therefore, dinotefuran technical is considered not to have altered ovarian anatomy at a dose level of 20000ppm. There was no effect of treatment on fertility and mating performance. All females in all experimental groups mated successfully and reared pups to weaning. The median pre-coital time was comparable in the treated and control groups. There was a treatment-related decrease in the number of implantations and increased post-implantation loss leading to a significantly ($p < 0.05$) reduced mean litter size at birth at 20000ppm (Table A6.8.2.1-3). Although the mean number of implantations and litter size at birth at 10000ppm were lower than control values, a treatment effect is not indicated because neither parameter was statistically significant ($p > 0.05$) or the control values were higher than normal.
4.1.3	F1 males	F1 offspring: Neonatal and pre-weaning viability, the weaning indices and sex ratios at 10000 and 20000ppm were not significantly different ($p > 0.05$) from the control group. There was a treatment- and dose-related decrease in the pre-weaning body weight gain of male and female pups at 10000 and 20000ppm (Table A6.8.2.1-3). The effect was statistically significant ($p < 0.05$ and 0.01, respectively) from day 14 *post partum*, and at weaning on day 21, the group mean pup weights for the sexes combined were reduced by 25.2 and 38.0%, respectively. F1 generation animals: The weight gain of F1 generation animals treated for 2 weeks after weaning continued to be depressed in both sexes at both dose levels (Table A6.8.2.1-4). The food consumption of both sexes at both dose levels was also markedly depressed, particularly during the first 4 days of treatment.
4.1.4	F1 females	As reported for F1 males above
4.1.5	F2 males	Not applicable
4.1.6	F2 females	Not applicable
4.2	**Other**	None
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Guidelines: No applicable EU guideline (dose range-finding study) Method: In a dose-range-finding study, groups of 6 male and 6 female P generation Wistar rats were treated orally with dinotefuran at concentrations of 0, 10000 or 20000 ppm in the diet for 2 weeks before mating, and throughout mating, gestation and lactation. Six F1 generation animals/sex/group were similarly treated from weaning for a further 2 weeks. Overall achieved dose levels were within the ranges 700 - 2145 and 1254 - 3534 mg/kg/day.
5.2	**Results and**	P parental animals: There was a treatment- and dose-related decrease in food consumption

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	discussion	and body weight gain throughout the treatment period in females treated at 10000 and 20000ppm. Thus, the group mean body weights were significantly lower than the control group at the end of the pre-pairing, gestation and lactation periods. Males treated at 20000ppm also showed reduced body weight gain, and significantly reduced food consumption during the first week of treatment. There was no effect of treatment on fertility and mating performance. There was a treatment-related decrease in the number of implantations and increased post-implantation loss leading to a significantly reduced mean litter size at birth at 20000ppm. Treatment-related gross findings at necropsy in the P generation were confined to an increased incidence of small thymus in females at 20000ppm. There were no treatment-related gross findings at necropsy in the surplus F1 generation pups culled on day 4 or 21 *post partum* or in animals selected for 2 weeks treatment after weaning. There were no treatment-related effects at either dose level in P generation males on sperm counts, motility and morphology. There was no treatment-related effect on the number and distribution of ovarian follicle types in females treated at 20000ppm. Although the chi-square test revealed a significant difference between the control and treated groups in the distribution of follicle types, the difference was due to a slightly higher number of primordial follicles in the treated group which is considered to be within the normal range of variation. Therefore, dinotefuran is considered not to have altered ovarian anatomy at a dose level of 20000ppm. <u>F1 offspring:</u> There were no effects of treatment on neonatal and pre-weaning viability, weaning indices and sex ratios at 10000 and 20000ppm. There was a treatment- and dose-related decrease in the pre-weaning body weight gain of male and female pups at 10000 and 20000ppm. <u>F1 generation animals:</u> The weight gain of F1 generation animals treated for 2 weeks after weaning continued to be depressed in both sexes at both dose levels. The food consumption of both sexes at both dose levels was also markedly depressed, particularly during the first 4 days of treatment. A no-observed-effect-level (NOEL) was not established in the study, based on the occurrence of reduced weight gain and food consumption in P generation animals and pre-weaning growth retardation in F1 generation progeny at ≥10000ppm, equivalent to a dose level of 637 - 2145mg/kg bw/day. Reduced litter size at birth was also evident at 20000ppm, equivalent to a dose level of 1254 - 1541mg/kg bw/day.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not determined
5.3.1.1	Parent males	Not applicable
5.3.1.2	Parent females	Not applicable
5.3.1.3	F1 males	Not applicable
5.3.1.4	F1 females	Not applicable
5.3.1.5	F2 males	Not applicable
5.3.1.6	F2 females	Not applicable
5.3.2	NO(A)EL	A no-observed-effect-level (NOEL) was not established in the study, based on the occurrence of reduced weight gain and food consumption

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	in P generation animals and pre-weaning growth retardation in F1 generation progeny at 10000ppm, equivalent to a dose level of 637 - 2145mg/kg bw/day. Reduced litter size at birth was also evident at 20000ppm, equivalent to a dose level of 1254 - 1541mg/kg bw/day.	
5.3.2.1 Parent males	As above	
5.3.2.2 Parent females	As above	
5.3.2.3 F1 males	As above	
5.3.2.4 F1 females	As above	
5.3.2.5 F2 males	As above	
5.3.2.6 F2 females	As above	
5.3.3 Reliability	2	
5.3.4 Deficiencies	Yes No applicable EU guideline (dose range-finding study)	

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Table A6.8.2.1-1: Group mean body weight gain and food consumption – P generation

Parameter	Study period	Males treated at (ppm):			Females treated at (ppm):		
		0	10000	20000	0	10000	20000
Body weight gain (g)	Weeks 1 - 2	36	25	14	16	11	6
	Weeks 3 - 7	54	45	41	-	-	-
	Gestation	-	-	-	122	102	82
	Lactation	-	-	-	52	26	7
Food consumed (g/day)	Week 1	24.4	22.6	20.3*	18.6	15.4**	14.6**
	Week 2	24.8	23.6	21.6	18.4	15.7**	16.1*
	Weeks 3 - 7	24.1	24.2	22.3	-	-	-
	Gestation	-	-	-	21.3	18.9	19.6
	Lactation	-	-	-	43.8	40.4	32.9

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

Table A6.8.2.1-2: Summary of varian histopathology

Follicle type	Group totalsa		
	0ppm	10000ppm	20000ppm
Primordial	749	NE	877
Growing	616	NE	665
Secondary/tertiary	1151	NE	1149

[a] total counted in 10 levels/ovary, both ovaries combined, in 6 animals;
NE not evaluated

Table A6.8.2.1-3: Group reproductive data

Reproductive parameter	0ppm	10000ppm	20000ppm
Mean duration of gestation (days)	21.5	22.0	22.0
Total no. of litters born	6	6	6
Mean no. implantations/dam	14.5	12.0	11.5*
Birth index[a]	95.4	95.8	79.7**
Post-implantation loss - total (% implantations)	4 (4.6)	3 (4.2)	14** (20.3)
Live pups at birth (mean/dam)	13.8	11.5	9.2*
Dead pups at birth (mean/dam)	0.0	0.0	0.0
Mean post-natal loss - days 0 - 4 (mean/dam)	0.3	0.0	0.2
Mean no. live pups on day 4	8.0	8.0	7.5
Viability index[b]	97.6	100.0	98.2
Post-natal loss - days 5 - 21 (mean/dam)	0.2	0.0	0.5
Mean no. live pups on day 21	7.8	8.0	7.0
Weaning index[c]	97.9	100.0	93.3
Sex ratio (% males)	49	54	48
Mean M + F body weight (g) on:			
- day 0	5.1	5.3	5.3
- day 4	7.7	7.9	8.1
- day 7	12.9	12.3	11.5
- day 14	29.5	22.4*	19.4**
- day 21	46.8	35.0*	29.0**

[a] no. pups live born x 100 / no. implantations;
[b] no. pups alive day 4 x 100 / no. live-born pups;
[c] no. pups alive day 21 x 100 / no. pups alive on day 4
* $p < 0.05$;
** $p < 0.01$

Table A6.8.2.1-4: Summary of mean body weights and food consumption – F1 generation

Parameter	Study period	Males treated at (ppm):			Females treated at (ppm):		
		0	10000	20000	0	10000	20000
Mean body weight (g)	Day 1	48	35**	31**	46	34**	29**
	Day 14	118	95*	76**	99	84*	70**
Weight gain (g)	Days 1 - 14	70	60	45	53	50	41
Food consumed (g/day)	Days 1 - 4	7.0	3.2**	2.9**	6.3	3.1**	2.8**
	Days 4 - 8	11.9	10.2	8.3**	10.6	9.6	8.2**
	Days 8 - 14	15.8	12.6*	11.0**	13.3	12.0	10.2**

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

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	7 February 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	There is a minor transcription error on Table A6.8.2.1-1: week 2 food consumption for females at 10000 ppm is 15.7 g/day, not 16.7 g.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

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			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 2002, MTI-446 two-generation reproduction study in the Han Wistar rat by oral (dietary) administration, [redacted], unpublished report no. 775192, February 20, 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 revised draft guideline no. 416 (1999) US-EPA OPPTS 870.3800 (1998) 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	5400810	
3.1.2	Specification		
3.1.2.1	Description	White solid	
3.1.2.2	Purity	98.9%	
3.1.2.3	Stability	Expiration date: 14 March 2005	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Hanlbm: WIST (SPF)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	5-6 weeks old, P animals weighing 130-161 g for males and 93-126 g for females	
3.2.6	Number of animals per group	25 males and 25 females per group (P generation)	
3.2.7	Mating	See Table A6_08_2-1	
3.2.8	Duration of mating	Up to 14 days	
3.2.9	Deviations from standard protocol	None	
3.2.10	Control animals	Yes	
3.3	**Administration/**	Oral	

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	Exposure	
3.3.1	Animal assignment to dosage groups	See Table A6.8.2.2-1
3.3.2	Duration of exposure before mating	10 weeks
3.3.3	Duration of exposure in general P, F1, F2 males, females	10 weeks prior to mating through to weaning of the F1 offspring. Groups of 25 male and 25 female F1 generation offspring were then similarly treated
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3.3.4	Type	In food
3.3.5	Concentration	P and F1 generation: 0, 300, 1000, 3000 or 10000 ppm (Overall achieved dose levels were within the ranges: 16.5 - 47.9, 54.7 - 161.8, 162.7- 477.7 and 525.2 - 1653.9 mg/kg/day.) See Table A6.8.2.2-2
3.3.6	Vehicle	Moistened with water after admixture to the diet
3.3.7	Total volume applied	A volume/weight ratio of approximately 1:5
3.3.8	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Clinical signs	Yes, both generations were observed at least twice daily for clinical signs of a reaction to treatment. Dams were observed daily for survival and abnormalities in nesting or nursing behaviour.
3.4.2	Body weight	Yes, body weights were recorded weekly except during pairing. After mating, females were weighed on days 0, 7, 14 and 21 of gestation and days 1, 4, 7, 14 and 21 *post partum*.
3.4.3	Food/water consumption	Yes, food consumption was measured weekly throughout the study until day 14 *post partum*, except during mating.
3.4.4	Oestrus cycle	Oestrous cycles were monitored by vaginal smear for at least 3 weeks before mating and mean oestrous cycle duration calculated. The vaginal smears were prepared on the day of necropsy and determined the stage of estrous. Mating, fertility and conception indices were calculated. The duration of gestation was recorded. The age and body weight at which vaginal patency or preputial separation occurred was recorded for F1 generation parental animals.
3.4.5	Sperm parameters	Testis weight, epididymis weight, sperm motility, cauda epididymal sample examined for motility (all groups) and morphology (0 and 10000ppm), and one epididymis and one testis were retained for the determination of homogenisation-resistant spermatids and caudal epididymal sperm reserve (0 and 10000ppm). Additional testicular histopathology, qualitative sperm staging, was performed on PAS-stained sections.

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3.4.6	Offspring	Pups were examined daily during the lactation period for clinical signs and mortality. Day 0 of lactation was the day of completion of parturition. Pregnant females were allowed to litter normally and the litters were examined for live births, stillbirths and external abnormalities. The sexes of pups were recorded on days 0, 4 and 21 of lactation. Litters were not standardised by culling. Pup weights were recorded on days 0/1, 4, 7, 14 and 21. Anogenital distance was measured in all F2 generation pups on day 1 of lactation. Litters were examined as soon as possible after birth for litter size, live births, still births and gross abnormalities. The sex ratio was determined on days 0, 4 and 21 of lactation. Pups were weighed on days 0/1, 4, 7, 14 and 21 of lactation. F1 animals for functional observation battery: Commencing at 6 weeks of age, the F1 animals selected for functional investigations (20 animals/sex/group) were subjected to a modified Irwin screen test battery comprising qualitative and semi-quantitative assessment of appearance, motor activity, behaviour, respiration, reflexes and general autonomic functional observations. Observations were made "blind" in a standard arena. Grip strength and locomotor activity were measured quantitatively.
3.4.7	Organ weights P and F1	P generation: major organs, including testes/ovaries, uterus, prostate, epididymides and seminal vesicles, were weighed. F1 and F2 generation: the brain, spleen and thymus from one pup/sex/litter (randomly selected) from both F1 and F2 generation weanlings were weighed at necropsy.
3.4.8	Histopathology P and F1	Histopathological examination of reproductive organs, pituitary and adrenal glands was performed on all both generation parental animals treated at 0 or 10000ppm. Additional testicular histopathology, qualitative sperm staging, was performed on PAS-stained sections. Additional ovarian histopathology, comprising quantitative primordial follicle counts in 10 levels/ovary and a comparison with secondary/tertiary follicles, was performed on 10 females/group in F1 parental animals treated at 0 or 10000ppm. Histopathology was also performed on the reproductive organs of any animals treated at 300, 1000 or 3000ppm that failed to mate.
3.4.9	Histopathology F1 not selected for mating, F2	Dead pups, except where excessively cannibalised, were subjected to necropsy. Excess F1 pups not selected for further study on day 21 were also subjected to necropsy, *post mortem* examination and retention of the carcass in fixative. All F2 generation pups were killed shortly after weaning and subjected to necropsy and the carcasses retained in fixative.
3.5	**Statistics**	Where appropriate, normally distributed variables were subjected to the Dunnett many-to-one t-test based on a pooled variance and the Steel many-to-one rank test was used for non-normally distributed variables. Fisher's exact test was applied if the data could be dichotomised without loss of information.

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4 RESULTS AND DISCUSSION

4.1 Effects

4.1.1 Clinical signs

One P generation females at 10000 ppm died prematurely on day 21 *post-partum (pp)* after showing clinical signs during lactation of soft feces, ruffled fur and blood-stained urine. Macroscopic and histopathological examination showed renal changes that were considered causal to death. Although death may have been incidental to treatment with dinotefuran, a treatment-related etiology cannot be precluded because of its occurrence at the highest dose level at a time of greatly increased dosage. All other P and F1 generation parental animals survived the scheduled treatment period. Treatment-related clinical signs were confined to soft feces during lactation in all P generation females at 10000ppm and one F1 generation female treated at 10000ppm. The observation is considered to be treatment-related since its occurrence was confined to the lactation period at which time high dose levels were ingested. No treatment-related clinical signs occurred in males at 10000ppm or in either sex at lower dose levels in either parental generation.

4.1.2 Body weight

Minor and transient effects occurred on food consumption during the pre-pairing period which were suggestive of reduced diet palatability. P and F1 generation parental animals of both sexes showed significantly reduced food consumption during weeks 1 and/or 2 of treatment (Table A6.8.2.2-3 and Table A6.8.2.2-4).

The group mean body weight gains during the pre-pairing period were reduced in P generation males and females at 10000ppm. Thus, group mean body weights at the start of mating were 5.8 and 4.0% lower than the controls, respectively (Table A6.8.2.2-5 and Table A6.8.2.2-6). The treatment-related reduction in pre-weaning body weight gains of F1 generation males and females at 10000ppm persisted during the pre-pairing treatment period. Thus, group mean body weights at the start of mating were 9.3 and 4.3% lower than the control values, respectively. The body weight gains of P and F1 generation animals of both sexes at 300 - 3000ppm were unaffected by treatment with dinotefuran.

The slightly lower body weights of P and F1 generation females at 10000ppm persisted during the gestation and lactation periods (Table A6.8.2.2-6). The group mean body weights at 21 days *pp* were 6.4 and 7.9% lower, respectively, than control values. The body weights of P and F1 generation females treated at 300 - 3000ppm were comparable to control values throughout gestation and lactation.

4.1.3 Food and water consumption

P generation males at 300 and 1000ppm also showed slightly lower food consumption than the controls during the first week of treatment only. Thereafter, there was no clear treatment-related effect on the food consumption of either sex at any dose level during the pre-pairing period. The food consumption during gestation of the P generation females was not affected by treatment at any dose level, but was significantly reduced by 11.3% during the first week of gestation in F1 generation females at 10000ppm. During lactation, the food consumption in P and F1 generation females treated at 3000 and 10000ppm was slightly reduced,

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		but since the effect at 3000ppm was not accompanied by an effect on body weight gain, the effect is considered not to be adverse at 3000ppm.
4.1.4	Mating performance	There was no effect of treatment at any dose level in either generation on the duration of the estrous cycle. The mean duration of the estrous cycle in P generation females was 5.3, 5.6, 4.8, 5.1 and 4.9 days, in order of ascending dose level, and 4.9, 4.9, 4.9, 5.0 and 5.0 days, in order of ascending dose level in the F1 generation. There were no treatment-related effects at any dose level in either generation on fertility and mating performance, duration of gestation, number of implantations, post-implantation loss, litter size at birth, pup mortality, litter size at weaning and sex ratio (Table A6.8.2.2-7). With the exception of two non-dose-related occurrences of statistical significance (higher neonatal pup mortality at 300ppm and higher number of empty implantation sites at 3000ppm), all reproductive data in the treated groups were comparable to, and not significantly different from, the control group. There were no treatment-related effects on sperm motility, morphology and counts in either P or F1 generation males at any dose level. The proportions of non-motile, stationary and progressively motile sperm in all treated P generation groups were similar to, and not significantly ($p > 0.05$) different from, the control group. In the F1 generation, statistically significant variation from the control values was observed for progressively motile and stationary sperm in the group treated at 10000ppm and for progressive sperm in the group treated at 1000ppm (Table A6.8.2.2-11). Males at 10000ppm showed 40% stationary and 50% progressively motile sperm compared with the control group that showed 32% stationary and 58% progressively motile sperm. The proportion of non-motile sperm in both groups was 10%. The group treated at 1000ppm also showed a significantly ($p < 0.05$) lower proportion of progressively motile sperm. Since the differences in the mean values were numerically small and showed no clear dose dependency, the small differences recorded are considered to be incidental to treatment. P generation males at 10000ppm showed a slightly, but significantly higher incidence of sperm abnormality type D (normal head but abnormally curved hook). However, the finding occurred in association with a high percentage incidence of normal sperm (95.0% versus 96.2% in the control) and the numerical difference from the control for this abnormality (type D) was small (2% affected versus 1.1% in the control). Furthermore, the F1 generation control incidence of type D abnormality was 1.7%. Therefore, the higher incidence in the P generation males at 10000ppm is considered to be incidental to treatment. In the F1 generation, there were minor differences from the control in the sperm morphology data, but the differences occurred in association with a high percentage incidence of normal sperm (95.4% versus 96.4% in the control) and the differences are considered to be incidental to treatment. There was no significant effect on epididymal sperm count for the P or F1 generation males, but the testicular sperm counts of the P generation males, showed a slight reduction at 10000ppm which was statistically significant ($p < 0.01$). However, as the magnitude of the difference was small, occurred in the absence of a significant effect on epididymal sperm count and was not repeated in the F1 generation males, the

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		difference is considered incidental to treatment.
4.1.5	Litter parameters	There were no treatment-related effects at any dose level in either generation on the nature and incidence of pup abnormalities during the pre-weaning period. The anogenital distance of F2 progeny of both sexes was unaffected by treatment at all dose levels (Table A6.8.2.2-7). The significantly greater anogenital distances of F2 pups at 1000ppm are considered incidental to treatment since a dose-relationship was not evident. Pre-weaning pup growth in both the F1 and F2 generations was retarded at 10000ppm. Group mean male and female pup weights were significantly ($p < 0.01$) reduced from day 14 *pp*, except for female F1 pups which were significantly ($p < 0.05$) reduced on day 21 *pp* only (Table A6.8.2.2-8). Thus at weaning, pup weights were 11.6 - 15.1% lower than control values. Pup weights were unaffected by treatment at lower dose levels. Sexual maturation of F1 generation pups, based on preputial separation or vaginal patency, was unaffected by treatment at all dose levels. The group mean age at which these events occurred was 27.9, 28.0, 27.8, 28.2 and 28.2 days (males) and 34.3, 34.1, 34.5, 33.9 and 35.5 days (females), in order of ascending dose level. None of the values was significantly ($p > 0.05$) different from the control values. Quantitative locomotor activity of F1 progeny at 6 weeks of age was not affected by treatment at any dose level. There were no statistically significant ($p > 0.05$) differences in the low beam counts recorded for control and treated groups of either sex. There were no direct treatment-related effects at any dose level on motor capability as assessed by grip strength. Quantitative measurement of grip strength showed significantly ($p < 0.05$) lower absolute values at 10000ppm for male forelimb grip strength and female hindlimb grip strength (Table A6.8.2.2-9). However, grip strength to body weight ratios, were not significantly ($p > 0.05$) different from the controls, suggesting the differences were due to lower body weight/smaller size at 10000ppm, rather than a specific effect of dinotefuran on motor capability. Absolute grip strength at 3000ppm and below were not affected by treatment. None of the animals at any dose level showed any behavioural, postural, motor, respiratory or reflex anomalies in the modified Irwin screen, and all animals were of normal appearance.
4.1.6	Gross findings at necropsy	The types and frequencies of gross lesions at necropsy in F1 and F2 pups shortly after weaning gave no indication of treatment-related effects. The most common finding in both generations was renal pelvic dilation, but the group incidences did not indicate an effect of treatment. The overall incidences of renal pelvic dilation were 11.0, 7.7, 11.5, 14.3 and 10.0% (F1 generation) and 8.0, 2.1, 17.0, 12.5 and 4.1% (F2 generation), in order of ascending dose level. All other gross lesions occurred at very low incidences and their distribution did not suggest an effect of treatment.
4.1.7	Organ weights	Direct treatment-related effects on organ weights were confined to the spleen in both the F1 and F2 generations. The mean absolute spleen weight (both sexes) and mean spleen weight relative to body weight (females only) were significantly ($p < 0.05$ or 0.01) reduced by up to 25.6% in F1 generation pups treated at 10000ppm (Table A6.8.2.2-10). The mean brain weight relative to body weight was also significantly (p

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		< 0.01) elevated in these animals, but is considered to be due to the lower body weights of the group. There was no effect on thymus weights at any dose level. Absolute and relative spleen weights were significantly ($p < 0.05$ or 0.01) reduced to a similar extent in both sexes of the F2 generation at 10000ppm. Absolute brain and thymus weights were significantly ($p < 0.05$ or 0.01) reduced and relative brain weights were significantly increased in F2 animals exposed to 10000ppm. However, the pattern of response is indicative of a body weight effect rather than a specific effect of dinotefuran on these organs. There were no effects on any of the measured organ weights in F1 and F2 generation male and female pups at 300 - 3000ppm. There were no treatment-related gross findings at necropsy in the male and female P and F1 generation parental animals at any dose level, but significantly ($p < 0.05$ or 0.01) reduced spleen weights (absolute and brain weight ratios, 10.0 - 16.0% reduced) occurred in both sexes of the P generation treated at 10000ppm. The effect was not evident at lower dose levels or in F1 generation parental animals at any dose level. Female F1 generation parental animals at 10000ppm showed significantly reduced thyroid weights (absolute, body weight and brain weight ratios, 20.0 - 25.0% reduced). Other minor, but statistically significant, differences in organ weights at 10000ppm are considered to be secondary to lower terminal body weights.
4.1.8	Histopathology	All histopathological findings recorded in the reproductive organs, pituitary and adrenal glands of P and F1 generation males and females were considered to be within the range of background lesions commonly observed in rats of this strain and age. The incidences of all individual findings at 10000ppm did not indicate an effect of treatment. There were no treatment-related findings during staging analysis of the testes. All cycles were complete and there were no indicators for maturation arrest. There were no treatment-related, biologically relevant effects on the quantitative evaluation of ovarian follicular stages. Although ovary staging revealed a significantly ($p<0.025$, $\chi 2$-test) lower number of primordial follicles in 10000ppm animals (Table A6.8.2.2-12), the finding is deemed to have no biological significance because the numbers of antral follicles were markedly higher at 10000ppm and the numbers of corpora lutea were also slightly higher than the controls. The numbers of pre-antral follicles were comparable in the treated and control groups.
4.2	**Other**	None
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Guidelines: OECD revised draft guideline no. 416 (1999), US-EPA OPPTS 870.3800 (1998), JMAFF 59 NohSan No. 4200 (1985). No relevant deviations from test guidelines Method: Groups of 25 male and 25 female P generation Wistar rats were treated orally, by diet administration, with dinotefuran at concentrations of0, 300, 1000, 3000 or 10000 ppm for 10 weeks prior to mating through to weaning of the F1 offspring. Groups of 25 male and 25 female F1

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Annex Point IIA6.8.2

Multigeneration Reproduction Toxicity Study
Rat
Oral

		generation offspring were then similarly treated. Overall achieved dose levels were within the ranges 16.5 - 47.9, 54.7 - 161.8, 162.7- 477.7 and 525.2 - 1653.9 mg/kg/day. In P and F1 parental animals, clinical signs were recorded daily, body weight and food consumption were recorded approximately weekly, oestrous cyclicity was monitored, the duration of gestation was recorded and the F1 animals were examined for sexual development landmarks. Pregnant females were allowed to litter normally and the litters were examined for live births, stillbirths and external abnormalities. The sexes and body weights of pups were recorded. Litters were not standardised by culling. Anogenital distance was measured in all F2 generation pups on day 1 of lactation. At 6 weeks of age, F1 animals selected for functional investigations were subjected to assessment of behavioural function, grip strength and locomotor activity.	
5.2	**Results and discussion**	P/F1 parental animals: There were no treatment-related deaths. Treatment-related clinical signs were confined to soft feces during lactation in all P generation females at 10000ppm and one F1 generation female treated at 10000ppm. Minor and transient effects occurred on food consumption during the pre-pairing period which were suggestive of reduced diet palatability. During lactation, the food consumption in P and F1 generation females treated at 10000ppm was slightly reduced, The body weight gain of P and F1 generation animals of both sexes at 10000ppm was reduced. There were no treatment-related effects at any dose level in either generation on fertility and mating performance, duration of gestation, number of implantations, post-implantation loss, litter size at birth, pup mortality, litter size at weaning and sex ratio. F1/F2 offspring: Pre-weaning pup growth in both the F1 and F2 generations was reduced at 10000ppm, resulting in lower pup weight at weaning. There were no treatment-related effects on the nature and incidence of pup abnormalities during the pre-weaning period and the anogenital distance of F2 progeny was unaffected by treatment at all dose levels. Sexual maturation of F1 generation pups was unaffected by treatment at all dose levels. There were no effects of treatment on behavioural function, locomotor activity and grip strength of 6 week old F1 progeny. Pathological examination (parental animals): There were no treatment-related gross findings at necropsy in the P and F1 generation parental animals at any dose level, but significantly reduced spleen weights occurred in both sexes of the P generation treated at 10000ppm. The effect was not evident at lower dose levels or in F1 generation parental animals at any dose level. Female F1 generation parental animals at 10000ppm showed significantly reduced thyroid weights. There were no treatment-related histopathological findings in the reproductive organs, pituitary and adrenal glands of P and F1 generation animals. There were no treatment-related findings during staging analysis of the testes. There were no treatment-related effects on sperm motility, morphology and counts in either P or F1 generation males at any dose level. There were no treatment-related, biologically	X1

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Multigeneration Reproduction Toxicity Study
Rat
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		relevant effects on the quantitative evaluation of ovarian follicular stages. Pathological examination (F1/F2 pups): There were no treatment-related gross lesions at necropsy in F1 and F2 pups. Direct treatment-related effects on organ weights were confined to lower spleen weight in both the F1 and F2 generations at 10000ppm. There was no effect on thymus weights at any dose level.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not specified in report
5.3.2	NOEL	
5.3.2.1	Parent males	241 mg/kg bw/day
5.3.2.2	Parent females	267.9 mg/kg bw/day
5.3.2.3	F1 males	269 mg/kg bw/day
5.3.2.4	F1 females	292.6 mg/kg bw/day
5.3.2.5	F2 males	Not specified in report
5.3.2.6	F2 females	Not specified in report
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Table A6.8.2.2-1: Animal assignment and treatment

Group number	Dose level of dinotefuran (ppm)	Number of P generation animals		Number of F1 generation animals	
		Male	Female	Male	Female
1	0	25	25	25	25
2	300	25	25	25	25
3	1000	25	25	25	25
4	3000	25	25	25	25
5	10000	25	25	25	25

Table A6.8.2.2-2: Mean achieved dose levels

Sex / generation	Study period	300ppm	1000ppm	3000ppm	10000ppm
		≡ mg/kg bw/day			
Male / P	Pre-mating	24.1	79.9	241.0	822.1
	Post-mating	16.9	56.6	166.7	577.3
Female / P	Pre-mating	26.8	90.1	267.9	907.0
	Gestation	21.9	75.1	226.1	767.5
	Lactation (weeks 1 & 2)	47.8	161.8	466.1	1628.8
Male / F1	Pre-mating	27.2	90.5	269.0	934.7
	Post-mating	16.5	54.7	162.7	575.3
Female / F1	Pre-mating	29.6	96.5	292.6	1004.8
	Gestation	21.1	70.5	211.9	725.2
	Lactation (weeks 1 & 2)	47.9	158.8	477.7	1653.9

Table A6.8.2.2-3: Summary of food consumption – P and F1 generation parental males

Generation	Study period	Group mean food consumption (g/day) at:				
		0ppm	300ppm	1000ppm	3000ppm	10000ppm
P	Week 1	22.9	21.9	21.0**	21.7*	20.6**
	Week 2	23.3	22.9	22.4	22.9	22.5
	Mean (weeks 1 - 10)	24.4	24.1	23.4	23.7	23.8
F1	Week 1	12.8	13.0	13.3	13.6	11.3
	Week 2	17.4	17.8	18.0	17.2	14.6**
	Mean (weeks 1 - 10)	20.3	21.3	21.4	21.2	19.4

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

Table A6.8.2.2-4: Summary of food consumption – P and F1 generation parental females

Generation	Study period	Group mean food consumption (g/day) at:				
		0ppm	300ppm	1000ppm	3000ppm	10000ppm
P	Week 1	15.7	16.2	15.3	15.5	14.6**
	Week 2	16.3	16.4	16.1	15.9	15.3*
	Mean (weeks 1 - 10)	17.0	16.8	16.7	16.5	16.4
	Mean during gestation	21.3	20.2	20.9	20.7	20.4
	Mean during lactation[a]	44.7	43.3	44.2	41.7	41.2
F1	Week 1	12.2	11.7	12.2	12.2	11.2
	Week 2	15.1	14.7	15.2	14.0*	13.3**
	Mean (weeks 1 - 10)	14.8	15.5	15.5	15.4	15.0
	Mean during gestation	19.6	18.5	18.9	18.6	18.5
	Mean during lactation[a]	41.2	40.4	42.2	40.6	40.0

[a] weeks 1 and 2 only;
* $p < 0.05$;
** $p < 0.01$

Table A6.8.2.2-5: Summary of bosy weights – P and F1 generation parental males

Generation	Study period	Group mean body weight (g) at:				
		0ppm	300ppm	1000ppm	3000ppm	10000ppm
P	Week 1 (study start)	145	145	143	145	144
	Week 10 (start of mating)	397	391	384	380	374*
	Week 14 (post-mating)	450	443	436	431	424*
F1	Week 1 (study start)	61	62	64	65	52*
	Week 10 (start of mating)	344	347	351	344	312**
	Week 14 (post-mating)	421	423	427	422	387*

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

Table A6.8.2.2-6: Summary of body weights – P and F1 generation parental females

Generation	Study period	Group mean body weight (g) at:				
		0ppm	300ppm	1000ppm	3000ppm	10000ppm
P	Week 1 (study start)	112	113	109	110	109
	Week 10 (start of mating)	225	227	226	223	216
	Day 0 (gestation)	224	225	225	222	217
	Day 7 (gestation)	245	243	244	240	233*
	Day 21 (gestation)	338	332	331	327	317**
	Day 1 (lactation)	245	244	246	243	230*
	Day 21 (lactation)	282	281	282	274	264**
F1	Week 1 (study start)	58	56	60	59	52*
	Week 10 (start of mating)	207	207	211	209	198
	Day 0 (gestation)	211	208	216	211	203
	Day 7 (gestation)	229	227	234	230	220
	Day 21 (gestation)	319	319	322	315	307
	Day 1 (lactation)	233	230	235	232	225
	Day 21 (lactation)	280	275	279	269	258**

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

Table A6.8.2.2-7: Summary of reproductive data – P and F1 generation

Generation	Reproductive parameter	Group mean value at:				
		0ppm	300ppm	1000ppm	3000ppm	10000ppm
P	No. paired / no. mated	25 / 25	25 / 25	25 / 25	25 / 25	25 / 25
	No. pregnant	24	25	25	25	24
	No. with viable litters	23[a]	25	22[b]	25	23[c]
	Mean pre-coital time (days)	2.8	2.8	2.8	2.6	2.6
	Duration of gestation (days)	21.5	21.4	21.5	21.5	21.8
	No. implantations/dam	13.1	13.2	12.6	12.5	12.9
	Post-implantation loss (%)	7.3	7.9	9.7	9.3	7.1
	Live litter size on day 0/1	12.2	12.2	11.4	11.3	12.0
	Litter size on day 4 *pp*	12.0	11.5[e]	11.1	11.2	11.6
	Pup mortality (group total) on days 5 - 21 *pp*	6	4	2	0	7
	Litter size on day 21 pp	11.7	11.4	11.0	11.2	11.3
	Sex ratio (% males)	51	49	47	47	45
F1	No. paired / no. mated	25 / 25	25 / 25	25 / 25	25 / 25	25 / 25
	No. pregnant	25	24	24	25	25
	No. with viable litters	25	24	24	25	25
	Mean pre-coital time (days)	2.7	2.8	2.5	2.6	2.4
	Duration of gestation (days)	21.5	21.4	21.5	21.5	21.5
	No. implantations/dam	11.8	11.8	11.5	11.4	12.3
	Post-implantation loss (%)	6.4	3.5	4.0	10.9[d]	5.2
	Live litter size on day 0/1	11.1	11.4	11.0	10.2	11.6
	Litter size on day 4 *pp*	10.9	11.1	11.0	10.1	11.4
	Pup mortality (group total) on days 5 - 21 *pp*	2	2	1	2	0
	Litter size on day 21 pp	10.8	11.0	11.0	10.0	11.4
	Sex ratio (% males)	52	46	49	55	46
	F2 male a/g distance (mm)	2.12	2.17	2.24**	2.16	2.12
	F2 female a/g distance (mm)	1.00	1.00	1.04*	1.02	1.01

[a] one animal with implantation sites only;
[b] two animals with implantation sites only and one animal with dead pups only at first examination;
[c] one animal with dead pups only at first examination;
[d] $p < 0.05$ for total no. of empty implantation sites;
[e] $p < 0.05$ for pup mortality days 1 - 4 *pp*;
* $p < 0.05$;
** $p < 0.01$

Table A6.8.2.2-8: Summary of pup body weights – F1 and F2 generations

Generation	Body weight (days *pp*):	Sex	Group mean value (g) at:				
			0ppm	300ppm	1000ppm	3000ppm	10000ppm
F1	0/1	Male	5.7	5.4	5.7	6.0	5.5
	4		8.3	8.1	8.5	8.7	8.2
	7		12.3	12.0	12.8	12.9	11.7
	14		23.9	24.0	25.0	24.7	21.2**
	21		37.4	37.3	38.8	38.1	32.0**
	0/1	Female	5.4	5.1	5.8	5.6	5.5
	4		8.0	7.6	8.3	8.2	8.0
	7		11.9	11.4	12.6	12.3	11.4
	14		23.4	22.7	24.5	23.7	21.3
	21		36.2	35.3	38.2	36.6	32.0*
F2	1	Male	5.9	5.9	6.2	6.2	5.9
	4		8.7	8.6	9.0	9.2	8.5
	7		12.7	12.4	13.2	13.5	12.1
	14		24.7	24.9	25.6	25.8	21.5**
	21		39.7	39.5	41.7	41.4	33.7**
	1	Female	5.6	5.6	5.8	5.8	5.7
	4		8.3	8.4	8.6	8.7	8.3
	7		12.2	12.2	12.7	12.8	12.0
	14		24.2	24.4	24.7	24.7	21.2**
	21		38.7	38.8	40.1	39.6	33.1**

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

Table A6.8.2.2-9: Summary of grip strrength data at 6 weeks of age – F1 generation

Sex	Parameter	Group mean value at:				
		0ppm	300ppm	1000ppm	3000ppm	10000ppm
Male	Forelimb grip strength (g)	618	576	557	554	505*
	Hindlimb grip strength (g)	387	416	350	379	343
	Forelimb GS/BW ratio	3.37	3.21	3.05	3.05	3.23
	Hindlimb GS/BW ratio	2.12	2.30	1.94	2.09	2.21
Female	Forelimb grip strength (g)	536	513	504	521	449
	Hindlimb grip strength (g)	428	401	364*	385	336*
	Forelimb GS/BW ratio	3.93	3.85	3.62	3.88	3.64
	Hindlimb GS/BW ratio	3.15	3.03	2.60*	2.88	2.73

GS/BW grip strength/body weight ratio;
* $p < 0.05$.

Table A6.8.2.2-10: Selected organ weight data – F1 and F2 generation weanlings

Sex and generation	Organ weight	Group mean value at:				
		0ppm	300ppm	1000ppm	3000ppm	10000ppm
Male / F1	Absolute spleen weight (g)	0.154	0.141	0.171	0.161	0.119*
	Relative spleen weight[a] (%)	0.408	0.379	0.437	0.429	0.373
Female / F1	Absolute spleen weight (g)	0.156	0.146	0.176	0.157	0.116**
	Relative spleen weight[a] (%)	0.436	0.417	0.462	0.435	0.369**
Male / F2	Absolute spleen weight (g)	0.173	0.173	0.193	0.190	0.125**
	Relative spleen weight[a] (%)	0.431	0.431	0.454	0.446	0.366**
Female / F2	Absolute spleen weight (g)	0.174	0.161	0.188	0.179	0.130**
	Relative spleen weight[a] (%)	0.445	0.410	0.465	0.448	0.392*

[a] relative to body weight;
* $p < 0.05$;
** $p < 0.01$

Table A6.8.2.2-11: Selected sperm analysis data – P and F1 parental animals

Generation	Sperm parameter	Group mean value at:				
		0ppm	300ppm	1000ppm	3000ppm	10000ppm
P	Motility:					
	- non-motile (%)	12	15	13	11	12
	- stationary (%)	35	35	37	37	34
	- progressive (%)	53	51	50	52	55
F1	Motility:					
	- non-motile (%)	10	12	15	10	10
	- stationary (%)	32	36	35	38	40*
	- progressive (%)	58	52	50*	53	50*
P	Morphology:					
	- type A (%)	96.2	-	-	-	95.0
	- type B (%)	1.6	-	-	-	1.7
	- type C (%)	0.9	-	-	-	1.0
	- type D (%)	1.1	-	-	-	2.0**
	- type E (%)	0.0	-	-	-	0.0
	- type F (%)	0.1	-	-	-	0.1
F1	Morphology:					
	- type A (%)	96.4	-	-	-	95.4*
	- type B (%)	1.3	-	-	-	1.8[a]
	- type C (%)	0.5	-	-	-	1.0*
	- type D (%)	1.7	-	-	-	1.7
	- type E (%)	0.0	-	-	-	0.0
	- type F (%)	0.0	-	-	-	0.1
P	Sperm count:					
	- testis (mio/g)	126.91	-	-	-	114.91**
	- epididymis (mio/g)	706.84	-	-	-	690.41
F1	Sperm count:					
	- testis (mio/g)	131.61	-	-	-	135.39
	- epididymis (mio/g)	677.66	-	-	-	710.58

* $p < 0.05$;
** $p < 0.01$;
[a] $p < 0.05$ for U-test and $p > 0.05$ for t-test;
- not examined;
A = normal sperm;
B = normal head with tail detached;
C = complete sperm with misshaped hook;
D = complete sperm with abnormally curved hook;
E = complete sperm with reversed hook;
F = abnormal head with tail detached

Table A6.8.2.2-12: Quantitative ovary data – F1 parental animals

Generation	Follicular stage	Group mean value at:				
		0ppm	300ppm	1000ppm	3000ppm	10000ppm
F1	No. follicles in 2 ovaries, 10 levels/ovary:					
	- primordial	62.0	-	-	-	37.2*
	- growing	123.5	-	-	-	120.2
	- antral	126.4	-	-	-	157.2
	- corpora lutea	239.3	-	-	-	274.0

* $p < 0.025$ ($\chi 2$-test); - not examined

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*7 February 2013*
Materials and Methods	*As described by Applicant*
Results and discussion	*As described by Applicant, except:* *X1 5.2, 3rd paragraph: the RMS does not consider that there is a treatment-related effect on thyroid weight for F1 parental females at 10000 ppm because a dose dependent response is not present for neither the absolute, bodyweight-related, or brain weight related thyroid weight.*
Conclusion	*As described by applicant*
Reliability	*As described by Applicant*
Acceptability	*As described by Applicant*
Remarks	*None*
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.9-1 **Acute Neurotoxicity**

Annex Point IIA6.9 **Rat**

Oral, acute

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 2001a, Acute oral gavage neurotoxicity study with MTI-446 in rats, [redacted], unpublished report no. [redacted] 6648-147, August 6, 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 No applicable EU guideline OECD guideline no. 424 (1997) US-EPA OPPTS 870.6200 (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	
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	**Reference Substance (positive control)**	None
3.3	**Test Animals**	
3.3.1	Species	Rat
3.3.2	Strain	Crl:CD®(SD) IGS BR
3.3.3	Source	[redacted]
3.3.4	Sex	Males and females
3.3.5	Rearing conditions	Animals were individually housed in suspended, stainless steel cage
3.3.6	Age/weight at study initiation	About 7 weeks old, weighing 212 -284 g for males and 142-208 g for females

Section A6.9-1 Acute Neurotoxicity
Annex Point IIA6.9 **Rat**
Oral, acute

3.3.7	Number of animals per group	10/sex/group
3.3.8	Control animals	Yes
3.4	**Administration**	Oral by gavage
3.4.1	Exposure	Single dose
3.4.2	Dose Levels	See table below
3.4.3	Vehicle	0.5% aqueous carboxymethylcellulose
3.4.4	Concentration in vehicle	Not applicable
3.4.5	Total volume applied	20 mL/kg
3.4.6	Postexposure period	14 days
3.4.7	Anticholinergic substances used	None
3.4.8	Controls	Vehicle
3.5	**Examinations**	
3.5.1	Body Weight	Body weights were recorded pre-dose and on days 1, 8 and 15.
3.5.2	Signs of Toxicity	All animals were subjected to a functional observation battery (FOB) pre-dose, 3 hours after treatment on day 1 (the estimated time of peak effect), and on days 8 and 15. The tests and observations were performed without knowledge of the treatment of each animal. The FOB comprised a series of qualitative and semi-quantitative observations made in the home cage, during handling, in an open arena and during manipulations to assess reflex responses and physiological parameters. The assessments included evaluation of posture, activity, gait, locomotor activity, unusual behavior, reactivity to handling, vocalisation, palpebral closure, exophthalmos, lacrimation, salivation, respiration, appearance of fur, piloerection, muscle tone, pupillary status, latency to first step in an open field, grooming and rearing activity, defecation, micturition, auditory reactivity, proprioceptive positioning, pinna response, approach response, righting reflex, corneal touch response, nociceptive reflexes and hind-limb foot splay. Quantitative measurements were made of rectal temperature, fore-limb and hind-limb grip strength, and motor activity counts for 2-minute intervals for 40 minutes.
3.5.3	Observation schedule	The animals were observed twice daily for morbidity/mortality and daily for clinical signs.

Group number	Dose level of dinotefuran (mg/kg)	Number of animals: Male	Female
1	0 (vehicle)	10	10
2	325	10	10
3	750	10	10
4	1500	10	10

Section A6.9-1 **Acute Neurotoxicity**
Annex Point IIA6.9 **Rat**
Oral, acute

3.5.4	Clinical Chemistry	No	
3.5.5	Pathology	Yes	
		Organs:	Brain and entire spinal cord.
3.5.6	Histopathology	Yes	
		Organs:	Olfactory bulb, forebrain, caudate nucleus, hypothalamus/thalamus, midbrain, cerebellum, medulla, pituitary gland, spinal cord, eye, anterior tibialis muscle, gastrocnemius muscle, macroscopic lesions.
3.5.7	Neuropathologic evaluation	Yes	
		Organs:	Cervical dorsal root ganglion, lumbar dorsal root ganglion, trigeminal ganglion, fibular nerve, optic nerve, sciatic nerve, tibial nerve, sural nerve.
3.6	**Further remarks**	None	

4 RESULTS AND DISCUSSION

4.1	**Body Weight**	There were no effects on body weight gain and food consumption at any dose level. The group mean body weights and food consumption of each treated group of each sex varied by less than 5% and 6%, respectively, from the controls.
4.2	**Clinical signs of toxicity**	There were no deaths or treatment-related clinical signs during the study at any dose level.
4.3	**Clinical Chemistry**	Not applicable
4.4	**Pathology**	Macroscopic examination at necropsy revealed no treatment-related lesions at any dose level.
4.5	**Histopathology**	There were few histopathological findings in the central and peripheral nervous tissues and other tissues examined and the nature and distribution between the groups did not indicate an effect of treatment at 1500mg/kg. All histopathological findings were considered incidental to treatment and common to animals of the strain and age used.
4.6	**Other**	There were some minor variations in the FOB observations, but none was considered to be treatment-related because they were not dose-related, or occurred also during the pre-dose evaluation, or occurred at very low incidence, or there were no other correlating behavioral changes. Therefore, there were no treatment-related effects in either sex at any dose level on the qualitative and semi-quantitative evaluation of FOB observations, reflexes and responses at any of the testing intervals. The motor activity of both sexes at 1500kg/kg, as measured quantitatively, was reduced on day 1 relative to pre-dose values and to the controls (Table A691-1). The effect was statistically significant ($p < 0.05$) for the females. The finding is considered to be treatment-related but not to be an adverse effect or indicative of neurotoxicity since the effect was transient and there were no correlating changes in the qualitative FOB

Section A6.9-1 **Acute Neurotoxicity**

Annex Point IIA6.9 **Rat**

Oral, acute

		observations. The effect did not occur subsequently at 1500mg/kg. Motor activity at lower dose levels was unaffected by treatment at all testing intervals. There were no other treatment-related effects in either sex at any dose level on the quantitative evaluation of grooming and rearing activity, defecation, micturition, grip strength, nociceptive reflex, foot splay and rectal temperature.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Guidelines: No applicable EU guideline, OECD guideline no. 424 (1997), US-EPA OPPTS 870.6200 (1998) No relevant deviations from test guidelines. Method: Four groups of 10 male and 10 female rats were treated once orally, by gavage, with 20 mL/kg of an aqueous suspension of dinotefuran at dose levels of 0, 325, 750 or 1500 mg/kg, and then maintained for a 14-day observation period. Food consumption and body weights were recorded, and a functional observation battery (FOB) of tests including a quantitative assessment of motor activity, was performed on all animals pre-dose, 3 hours after treatment on day 1 (the estimated time of peak effect), and on days 8 and 15. All animals were subjected to necropsy, *post mortem* examination, brain weight and dimensions recording, perfusion fixation and preservation of brain, dorsal root fibres and ganglia, ventral root fibres, spinal cord, eyes, optic, tibial and sciatic nerves, skeletal muscle and gross lesions. Six animals/sex from the control and high dose groups were subjected to histopathological evaluation.	
5.2	**Results and discussion**	There were no deaths, treatment-related clinical signs, or effects on body weight gain and food consumption at any dose level. There were some minor variations in the FOB observations, but none was considered to be treatment-related because they were not dose-related, or occurred also during the pre-dose evaluation, or occurred at very low incidence, or there were no other correlating behavioral changes. Therefore, there were no treatment-related effects in either sex at any dose level on the qualitative and semi-quantitative evaluation of FOB observations, reflexes and responses at any of the testing intervals. Although quantitative motor activity at 1500kg/kg was reduced on day 1, the finding is considered not to be an adverse effect or indicative of neurotoxicity since the effect was transient and there were no correlating changes in the qualitative FOB observations. The effect did not occur subsequently at 1500mg/kg. Motor activity at lower dose levels was unaffected by treatment at all testing intervals. There were no other treatment-related effects in either sex at any dose level on the quantitative evaluation of grooming and rearing activity, defecation, micturition, grip strength, nociceptive reflex, foot splay and rectal temperature. There were no macroscopic observations at necropsy in any of the animals. All histopathological findings were considered	X1

Section A6.9-1 **Acute Neurotoxicity**

Annex Point IIA6.9 **Rat**

Oral, acute

		incidental to treatment and common to animals of the strain and age used. A no-observed-effect-level (NOEL) for neurotoxicity and a no-observed-adverse-effect-level (NOAEL) for all effects were established as > 1500mg/kg, based on the absence of adverse effects including functional and histopathological evidence of neurotoxicity at this dose level.	
5.3	**Conclusion**		
5.3.1	NOEL	1500mg/kg, based on the absence of adverse effects including functional and histopathological evidence of neurotoxicity at this dose level.	
5.3.2	NOAEL	1500mg/kg, based on the absence of adverse effects including functional and histopathological evidence of neurotoxicity at this dose level.	X2
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Table A6.9.1-1: Summary of quantitative motor activity evaluation

Testing interval	Group mean no. of beam breaks/40 minute test period in:							
	Males treated at (mg/kg):				Females treated at (mg/kg):			
	0	325	750	1500	0	325	750	1500
Pre-dose	1369	1365	1377	1323	1147	1016	1061	1151
Day 1	809	1074	837	567[a]	1006	940	682	531*
Day 8	1302	1529	1471	1435	1400	1105	1118	1175
Day 15	1389	1662	1650	1363	1375	1258	1157	1068

* $p < 0.05$;

[a] $p < 0.05$ for the first 10-minute interval only