Section A6.1.1-2 Acute Toxicity
Annex Point IIA6.1 Oral
Mouse

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Guidelines: OECD 401 (1981), which is equivalent to 92/69/EEC (method B1); EPA-FIFRA, Subdivision F, § 81-1 (1982); JMAFF 59 NohSan No. 4200 (1985) No relevant deviations from test guidelines. Method: Dose range-finding study: 1 male and 1 female per group, administered dinotefuran at dose levels of 500, 1000, 3000 and 5000mg/kg bw. Main study: 5 males and 5 females per group administered dinotefuran at dose levels of 1000, 2000 or 3000mg/kg bw. Dinotefuran administered orally by gavage as suspension in CMC, 14-day observation period.
5.2	**Results and discussion**	Mouse, dinotefuran: oral LD_{50} 2450 mg/kg bw for males, 2275 mg/kg bw for females and 2371 mg/kg bw for the sexes combined.
5.3	**Conclusion**	Non-entry field
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A6.1.1.2-1 Mortality and time of death

Dose level (mg/kg bw)	Number dying / number tested			
	Dose range-finding study		Main study	
	Male	Female	Male	Female
500	0 / 1	0 / 1	-	-
1000	0 / 1	0 / 1	0 / 5	0 / 5
2000	-	-	1[a] / 5	2[a] / 5
3000	1[a] / 1	0 / 1	4[a] / 5	4[a] / 5
5000	1[a] / 1	1[a] / 1	-	-

[a] died on the day of treatment;
- not tested

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*10/09/12*
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	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.1.2 Acute Toxicity
Annex Point IIA6.1 Dermal
Rat, limit test

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1997, Acute dermal toxicity study of MTI-446 in rats, [redacted], unpublished report no. [redacted] 6648-120, December 9, 1997
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 402 (1987), which is equivalent to 92/69/EEC (method B3) EPA-FIFRA, Subdivision F, § 81-2 (1982) JMAFF 59 NohSan No. 4200 (1985)
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	22-00110
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	96.5% + 2% water, purity of dried material 99.1%
3.1.2.3	Stability	Expiration date: May 14, 2001
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Crl:CD[SD]BR (SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	8 - 16 weeks old, weighing 254 to 290g
3.2.6	Number of animals per group	5 males and 5 females per group
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Dermal
3.3.1	Post-exposure period	14 days

Section A6.1.2 **Acute Toxicity**
Annex Point IIA6.1 **Dermal**
Rat, limit test

		Dermal
3.3.2	Area covered	16 cm^2
3.3.3	Occlusion	Occluded
3.3.4	Vehicle	0.5% (w/v) solution of carboxymethylcellulose in distilled water
3.3.5	Concentration in vehicle	1.02 to 1.16 g/mL
3.3.6	Total volume applied	0.5 mL
3.3.7	Duration of exposure	24 h
3.3.8	Removal of test substance	Tap water
3.3.9	Controls	No
3.4	**Examinations**	Morbidity/mortality, clinical observations, body weights, dermal irritation reactions, necropsy and abbreviated *post mortem* examination of major organs and tissues.
3.5	**Method of determination of LD_{50}**	Estimated based on the absence of mortality.
3.6	**Further remarks**	Dermal irritation reactions were evaluated by the Draize technique.
		4 RESULTS AND DISCUSSION
4.1	**Mortality**	No deaths
4.2	**Clinical signs**	There were no treatment-related clinical signs of toxicity, although 2 females showed red-stained face on the day of treatment. Transient slight to moderate erythema, associated with slight edema in one animal, occurred in 8 of the 10 animals on the day of patch removal. Slight erythema persisted in 2 animals until day 7, but thereafter no dermal reactions were evident. See Table A6.1.2-1
4.3	**Pathology**	There were no macroscopic findings at necropsy in any animal.
4.4	**Body weight**	All male animals gained weight throughout the study, but 4 females during the first week and 2 females during the second week showed minor weight losses of up to 9g.
4.5	**LD_{50}**	The acute dermal median lethal dose (LD_{50}) was estimated to be greater than 2000 mg/kg bw in both sexes.

Section A6.1.2 **Acute Toxicity**
Annex Point IIA6.1 **Dermal**
Rat, limit test

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Guidelines: OECD 402 (1987), which is equivalent to 92/69/EEC (method B3); EPA-FIFRA, Subdivision F, § 81-2 (1982); JMAFF 59 NohSan No. 4200 (1985) No relevant deviations from test guidelines. Method: A group of 5 male and 5 female rats exposed to a limit dose of 2000mg formulated as a paste in aqueous carboxymethylcellulose solution /kg bw, by occluded dermal application for 24 hours to an area of 16 cm^2 intact clipped dorsal skin. 14-day observation period.
5.2	**Results and discussion**	Rat, dinotefuran limit test: dermal median lethal dose (LD_{50}) was estimated to be greater than 2000 mg/kg bw in both sexes.
5.3	**Conclusion**	Non-entry field
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A6.1.2-1 Group mean dermal irritation scores

Sex	Observation	Group mean dermal irritation scores on day:				
		1	**3**	**7**	**10**	**14**
Male	Erythema	1.60	1.0	0.40	0	0
	Edema	0.20	0.40	0	0	0
	Atonia	0	0	0	0	0
	Desquamation	0	0	0	0	0
	Coriaceousness	0	0	0	0	0
	Fissuring	0	0	0	0	0
Female	Erythema	0.60	0.20	0	0	0
	Edema	0	0	0	0	0
	Atonia	0	0	0	0	0
	Desquamation	0	0	0	0	0
	Coriaceousness	0	0	0	0	0
	Fissuring	0	0	0	0	0

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*10/09/12*
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	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.1.3 Acute Toxicity
Annex Point IIA6.1 Inhalation
Rat

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1999, MTI-446: Acute inhalation (nose only) toxicity study in the rat, [redacted], unpublished report no. 1300/3-D6154, August 3, 1999. [redacted] 2000a, First amendment to report - MTI-446: Acute inhalation (nose only) toxicity study in the rat, [redacted], unpublished report no. 1300/3-D6154, April 11, 2000. [redacted] 2000b, Second amendment to report - MTI-446: Acute inhalation (nose only) toxicity study in the rat, [redacted], unpublished report no. 1300/3-D6154, April 20, 2000.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes 92/69/EEC, method B2 (1992) OECD 403 (1981) OPPTS 870.1300 (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	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

Section A6.1.3	**Acute Toxicity**	
Annex Point IIA6.1	**Inhalation**	
	Rat	
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Crl:WI[Glx/BRL/Han]BR (SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	About 12 weeks old, weighing 321-378 g for males. 188-207 g for females
3.2.6	Number of animals per group	5 male and 5 females per group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Inhalation
3.3.1	Post-exposure period	14 days
		Inhalation
3.3.2	Concentrations	Nominal concentration: 0 mg/L Nominal concentration: 29.1 mg/L Analytical concentration: 0 mg/L Analytical concentration: 4.09 mg/L
3.3.3	Particle size	MMAD (mass median aerodynamic diameter) 4.74 µm ± GSD (geometric standard deviation) ±2.79 µm
3.3.4	Type of exposure	Nose only
3.3.5	Vehicle	Air
3.3.6	Concentration in vehicle	4.09 mg/L
3.3.7	Duration of exposure	4 h
3.3.8	Controls	Exposure to chamber air only
3.4	**Examinations**	Morbidity/mortality, clinical observations, body weights, necropsy and a full internal and external *post mortem* examination. The nasal cavity and respiratory tract were assessed for evidence of irritation and the weight of the lungs with trachea was recorded.
3.5	**Method of determination of LD_{50}**	Estimated based on the absence of mortality.
3.6	**Further remarks**	The concentration employed is less than the specified limit concentration of 5mg/L, since 4.09mg/L is the highest technically achievable concentration with a particle size of approximately 5µm (MMAD of 4.74µm)

Section A6.1.3 Acute Toxicity
Annex Point IIA6.1 Inhalation
Rat

4 RESULTS AND DISCUSSION

4.1	**Mortality**	No deaths occurred during the exposure or observation periods. See Table A6.1.3-1
4.2	**Clinical signs**	No clinical signs of an adverse reaction to treatment occurred during the exposure period and no treatment-related clinical signs of an adverse reaction to treatment were apparent.
4.3	**Pathology**	Necropsy and *post mortem* examination did not reveal any treatment-related lesions in either sex. The group mean absolute and relative lung weights of the male treated group were 11 and 14%, respectively, higher than the control group. However, the differences are considered to be incidental to treatment with dinotefuran since one control animal had an unusually low lung weight of 1.196g. The lung weights of the treated males were comparable to the lung weights of the other control males. See Table A6_01_3-01
4.4	**Body weight**	Body weight gains were not affected by exposure to dinotefuran. See Table A6_01_3-01
4.5	**LD_{50}**	The 4-hour inhalation lethal concentration (LC_{50}) value for respirable dinotefuran in male and female rats is > 4.09 mg/L.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Guidelines: 92/69/EEC, method B2 (1992); OECD 403 (1981); OPPTS 870.1300 (1998); JMAFF 59 NohSan no. 4200 (1985) No relevant deviations from test guidelines. Methods: A limit test was performed using 2 groups of 5 male and 5 female rats. Animals were exposed once for 4 hours by nose-only, flow-past inhalation to an atmosphere of dinotefuran as a dust in air at nominal concentration of 0 and 29.1 mg/L. 14-day observation period.
5.2	**Results and discussion**	Rat, dinotefuran limit test: inhalation lethal concentration (LC_{50}) value for respirable dinotefuran in male and female rats is > 4.09 mg/L.
5.3	**Conclusion**	Non-entry field
5.3.1	Reliability	1
5.3.2	Deficiencies	No

See Table A6.1.3-1 Mortality, body weight and lung weight

Sex	Exposure (mg/L)	Mortality (dying / tested)	Group mean body weight (g):				Mean lung weight	
			Pre-test	Day 2	Day 8	Day 15	(g)	(%)
Male	0	0 / 5	352	348	356	373	1.68	0.454
	4.09	0 / 5	342	336	350	366	1.87	0.516
Female	0	0 / 5	197	196	197	204	1.18	0.585
	4.09	0 / 5	199	198	201	208	1.26	0.614

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*10/09/12*
Materials and Methods	*As described by Applicant.*
Results and discussion	*As described by Applicant.*
Conclusion	*As described by Applicant.*
Reliability	*As described by Applicant.*
Acceptability	*Acceptable.*
Remarks	*The relative humidity range in the exposure chamber was 5-23% which is below the OECD recommended range of 30-70% but this deviation was not considered to affect the integrity of the study.*
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.1.4.d Acute Dermal Irritation
Annex Point IIA6.4 Rabbit

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1998a, Primary dermal irritation study of MTI-446 in rabbits, [redacted], unpublished report no. [redacted] 6648-121, March 17, 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. 404 (1992), which is equivalent to 92/69/EEC (method B4) EPA FIFRA, Subdivision F, 81-5 (1982) JMAFF 59 NohSan no. 4200 (1985) Japan Ministry of International Trade and Industry Guidelines
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Six rabbits instead of 3 (92/69/EEC B.4) were used since this is a regulatory requirement of EPA-FIFRA
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	22-00110
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	96.5% + 2% water, purity of dried material 99.1%
3.1.2.3	Stability	Expiration date: May 14, 2001
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White (Hra:(NZW)SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	14-18 weeks old, weighing 2260 – 2602 g
3.2.6	Number of animals per group	5 males and 1 female
3.2.7	Control animals	No

Section A6.1.4.d
Annex Point IIA6.4

Acute Dermal Irritation
Rabbit

3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	Non entry field
3.3.1.1	Preparation of test substance	Test substance was prepared by mixing 0.5 grams of test substance with 0.3 mL of distilled water.
3.3.1.2	Test site and Preparation of Test Site	Shaved intact dorsal and/or flank skin (2.5 x 2.5cm)
3.3.2	Occlusion	Semi-occlusive dressing
3.3.3	Vehicle	Distilled water
3.3.4	Concentration in vehicle	Not applicable
3.3.5	Total volume applied	0.3 mL
3.3.6	Removal of test substance	water
3.3.7	Duration of exposure	4 h
3.3.8	Post-exposure period	72 h
3.3.9	Controls	None
3.4	**Examinations**	
3.4.1	Mortality	Yes
3.4.2	Dermal examination	Yes
3.4.2.1	Scoring system	Dermal erythema & eschar formation and edema were graded according to the Draize scoring method[1] and the primary dermal irritation index (PDII) was calculated. EPA and EU index scores were calculated for each animal.
3.4.2.2	Examination time points	At 30 minutes after patch removal, and subsequently at 24, 48 and 72 hours.
3.4.3	Other examinations	None
3.5	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Average score**	Non-entry field
4.1.1	Erythema	See Table A6_1-4d
4.1.2	Edema	See Table A6_1-4d
4.2	**Reversibility**	Yes

[1] Draize, J. H. (1959): "Primary Irritation of the Skin," In: *Appraisal of the safety of chemicals in foods, drugs and cosmetics* - Dermal Toxicity, Assoc. of Food and Drug Officials of the United States., pp 46 - 47

Section A6.1.4.d **Acute Dermal Irritation**

Annex Point IIA6.4 **Rabbit**

4.3	**Other examinations**	Mortality: no deaths Clinical observations: not reported Body weights: not reported
4.4	**Overall result**	Dinotefuran does not require classification as a skin irritant in the EU according to Annex VI of Commission Directive 93/21/EEC (4 May 1993) since neither the overall mean index score nor any individual score was greater than 2. Dinotefuran is classified as a slight skin irritant according to EPA criteria, based on a PDII of 0.2. (category IV according to EPA classification criteria and not classified according to GHS criteria).
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Guidelines: OECD guideline no. 404 (1992), which is equivalent to 92/69/EEC (method B4); EPA FIFRA, Subdivision F, 81-5 (1982); JMAFF 59 NohSan no. 4200 (1985); Japan Ministry of International Trade and Industry Guidelines No relevant deviations from test guidelines. Methods: 0.5g dinotefuran moistened with 0.3 mL of distilled water was applied once for 4 hours, under semi-occlusive dressing, to shaved intact dorsal and/or flank skin (2.5 x 2.5cm), to 6 NZW rabbits (5 male and 1 female). 72 h observation period.
5.2	**Results and discussion**	Rabbit, dinotefuran: 5 of the 6 animals had EU index scores of 0.0 and the remaining animal had an index score of 0.33.
5.3	**Conclusion**	Dinotefuran does not require classification as a skin irritant in the EU according to Annex VI of Commission Directive 93/21/EEC (4 May 1993) since neither the overall mean index score nor any individual score was greater than 2.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A6_1-4d **Individual skin irritation and index scores**

Animal	Individual erythema / edema scores at:				EPA index score*	EU index score**
	30 minutes	24 hours	48 hours	72 hours		
1	1 / 0	0 / 0	0 / 0	0 / 0	0.25	0.0
2	0 / 0	0 / 0	0 / 0	0 / 0	0	0.0
3	0 / 0	0 / 0	0 / 0	0 / 0	0	0.0
4	1 / 0	0 / 0	0 / 0	0 / 0	0.25	0.0
5	0 / 0	0 / 0	0 / 0	0 / 0	0	0.0
6	1 / 0	1 / 0	0 / 0	0 / 0	0.50	0.33
Total score (erythema + edema)	3	1	0	0	PDII*** 0.2	Mean 0.06

* EPA index score = total erythema & edema score at all observation intervals / no. of observation intervals

** EU index score = total erythema & edema score at the 24, 48 and 72hr intervals / no. of observation intervals

*** PDII = sum of individual index scores / no. of animals, rounded to nearest 0.1

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *11/09/12*

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

COMMENTS FROM ...

Date

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

Section 6.1.4.e Acute Eye Irritation
Annex Point IIA6.1.4 Rabbit

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1998b, Primary eye irritation study of MTI-446 in rabbits, [redacted], unpublished report no. [redacted] 6648-122, June 11, 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. 405 (1987), which is equivalent to 92/69/EEC (method B5) EPA FIFRA, Subdivision F, 81-4 (1982) JMAFF 59 NohSan no. 4200 (1985) Japan Ministry of International Trade and Industry Guidelines
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Nine rabbits instead of 3 (92/69/EEC B.5) were used, the eyes of 6 rabbits remaining unwashed (regulatory requirement in US) and the eyes of 3 animals washed 30 seconds after instillation of test article.
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	22-00110
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	96.5% + 2% water, purity of dried material 99.1%
3.1.2.3	Stability	Expiration date: May 14, 2001
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White (Hra:(NZW)SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	14-18 weeks old, weighing 2334 – 2694 g
3.2.6	Number of animals per group	6 males and 3 females
3.2.7	Control animals	No

Section 6.1.4.e Acute Eye Irritation
Annex Point IIA6.1.4 Rabbit

3.3	**Administration/ Exposure**	
3.3.1	Preparation of test substance	Test substance was used as delivered
3.3.2	Amount of active substance instilled	0.1 grams dry dinotefuran solid
3.3.3	Exposure period	Washed group: 3 rabbits 30-second exposure Unwashed group: 6 rabbits 96 h
3.3.4	Post-exposure period	14-days
3.4	**Examinations**	
3.4.1	Mortality	Yes
3.4.2	Ophthalmoscopic examination	Yes
3.4.2.1	Scoring system	Irritation reactions were graded and scored according to the Draize technique. Sodium fluorescein examinations were performed to assist the visualisation of possible corneal lesions.
3.4.2.2	Examination time points	Irritation reactions: 1, 24, 48, 72 and 96 hours and 7 and 14 days after instillation. Sodium fluorescein examinations: at 24, 48, 72 and 96 hours or until a negative response was evident.
3.4.3	Other investigations	None
3.5	**Further remarks**	None

Section 6.1.4.e **Acute Eye Irritation**
Annex Point IIA6.1.4 **Rabbit**

4 RESULTS AND DISCUSSION

4.1	**Average score**	
4.1.1	Cornea	Unwashed group: 0.2, 0.7, 0.2, 0.2 and 0.2 at 1, 24, 48, 72 and 96 h respectively. Washed group: 0.0, 1.0, 0.0, 0.0 and 0.0 at 1, 24, 48, 72 and 96 h respectively. See Table A6.1.4.e-1 See Tables A6.1.4.e-2 and A6.1.4.e-3 for mean primary eye irritation scores and individual irritation scores, respectively.
4.1.2	Iris	Unwashed group: 0.2, 0.3, 0.2, 0.0 and 0.0 at 1, 24, 48, 72 and 96 h respectively. Washed group: 0.0, 0.0, 0.0, 0.0 and 0.0 at 1, 24, 48, 72 and 96 h respectively. See Table A6.1.4.e-1 See Tables A6.1.4.e-2 and A6.1.4.e-3 for mean primary eye irritation scores and individual irritation scores, respectively.
4.1.3	Conjunctiva	
4.1.3.1	Erythema	Unwashed group: 2.0, 2.0, 1.3, 0.8 and 0.3 at 1, 24, 48, 72 and 96 h respectively. Washed group: 2.0, 2.0, 1.7, 1.3 and 1.0 at 1, 24, 48, 72 and 96 h respectively. See Table A6.1.4.e-1 See Tables A6.1.4.e-2 and A6.1.4.e-3 for mean primary eye irritation scores and individual irritation scores, respectively.
4.1.3.2	Edema	Unwashed group: 2.0, 1.5, 1.2, 0.3 and 0.2 at 1, 24, 48, 72 and 96 h respectively. Washed group: 2.3, 1.7, 1.3, 0.7 and 0.7 at 1, 24, 48, 72 and 96 h respectively. See Table A6.1.4.e-1 See Tables A6.1.4.e-2 and A6.1.4.e-3 for mean primary eye irritation scores and individual irritation scores, respectively.
4.2	**Reversibility**	Yes Unwashed eyes: returned to normal appearance 14-days after instillation. Washed eyes: returned to normal appearance by 96-hours after instillation.

X

Section 6.1.4.e **Acute Eye Irritation**

Annex Point IIA6.1.4 **Rabbit**

4.3	**Other**	Mortality: no deaths Clinical observations: not reported Body weights: not reported
4.4	**Overall result**	Dinotefuran is slightly irritating to the eyes of the rabbit but does not require classification in the EU under the provisions of Commission Directive 93/21/EEC, Annex VI (1993), and no risk phrase is required in respect of ocular irritation. Dinotefuran is slightly irritating to both irrigated and unirrigated rabbit eyes, based on the classification system of Kay & Calandra (1962)[1] (category IV according to EPA classification criteria and not classified according to GHS criteria).
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Guidelines: OECD guideline no. 405 (1987), which is equivalent to 92/69/EEC (method B5); EPA FIFRA, Subdivision F, 81-4 (1982); JMAFF 59 NohSan no. 4200 (1985); Japan Ministry of International Trade and Industry Guidelines No relevant deviations from test guidelines. Methods: 0.1g dry dinotefuran solid was introduced into the right conjunctival sac of 9 New Zealand White rabbits (6 male and 3 female). The left eye remained untreated as a reference control. Both eyes of 3 animals were flushed with water for one minute starting 30 seconds after instillation of the test article. The eyes of the other 6 animals remained unwashed. 14-days observation period.
5.2	**Results and discussion**	Rabbit, dinotefuran: None of the individual animal or group mean irritation scores exceeded the EU criteria for classification as "irritating to eyes".
5.3	**Conclusion**	Dinotefuran is slightly irritating to the eyes of the rabbit but does not require classification in the EU under the provisions of Commission Directive 93/21/EEC, Annex VI (1993), and no risk phrase is required in respect of ocular irritation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

[1] Kay, J. H. and Calandra, J. C. (1962): "Interpretation of eye irritation test," *Journal of the Society of Cosmetic Chemists,* 13(6): pp. 281-289.

Table A6.1.4.e-1 Group mean irritation scores according to Commission Directive 93/21/EEC (Annex VI)

Observation	Mean irritation scores in the:									
	Unwashed group at:					Washed group at:				
	1hr	24hr	48hr	72hr	96hr	1hr	24hr	48hr	72hr	96hr
Corneal opacity	0.2	0.7	0.2	0.2	0.2	0.0	1.0	0.0	0.0	0.0
Iris lesion	0.2	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erythema	2.0	2.0	1.3	0.8	0.3	2.0	2.0	1.7	1.3	1.0
Edema	2.0	1.5	1.2	0.3	0.2	2.3	1.7	1.3	0.7	0.7

Table A6.1.4.e-2 Mean primary eye irritation scores

Group	Mean primary eye irritation scores* at:						
	1hr	24hr	48hr	72hr	96hr	7 days	14 days
Unwashed group	13.0	14.8	6.7	3.2	1.8	2.2	0.0
Washed group	12.0	14.7	8.3	4.0	3.3	1.3	0.0

* Mean score = total eye irritation score at each interval for all animals / no. animals in the group
Individual irritation scores = (A x B x 5) + (C x 5) + ([D + E + F] x 2)

Table A6.1.4.e-3 Individual irritation scores

Observation	Time	Individual scores								
		unwashed group						washed group		
		1	2	3	4	5	6	7	8	9
Cornea (severity/area) (A / B)	1hr	0 / 0	0 / 0	1 / 1	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
	24hr	0 / 0	0 / 0	1[a] / 1	1[a] / 1	1[a] / 1	1[a] / 2	1[a] / 1	1[a] / 1	1[a] / 2
	48hr	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	1[a] / 1	0 / 0	0 / 0	1[a] / 1
	72hr	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	1[a] / 1	0 / 0	0 / 0	0 / 0
	96hr	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	1 / 1	0 / 0	0 / 0	0 / 0
	7days	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	1 / 1	0 / 0	0 / 0	0 / 0
	14days	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
Iris (C)	1hr	0	0	1[b]	0	0	0	0	0	0
	24hr	0	0	1[b]	0	0	1[b]	0	0	0
	48hr	0	0	0	0	0	1[b]	0	0	0
	72hr	0	0	0	0	0	0	0	0	0
	96hr	0	0	0	0	0	0	0	0	0
	7days	0	0	0	0	0	0	0	0	0
	14days	0	0	0	0	0	0	0	0	0
Redness (D)	1hr	2[c]	2[c]	2[c]	2[c]	2[c]	2[c]	2[c]	2[c]	2[c]
	24hr	2[c]	2[c]	2[c]	2[c]	2[c]	2[c]	2[c]	2[c]	2[c]
	48hr	1	1	1	1	2[c]	2[c]	2	1	2[c]
	72hr	0	1	0	1	1	2	1	1	2[c]
	96hr	0	0	0	1	0	1	1	0	2
	7days	0	0	0	1	0	2	0	0	1
	14days	0	0	0	0	0	0	0	0	0
Chemosis (E)	1hr	2	2	2	2	2	2	2	2	3
	24hr	1	2	1	1	1	3	2	1	2
	48hr	1	1	1	1	1	2	1	1	2
	72hr	0	0	0	0	1	1	0	0	2
	96hr	0	0	0	0	0	1	0	0	2
	7days	0	0	0	0	0	1	0	0	1
	14days	0	0	0	0	0	0	0	0	0
Discharge (F)	1hr	1[d]	1[d]	2[d]	2[e]	2[e]	2[d]	1[d]	2[d]	2[d]
	24hr	0	1[d]	1[d]	1[d]	1[d]	2[d]	0	0	1[e]
	48hr	0	0	0	0	0	0	0	0	1[e]
	72hr	0	0	0	0	0	0	0	0	0
	96hr	0	0	0	0	0	0	0	0	0
	7days	0	0	0	0	0	0	0	0	0
	14days	0	0	0	0	0	0	0	0	0

a – corneal epithelial peeling

b – injected

c – blanching

d – clear discharge

e – purulent discharge

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*11/09/12*
Materials and Methods	*As described by Applicant.*
Results and discussion	*As described by Applicant but with the highlighted amendments/additions to Table A6.1.4e-3 (see above). Plus in Section 4.2it is stated that the irritant effects observed in the washed group had reversed by 96 hours post-exposure but effects on the conjunctiva were apparent up to 7 days post-exposure in at least 1 animal.*
Conclusion	*As described by Applicant.*
Reliability	*As described by Applicant.*
Acceptability	*Acceptable.*
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Statement on the dose setting for
the Dermal Sensitization Study in Guinea Pigs with Dinotefuran (6648-123)
Mitsui Chemicals Agro, Inc

Introduction

The skin sensitization potential of dinotefuran was evaluated in a guinea-pig maximization test according to the method of Magnusson and Kligman[1]: [redacted] 1997d, Dermal sensitization study of MTI-446 in guinea pigs - maximisation test, [redacted], unpublished report no. [redacted] 6648-123, December 9, 1997. Dinotefuran concentrations were based on the results of a preliminary study.

The Competent Authority for the evaluation of the data submitted in support of the application for Annex I inclusion of dinotefuran have expressed the following: "The challenge concentration of 25% w/w dinotefuran may not be sufficiently high to assess the full sensitisation potential of dinotefuran", since the concentration used for topical induction (25% w/w) did not cause irritation in the preliminary or final study.

Discussion

[redacted] (1997d) was conducted in accordance with OECD Health Effects Test Guideline 406 "Skin Sensitization" adopted 17 July 1992.

The original Magnusson and Kligman maximization publication[1] specifies the procedure to prepare and evaluate the maximum test item concentration; the methodology has been a long-standing industry practice and is known to be widely accepted by the US EPA and OECD registration officials.

The Magnusson and Kligman procedure includes an approach to incorporate the pulverized solid test item into petrolatum at a concentration not exceeding 25% w/w, based on the rationale that solid test item concentrations greater than 25% w/w in petrolatum are generally not homogeneous and do not allow for good contact of the test item with animal skin under the conditions of the test. Good contact of the test item with animal skin is necessary to ensure potential absorption into the skin.

Therefore, the dinotefuran concentration for topical induction application in study [redacted] (1997d) is considered appropriate and in accordance with the regulatory test guideline and industry standards.

Additionally, dinotefuran technical is a powder with low solubility in organic solvents; 25% w/w dinotefuran in petrolatum is the maximum technically achievable concentration possible to prepare a homogeneous solution.

Conclusion

[redacted] (1997d) has been performed in accordance with scientifically sound and valid methodology in accordance with OECD 406, which is widely accepted by industry and regulatory authorities. On this basis, dinotefuran should be considered as a non-sensitiser.

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[1] Magnusson B. and Kligman A.M. (1969). The identification of contact allergens by animal assay. The guinea pig maximization test. J. Invest. Dermatol., 52, 268.

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 1997d, Dermal sensitization study of MTI-446 in guinea pigs - maximisation test, [redacted], unpublished report no. [redacted] 6648-123, December 9, 1997.	
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. 406 (1992), which is equivalent to 92/69/EEC (method B6) EPA-FIFRA, Subdivision F, 81-6 (1982) JMAFF 59 NohSan no. 4200 (1985)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	22-00110	
3.1.2	Specification		
3.1.2.1	Description	White powder	
3.1.2.2	Purity	96.5% + 2% water, purity of dried material 99.1%	
3.1.2.3	Stability	Expiration date: May 14, 2001	
3.1.2.4	Preparation of test substance for application	a) <u>for induction:</u> A series of 3 preparations for dinotefuran for intradermal induction was prepared as follows: 1. 1:1 dilution of FCA in sterile water, without dinotefuran 2. 5% suspension of dinotefuran in 0.5% CMC/distilled water 3. 1:1 dilution of 10% suspension of dinotefuran in 0.5% CMC/distilled water and FCA b) <u>for challenge:</u> A 25% (w/w) mixture of dinotefuran in petrolatum.	X
3.1.2.5	Pretest performed on irritant effects	Yes, a preliminary irritation study in which 2 groups of 4 guinea pigs were exposed by occluded topical application for 24 hours to dinotefuran at concentrations of 5, 10, 15 and 25% w/w in petrolatum, or intradermally at concentrations of 1, 5, 10 and 15% in aqueous carboxymethylcellulose. Dermal reactions evaluated 24 and 48 hours after treatment. .	

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT)**

3.2	**Test Animals**	Non-entry field
3.2.1	Species	Albino guinea pig
3.2.2	Strain	Crl:[HA]BR
3.2.3	Source	[redacted]
3.2.4	Sex	male
3.2.5	Age/weight at study initiation	4-8 weeks old, weighing 372 – 500 g
3.2.6	Number of animals per group	20 test animals (dinotefuran): exposed to the test substance at inductions and challenge.
3.2.7	Control animals	20 irritation control animals (for dinotefuran): exposed to the test substance only at challenge.
3.3	**Administration/ Exposure**	State study type: Adjuvant
3.3.1	Induction schedule	- Day 1: intradermal injections - Day 7: the application sites of both groups of animals were treated topically with 10% sodium lauryl sulfate which was massaged into the skin. - Day 8: the animals were treated topically, over the injection sites, under occlusive dressing for 48 hours.
3.3.2	Way of Induction	Intradermal and topical (occlusive)
3.3.3	Concentrations used for induction	-.....Intradermal induction: the test group received 3 pairs of intradermal injections o.....50% FCA in water. o.....5% w/v dinotefuran in aqueous carboxymethylcellulose. o.....10% w/v dinotefuran in aqueous carboxymethylcellulose diluted 1:1 with FCA. The control group received similar injections but without dinotefuran. -.....Topical induction: the animals were treated topically, over the injection sites, under occlusive dressing for 48 hours. o.....25% w/w dinotefuran in petrolatum (treated group) o.....petrolatum alone (irritation control group)
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	50% in water
3.3.5	Challenge schedule	Day 22
3.3.6	Concentrations used for challenge	Both groups were challenged topically, under occlusive dressing for 24 hours, with 25% w/w dinotefuran in petrolatum (right side) and petrolatum alone (left side)
3.3.7	Rechallenge	No
3.3.8	Scoring schedule	24 h and 48 h after challenge
3.3.9	Removal of the test substance	24 h after challenge

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT)**

3.3.10	Positive control substance	α-hexylcinnamaldehyde
3.4	**Examinations**	Non-entry field
3.4.1	Pilot study	No
3.5	**Further remarks**	- Following removal of the dressings, the challenge sites were shaved and then scored for dermal reactions 24 and 48 hours after removal of the challenge dressings. - Clinical observations were recorded daily and individual body weights were recorded pre-test and at termination. - The main study was performed according to the method of Magnusson & Kligman[1]
		4 RESULTS AND DISCUSSION
4.1	**Results of pilot studies**	Not applicable
4.2	**Results of test**	
4.2.1	Preliminary study	No dermal irritation occurred at any concentration of dinotefuran administered by topical application, up to 25% w/v. Therefore, all dermal reactions were scored as zero. In the 4 animals treated by intradermal injection, dinotefuran produced mild erythema (grade 1) at 1% w/v, mild-moderate diffuse erythema (grade 1 - 2) reactions at 5% w/v and moderate-marked erythema (grade 2 - 3) at 10 and 15% w/v. See Table A6.1.5-1
4.2.2	Main study	There were no treatment-related clinical signs or adverse effects on body weight. None of the test and control group animals exhibited a dermal response to the challenge application of the test or control articles either 24 or 48 hours after patch removal. Therefore, all dermal reaction scores were zero. The positive control study was conducted within six months of the conduct of this study. The positive reaction of the challenge skin of all 10 positive control (hexylcinnamaldehyde) animals was observed, whereas all scores in the negative control group were zero. Therefore, hexylcinnamaldehyde was considered to be an extreme dermal sensitizer
4.2.3	Other findings	- Mortality: No deaths - Clinical observations: No clinical signs reported - Body weight: All animals gained weight during the observation period (range of weight gain: 83 - 159 g).
4.3	**Overall result**	Based on a skin reaction incidence of 0%, which is below the 30% threshold of significance specified in Commission Directive 93/21/EEC, dinotefuran is not a dermal sensitiser in the guinea pig, and no EU classification is required (category IV according to EPA classification criteria and not classified according to GHS criteria).

[1] Magnusson, B. and Kligman, A. (1970): Allergic Contact Dermatits in the Guinea Pig, Charles C. Thomas, pp. 113-117, 120.

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT)**

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Guidelines: OECD guideline no. 406 (1992), which is equivalent to 92/69/EEC (method B6), EPA-FIFRA, Subdivision F, 81-6 (1982), JMAFF 59 NohSan no. 4200 (1985) No relevant deviations from test guidelines. Method: Groups of 20 test and 20 negative control male guinea pigs were subjected to a maximisation test. Skin reactions to the challenge applications were evaluated 24 and 48 hours after patch removal. The concentrations of dinotefuran applied, 5 and 10% intradermally and 25% topically, were determined in preliminary irritation studies.	X
5.2	**Results and discussion**	There were no treatment-related clinical signs or adverse effects on body weight. None of the test and control group animals exhibited a dermal response to the challenge application of the test or control articles either 24 or 48 hours after patch removal. Therefore, all dermal reaction scores were zero. The positive control study was conducted within six months of the conduct of this study. The positive reaction of the challenge skin of all 10 positive control (hexylcinnamaldehyde) animals was observed, whereas all scores in the negative control group were zero. Therefore, hexylcinnamaldehyde was considered to be an extreme dermal sensitizer. Based on a skin reaction incidence of 0%, which is below the 30% threshold of significance specified in Commission Directive 93/21/EEC, dinotefuran is not a dermal sensitiser in the guinea pig, and no EU classification is required (category IV according to EPA classification criteria and not classified according to GHS criteria).	X
5.3	**Conclusion**		
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	X

Table A6.1.5-1 Individual dermal reaction scores in the irritation screening study – intradermal injection

Animal number	Dermal reaction score at:							
	1% w/v		5% w/v		10% w/v		15% w/v	
	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
1	1	1	2	2	3	3	3	3
2	1	1	2	2	2	2	3	3
3	1	1	1	1	2	2	2	2
4	1	1	1	1	2	2	3	3

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*12/09/12*
Materials and Methods	*As described by the Applicant with the following additions/amendments,* *Section 3.1.2.4 – A topical induction with 25% w/w dinotefuran in petrolatum was also applied.* *Section 5.1 – The Applicant states that dinotefuran was applied at 5 and 10% intradermally but the 10% dinotefuran preparation was diluted 1:1 with FCA before application. Therefore only 5% dinotefuran was applied intradermally with and without FCA.*
Results and discussion	*As described by the Applicant with the following amendment,* *Section 5.2 – One animal in the test group appeared thin from day 20.*
Conclusion	*Dinotefuran does not induce skin sensitisation at concentrations ≤ 25%.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*None.*
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.10-1 Immunotoxicity
Annex Point IIA6.10 Rat
Oral

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 2011, Dinotefuran: 4-week dietary immunotoxicity study in the CD rat, [redacted], unpublished report no. [redacted] 0018, 29 March, 2011.	
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 OPPTS 870.7800 (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	K09C3718	
3.1.2	Specification		
3.1.2.1	Description	White crystalline solid	
3.1.2.2	Purity	97.9%	
3.1.2.3	Stability	Expiry date: December 27, 2012	
3.2	**Reference Substance (positive control)**	Group 5 animals received one intraperitoneal administration of 50mg/kg Cyclophosphamide as a 5 mg/mL solution at 10 mL/kg.	
3.3	**Test Animals**		
3.3.1	Species	Rat	
3.3.2	Strain	Crl:CD® (SD)	
3.3.3	Source	[redacted]	
3.3.4	Sex	Males and females	
3.3.5	Housing	5 of one sex/cage (and 4 of one sex/cage – group 5, positive controls)	
3.3.6	Age/weight at study initiation	47 – 56 days old, weighing 265 - 333 g (males) and 193 - 263 g (females)	

Section A6.10-1 **Immunotoxicity**

Annex Point IIA6.10 **Rat**

Oral

3.3.7	Number of animals per group	The animals were randomly assigned to single sex treatment groups on arrival:

Group number / treatment	Dose level (ppm)*	Number of animals	
		Male	Female
1 – Control	0 (vehicle)	10	10
2 – Dinotefuran	2400	10	10
3 – Dinotefuran	5600	10	10
4 – Dinotefuran	14000	10	10
5 – Cyclophosphamide	50 mg/kg†	8	8

* Expressed in terms of the active substance; a conversion factor of 1.021 was applied to allow for purity.
† Cyclophosphamide administered by intraperitoneal injection on Day 27.

3.3.8	Control animals	Yes
3.4	**Administration**	Oral by admixture to the diet
3.4.1	Exposure	Administration daily for 28 days
3.4.2	Concentration	See 3.3.7 above. Overall achieved dosage: 0, 164, 425 and 992 mg/kg/day for males and 0, 179, 430 and 1018 mg/kg/day for females.
3.4.3	Vehicle	No vehicle, added to basal diet
3.4.4	Concentration in vehicle	Not applicable
3.4.5	Total volume applied	Not applicable
3.4.6	Postexposure period	None.
3.4.7	Anticholinergic substances used	None
3.4.8	Controls	Vehicle
3.5	**Examinations**	
3.5.1	Body Weight	Body weights of Groups 1 – 4 were recorded twice during the week before treatment (Day -7 and -4), on the first day of test (Day 1), twice weekly during the treatment period and at necropsy. Group 5 animals were weighed on the day of cyclophosphamide treatment only (Day 27).
3.5.2	Signs of Toxicity	The spleen from each animal in all groups was used as the source of splenocytes for conducting a plaque forming cell (PFC) assay using a modification of the Jerne plaque-forming cell (PFC) assay[1,2] which had been fully validated at the conducting[1] laboratory in addition to the concurrent positive control.
3.5.3	Observation schedule	Animals were examined twice daily for morbidity or mortality, and a detailed clinical examination was performed weekly.

[1] Jerne, N. K. and Nordin, A. A.(1963): Science, 140, 405.

[2] Holsapple, M. P. (1995): In Methods in Immunotoxicity, vol. 1, 71 – 108. Eds. Burlinson, Dean and Monson, Wiley Liss, New York.

Section A6.10-1 **Immunotoxicity**

Annex Point IIA6.10 **Rat**

Oral

		Food consumption was recorded weekly and water consumption was recorded for a 3-day period each week throughout the treatment period for groups 1 - 4.
3.5.4	Clinical Chemistry	No
3.5.5	Pathology	Yes Organs: Spleen and thymus were weighed and were also adjusted for terminal body weight. Spleen tissues required for immunotoxicology investigations were retained from Groups 1 – 5 for assessment of the acquired or adaptive immune response using a modification of the Jerne plaque-forming cell assay which had been fully validated by the conducting laboratory, in addition to the concurrent positive control.
3.5.6	Histopathology	No
3.6	**Further remarks**	All animals received a single, intravenous dose (bolus injection) of 2 x 10^8 sheep washed red blood cells (SRBCs) in physiological saline at a dose volume of 1.0 mL/animal on Day 25 of the study. Statistics: All statistical analyses were conducted separately for males and females using the individual animal as the basic experimental unit. Body weight, organ weight and plaque forming cell data were analysed as follows: If Bartlett's test for variance homogeneity was not significant at the 1% level, then parametric analysis was applied. The F_1 approximate test was applied. If the F_1 approximate test for monotonicity of dose-response was not significant at the 1% level, Williams' test for a monotonic trend was applied. If the F_1 approximate test was significant, suggesting that the dose response was not monotone, Dunnett's test was performed instead. If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. The H_1 approximate test, the non-parametric equivalent of the F_1 test described above, was applied. If the H_1 approximate test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend was applied. If the H_1 approximate test was significant, suggesting that the dose-response was not monotone, Steel's test was performed instead. For organ weight data, analysis of covariance was performed using terminal bodyweight as covariate. The treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.

Section A6.10-1 **Immunotoxicity**
Annex Point IIA6.10 **Rat**
Oral

4 RESULTS AND DISCUSSION

4.1 Body Weight

The body weight gain of males receiving 14000 ppm was persistently lower than control throughout the treatment period resulting in an overall (Days 1 to 29) weight gain that was statistically significantly lower than control ($p<0.01$) by approximately 28%. On Day 29, the group mean body weight of males receiving 14000 ppm was 89.7% of the control value. The body weights for both sexes receiving 2400 or 5600 ppm and females receiving 14000 ppm were considered to have been unaffected by treatment. Although the overall weight gain among females receiving 14000 ppm was 10% lower than control, it did not attain statistical significance ($p>0.5$) and was due to the lower weight gain that occurred between Days 1 to 4 of treatment, with the subsequent weight gain being similar to controls (Table A6.10.1-1).Thus, the group mean body weight of females receiving 14000 ppm on Day 29 remained marginally higher than the control value.

4.2 Clinical signs of toxicity

There were no treatment-related clinical signs at any dose level or in the cyclophosphamide-treated group and no animals died prematurely.

Food consumption was persistently lower than control for both sexes receiving 14000 ppm, resulting in an overall reduction, compared to controls, of 12% in males and 10% in females. The food consumption of both sexes receiving 2400 or 5600 ppm were considered to be unaffected by treatment. The slightly low food consumption of females receiving 5600 ppm represented a trend that was present before treatment commenced and was therefore considered not to be due to treatment with dinotefuran.

Water consumption among all treated groups of both sexes, although variable, was similar to control or pre-treatment values and were therefore considered unaffected by treatment.

The macroscopic examination performed after 4 weeks of treatment revealed no lesions attributable to treatment with dinotefuran. The nature and incidence of all the findings were consistent with the commonly seen background of macroscopic changes in Crl: CD (SD) strain rats.

4.3 Immunotoxicology Investigations

There was no effect of treatment at any dose level on the humoral T-lymphocyte dependent antibody response to sheep red blood cells, as measured using the modified plaque forming cell (PFC) assay. There were no statistically significant changes in the number of cells/spleen, PFC/10^6 viable cells or PFC/spleen, when compared to the control, for the treated groups which received dinotefuran at 2400, 5600 or 14000 ppm (Table A6.10.1-3).

Treatment with a single 50 mg/kg dose of the immunosuppressant cyclophosphamide on Day 27 resulted in a very marked and statistically significant reduction of the PFC response. The number of cells/spleen, PFC/10^6 viable cells and PFC/spleen ($p<0.001$) were all statistically significantly reduced for males and females when compared to the control, demonstrating the sensitivity of the PFC assay

4.4 Pathology

Absolute and bodyweight adjusted spleen and thymus weights were similar to, and not significantly different ($p>0.05$) from the controls for all dinotefuran treated groups of both sexes and were therefore not affected by treatment (Table A6.10.1-2).

Section A6.10-1 **Immunotoxicity**
Annex Point IIA6.10 **Rat**
Oral

4.5	**Histopathology**	Not applicable
4.6	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Guidelines: OPPTS 870.7800 (1998) No relevant deviations from test guidelines. Method: Four groups of 10 male and 10 female SD rats were treated orally, by diet admixture, with dinotefuran at concentrations of 0, 2400, 5600 and 14000 ppm (overall achieved dosage: 0, 164, 425 and 992 mg/kg/day for males and 0, 179, 430 and 1018 mg/kg/day for females) for 4 weeks. A group of 8 rats/sex, given a single intraperitoneal injection of 50 mg/kg cyclophosphamide (CP) 2 days before termination, acted as a positive control group. All animals received a sensitising intravenous dose of sheep red blood cells (SRBCs) 4-days prior to termination. Body weights were recorded twice weekly, and food and water consumption were recorded weekly throughout the treatment period. All animals were subjected to detailed necropsy after 4-weeks treatment, and the weights of the spleen and thymus were recorded. Splenic tissue from all test, control and positive control animals, was used as a source of splenocytes for assessment of the adaptive or acquired immune response to the T-cell-dependent immunogen, SRBCs, using a modification of the Jerne Plaque Forming Cell (PFC) assay. The number of lytic plaques for each animal was determined and group mean responses were calculated and expressed as group mean number of PFC/spleen and per 10^6 splenocytes.
5.2	**Results and discussion**	All animals survived the scheduled treatment period and there were no treatment-related clinical signs. Body weight gain was reduced by treatment in males receiving 14000 ppm. Body weight gain for both sexes receiving 2400 or 5600 ppm and females receiving 14000 ppm was considered to have been unaffected by treatment. Food consumption was persistently lower than control for both sexes receiving 14000 ppm. The food consumption of both sexes receiving 2400 or 5600 ppm was considered to be unaffected by treatment. Treatment with a single 50 mg/kg dose of the immunosuppressant cyclophosphamide on Day 27 resulted in a very marked and statistically significant reduction of the PFC response. The number of cells/spleen, PFC/10^6 viable cells and PFC/spleen were significantly reduced for males and females when compared to the control. To determine the effect of dietary administration of dinotefuran on the antigen-specific activity of the immune system, the ability to produce a primary antibody response was assessed in a plaque forming cell assay. This investigation demonstrated that there was no immunotoxicologically relevant effect of dinotefuran on the humoral T-lymphocyte-dependent response against antigen on sheep red blood cells. There were no statistically significant differences in the number of cells/spleen, PFC/10^6 viable cells or PFC/spleen, when compared to the control, for the treated groups which received dinotefuran at 2400,

Section A6.10-1 **Immunotoxicity**
Annex Point IIA6.10 **Rat**
Oral

		5600 or 14000 ppm The magnitude of the effect on body weight in males demonstrated that 14000 ppm was the maximum tolerated dose for this study type and duration. There was no effect on immune function, as assessed by the measurement of antigen-specific, T-cell dependant antibody formation. The no-observed-effect-level (NOEL) for functional immunotoxicity by dinotefuran was therefore greater than 14000 ppm, equivalent to dose levels of >992 mg/kg/day in males and >1018 mg/kg/day in females. The no-observed-adverse-effect-level (NOAEL) for all effects was 5600 ppm, equivalent to dose levels of 425 and 430 mg/kg/day in males and females, respectively.
5.3	**Conclusion**	
5.3.1	LOAEL (All effects)	The lowest-observed-adverse-effect level (LOAEL) for all effects was 14000 ppm, equivalent to dose levels of 992 and 1018 mg/kg/day in males and females, respectively.
5.3.2	NOAEL (All effects)	The no-observed-adverse-effect-level (NOAEL) for all effects was 5600 ppm, equivalent to dose levels of 425 and 430 mg/kg/day in males and females, respectively.
5.3.3	NOEL (Immunotoxicity)	14000 ppm, equivalent to dose levels of 992 mg/kg/day in males and 1018 mg/kg/day in females, based on no effects in the PFC assay at the highest dose level employed.
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Table A6.10.1-1: Selected group mean body weight data

Treatment	Group mean body weight (g) on day:				Group mean change Day 1–29:	
(ppm)	1	8	22	29	(g)	(% of control)
Males						
Control	295	343	412	447	152	-
2400	298	342	408	439	141	93
5600	303	355	421	460	157	103
14000	292	322	375	401	109**	72
CP – 50 †	-	-	-	-	-	-
Females						
Control	210	223	244	253	44	-
2400	210	224	248	255	45	103
5600	208	220	237	249	40	93
14000	219	227	248	258	39	90
CP – 50 †	-	-	-	-	-	-

** $p < 0.01$; CP cyclophosphamide
† Dosed on one occasion (intraperitoneal injection, 50 mg/kg) 2 days after administration of SRBC.

Table A6.10.1-2: Selected group mean organ weight data (unadjusted)

Treatment	Mean body	Group mean organ weight:	
(ppm)	weight (g)	Spleen (g)	Thymus (g)
Males			
Control	447	0.872	0.530
2400	439	0.901	0.527
5600	458	0.925	0.486
14000	399**	0.772	0.455
CP – 50 †	-	-	-
Females			
Control	252	0.614	0.385
2400	254	0.599	0.363
5600	248	0.528	0.343
14000	257	0.611	0.387
CP – 50 †	-	-	-

** $p < 0.01$
† Dosed on one occasion (intraperitoneal injection, 50 mg/kg) 2 days after administration of SRBC.

Table A6.10.1-3: Summary of findings in the PFC assay

Group Level (ppm)	1 0	2 2400	3 5600	4 14000	5 Cyclophosphamide #
Males					
Cells/spleen ($x10^7$)	48.32	45.85	48.15	45.29	9.74***
PFC/10^6 viable cells	291.0	561.3	609.3	405.0	9.6***
PFC/spleen	147252	256969	290493	186292	672***
Females					
Cells/spleen ($x10^7$)	37.01	40.44	29.8	34.04	8.03***
PFC/10^6 viable cells	813.5	756.8	763.0	625.5	29.4***
PFC/spleen	334615	296590	224822	230603	2339***

50 mg/kg given by intraperitoneal injection on Day 27
*** statistically significantly different from Group 1 ($p<0.001$)

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	19 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	None
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.10-2 Immunotoxicity
Annex Point IIA6.10 Mouse
Oral

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 2011, Dinotefuran: 4-week dietary immunotoxicity study in the CD-1 mouse, [redacted], unpublished report no. [redacted] 0019, March 10, 2011.	
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 OPPTS 870.7800 (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	K09C3718	
3.1.2	Specification		
3.1.2.1	Description	White crystalline solid	
3.1.2.2	Purity	97.9%	
3.1.2.3	Stability	Expiry date: December 27, 2012	
3.2	**Reference Substance (positive control)**	Group 5 animals received 5 oral doses of 20 mg/kg/day cyclophosphamide by gavage as a 2 mg/mL solution at 10 mL/kg on days 22 - 26.	

Section A6.10-2 Immunotoxicity
Annex Point IIA6.10 Mouse
Oral

3.3	**Test Animals**	
3.3.1	Species	Mouse
3.3.2	Strain	Crl:CD1 (ICR)
3.3.3	Source	[redacted]
3.3.4	Sex	Males and females
3.3.5	Housing	2 of one sex/cage
3.3.6	Age/weight at study initiation	43 – 54 days old, weighing 30.8 – 41.2 g (males) and 22.5 – 31.5 g (female)
3.3.7	Number of animals per group	The animals were randomly assigned to single sex treatment groups on arrival:

Group number / treatment	Dose level (ppm)*	Number of animals	
		Male	Female
1 – Control	0 (vehicle)	10	10
2 – Dinotefuran	1120	10	10
3 – Dinotefuran	2800	10	10
4 – Dinotefuran	7000	10	10
5 – Cyclophosphamide	20 mg/kg†	8	8

* Expressed in terms of the active substance; a conversion factor of 1.021 was applied to allow for purity.
† Cyclophosphamide administered by gavage for 5 days on Days 22 - 26.

3.3.8	Control animals	Yes
3.4	**Administration**	Oral by admixture to the diet
3.4.1	Exposure	Administration daily for 28 days
3.4.2	Concentration	See 3.3.7 above. Overall achieved dose levels were 0, 153, 405 and 1053 mg/kg/day for males and 0, 223, 581 and 1438 mg/kg/day for females
3.4.3	Vehicle	No vehicle, added to basal diet
3.4.4	Concentration in vehicle	Not applicable
3.4.5	Total volume applied	Not applicable
3.4.6	Postexposure period	None.
3.4.7	Anticholinergic substances used	None
3.4.8	Controls	Vehicle
3.5	**Examinations**	
3.5.1	Body Weight	Body weights of Groups 1 – 4 were recorded twice during the week before treatment (Day -7 and -4), on the first day of test (Day 1), twice weekly during the treatment period and at necropsy. Group 5 animals were weighed on the first day of cyclophosphamide treatment only (Day 22).

Section A6.10-2		**Immunotoxicity**
Annex Point IIA6.10		**Mouse**
		Oral
3.5.2	Signs of Toxicity	The spleen from each animal in all groups was used as the source of splenocytes for conducting a plaque forming cell (PFC) assay using a modification of the Jerne plaque-forming cell (PFC) assay[1,2] which had been fully validated at the conducting laboratory in addition to the concurrent positive controls.
3.5.3	Observation schedule	Animals were examined twice daily for morbidity or mortality, and a detailed clinical examination was performed weekly. Food consumption was recorded weekly and water consumption was recorded for a 3-day period each week throughout the treatment period for groups 1 - 4.
3.5.4	Clinical Chemistry	No
3.5.5	Pathology	Yes Organs: Spleen and thymus were weighed and were also adjusted for terminal body weight. Spleen tissues required for immunotoxicology investigations were retained from Groups 1 – 5 for assessment of the acquired or adaptive immune response using a modification of the Jerne plaque-forming cell assay which had been validated by the conducting laboratory in addition to the concurrent positive control.
3.5.6	Histopathology	No
3.6	**Further remarks**	All animals received a single, intravenous dose (bolus injection) of 4 x 10^8 sheep washed red blood cells (SRBCs) in physiological saline in a dose volume of 0.2 mL/animal on Day 25 of the study. Statistics: All statistical analyses were conducted separately for males and females using the individual animal as the basic experimental unit. Body weight, organ weight and plaque forming cell data were analysed as follows: If Bartlett's test for variance homogeneity was not significant at the 1% level, then parametric analysis was applied. The F_1 approximate test was applied. If the F_1 approximate test for monotonicity of dose-response was not significant at the 1% level, Williams' test for a monotonic trend was applied. If the F_1 approximate test was significant, suggesting that the dose-response was not monotone, Dunnett's test was performed instead. If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. The H_1 approximate test, the non-parametric equivalent of the F_1 test described above, was applied. If the H_1 approximate test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend was applied. If the H_1 approximate test was significant, suggesting that the dose-response was not monotone, Steel's test was performed instead. For organ weight data, analysis of covariance was performed using terminal bodyweight as covariate. The treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.

Section A6.10-2 **Immunotoxicity**

Annex Point IIA6.10 **Mouse**

Oral

4 RESULTS AND DISCUSSION

4.1 Body Weight

There were no treatment-related effects on body weight gain in either sex at any dose level (Table A6.10.2-1). Although the overall mean body weight gain of males receiving 7000 ppm (2.3 g) was lower than the mean control gain (3.8 g) the difference was not statistically significant ($p > 0.05$). Some males from all treated and control groups lost weight following the administration of antigen on day 25, but the loss in males at 7000 ppm was greater than in other groups, and largely accounted for the observed difference.

4.2 Clinical signs of toxicity

There were no treatment-related clinical signs at any dose level or in the cyclophosphamide-treated group and no animals died prematurely.

The food and water consumption of all treated groups were unaffected by treatment with dinotefuran at all dose levels. The mean overall food consumption of the treated groups was within the range 96 – 109% of control values, and overall mean water consumption was within the range 86 – 114%.

The macroscopic examination performed after 4 weeks of treatment revealed no lesions attributable to treatment with dinotefuran. The nature and incidence of all the findings were consistent with the commonly seen background of macroscopic changes in CD-1 strain mice.

4.3 Immunotoxicology Investigations

There was no effect of treatment at any dose level on the humoral T-lymphocyte dependent antibody response to sheep red blood cells, as measured using the modified PFC assay. There were no statistically significant differences in the dinotefuran-treated groups ($p > 0.05$) at any dose level in the number of cells/spleen, PFC/10^6 viable cells and PFC/spleen, when compared to the vehicle control group (Table A6.10.2-3).

Treatment with 5 daily oral doses of 20 mg/kg/day of the immunosuppressant cyclophosphamide on days 22 - 26 resulted in a very marked and statistically significant reduction of the PFC response. The number of cells/spleen, PFC/10^6 viable cells and PFC/spleen were all statistically significantly reduced ($p < 0.05$, < 0.01 or < 0.001) for males and females when compared to the control, demonstrating the sensitivity of the PFC assay

4.4 Pathology

Absolute and bodyweight-adjusted spleen and thymus weights were not significantly different ($p>0.05$) from the controls values for all dinotefuran-treated groups of both sexes and were therefore considered not to have been affected by treatment (Table A6.10.2-2).

4.5 Histopathology

Not applicable

4.6 Other

None

Section A6.10-2 **Immunotoxicity**

Annex Point IIA6.10 **Mouse**

Oral

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

OPPTS 870.7800 (1998)

No relevant deviations from test guidelines.

Method:

Four groups of 10 male and 10 female CD-1 mice were treated orally, by diet admixture, with dinotefuran at concentrations of 0, 1120, 2800 and 7000 ppm for 4 weeks. The overall achieved dose levels were 0, 153, 405 and 1053 mg/kg/day for males and 0, 223, 581 and 1438 mg/kg/day for females. A group of 8 mice/sex, given 5 daily oral, gavage, doses of 20 mg/kg/day cyclophosphamide on days 22 - 26, acted as a positive control group. All animals received a sensitising intravenous dose of sheep red blood cells (SRBCs) 4-days prior to termination.

Body weights were recorded twice weekly, and food and water consumption were recorded weekly throughout the treatment period. All animals were subjected to detailed necropsy after 4-weeks treatment, and the weights of the spleen and thymus were recorded.

Splenic tissue from all test, control and positive control animals, was used as a source of splenocytes for assessment of the adaptive or acquired immune response to the T-cell-dependent immunogen, SRBCs, using a modification of the Jerne Plaque Forming Cell (PFC) assay. The number of lytic plaques for each animal was determined and group mean responses were calculated and expressed as group mean number of PFC/spleen and per 10^6 splenocytes.

5.2 Results and discussion

All animals survived the scheduled treatment period and there were no treatment-related clinical signs. Body weight gain, and food and water consumption, were unaffected by treatment at all dose levels of dinotefuran.

Treatment with the immunosuppressant cyclophosphamide on days 22 - 26 resulted in a very marked and statistically significant reduction of the PFC response. The number of cells/spleen, PFC/10^6 viable cells and PFC/spleen were significantly reduced for males and females when compared to the control.

The PFC assay demonstrated there was no immunotoxicologically relevant effect of dinotefuran on the humoral T-lymphocyte-dependent response against antigen on sheep red blood cells. There were no statistically significant differences in the dinotefuran-treated groups at any dose level in the number of cells/spleen, PFC/10^6 viable cells and PFC/spleen, when compared to the vehicle control group.

The no-observed-effect-level (NOEL) for functional immunotoxicity by dinotefuran was greater than 7000 ppm, equivalent to dose levels of 1053 mg/kg/day in males and 1438 mg/kg/day in females, based on no effect in the PFC assay at the highest dose level employed.

Dinotefuran was well tolerated at the highest dose level employed and the no-observed-adverse-effect level (NOAEL) for all effects was also 7000 ppm, equivalent to dose levels of 1053 and 1438 mg/g/day in males and females, respectively.

Section A6.10-2 Immunotoxicity
Annex Point IIA6.10 Mouse
Oral

5.3	**Conclusion**	
5.3.1	LOAEL (All effect)	Not determined
5.3.2	NOAEL (All effect)	The no-observed-adverse-effect level (NOAEL) for all effects was also 7000 ppm, equivalent to dose levels of 1053 and 1438 mg/g/day in males and females, respectively.
5.3.3	NOEL (Immunotoxicity)	The no-observed-effect-level (NOEL) for functional immunotoxicity by dinotefuran was greater than 7000 ppm, equivalent to dose levels of 1053 mg/kg/day in males and 1438 mg/kg/day in females, based on no effect in the PFC assay at the highest dose level employed.
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Table A6.10.2-1: Selected group mean body weight data

Treatment	Group mean body weight (g) on day:				Group mean change Day 1–29:	
(ppm)	1	8	22	29	(g)	(% of control)
Males						
Control	35.7	37.1	39.1	39.5	3.8	-
1120	36.0	37.5	40.6	39.3	3.3	87
2800	36.0	37.6	40.1	40.4	4.4	116
7000	33.8	34.8	36.4	36.1	2.3	62
CP – 20 mg/kg/day †	-	-	-	-	-	-
Females						
Control	27.3	28.5	30.4	31.0	3.7	-
1120	26.4	27.9	29.8	30.2	3.8	102
2800	27.4	28.3	31.2	31.3	3.9	105
7000	26.7	28.1	28.7	30.1	3.5	93
CP – 20 mg/kg/day †	-	-	-	-	-	-

** $p < 0.01$; CP cyclophosphamide
† Dosed by gavage on 5 consecutive days from day 22 - 26.

Table A6.10.2-2: Selected group mean organ weight data (unadjusted)

Treatment (ppm)	Mean body weight (g)	Group mean organ weight: Spleen (g)	Thymus (g)
Males			
Control	39.8	0.148	0.0416
1120	39.1	0.128	0.0365
2800	40.3	0.151	0.0364
7000	36.2*	0.144	0.0310
CP – 20 mg/kg/day †	-	-	-
Females			
Control	30.9	0.162	0.0540
1120	30.0	0.174	0.0496
2800	31.1	0.178	0.0511
7000	29.9	0.170	0.0519
CP – 20 mg/kg/day †	-	-	-

*** $p < 0.01$
† Dosed by gavage on 5 consecutive days from day 22 - 26.

Table A6.10.2-3: Summary findings in the PFC assay

Group Level (ppm)	1 0	2 1120	3 2800	4 7000	5 Cyclophosphamide #
Males					
Cells/spleen ($x10^7$)	9.29	6.40	7.74	7.65	4.84*
PFC/10^6 viable cells	1455.8	1729.0	1547.0	1519.3	16.6***
PFC/spleen	138221	114871	125781	114221	839***
Females					
Cells/spleen ($x10^7$)	7.01	8.35	8.20	7.72	2.91**
PFC/10^6 viable cells	1196.8	1588.0	1135.3	1602.8	195.6***
PFC/spleen	96605	140864	106564	129998	7091***

20 mg/kg/day given by intraperitoneal injection on Days 22 - 26
* statistically significantly different from Group 1 ($p<0.05$)
** statistically significantly different from Group 1 ($p<0.01$)
*** statistically significantly different from Group 1 ($p<0.001$)

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*20 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	*None*
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 6.11 **Annex Point IIIA, III-0§**	**Studies on other routes of administration**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The oral route is the primary route of administration of dinotefuran therefore additional studies for other routes e.g. intraperitoneal, intravenous subcutaneous and intramuscular routes are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section IIIA 6.12.1 Annex Point IIA, VI.6.12	**Medical surveillance data on manufacturing plant personnel if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Dinotefuran is a new substance in the EU. In Japan and the United States, no symptoms have been reported in connection with the handling of dinotefuran in the synthesis, formulation laboratory or during process development.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section IIIA 6.12.2 Annex Point IIA, VI.6.12	**Direct observation, e.g. clinical cases, poisoning incidents if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Dinotefuran is a new substance in the EU. There are no known clinical cases and poisoning incidents.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section IIIA 6.12.3 Annex Point IIA, VI.6.12	**Health records, both from industry and any other available sources**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Dinotefuran is a new substance in the EU. The registration for use of dinotefuran in Japan and in the United States is recent (April 2002 and September 2004, respectively) and it has not been used commercially in Europe. There have been no epidemiological studies conducted with this compound. There have been no reports of adverse health effects associated with the manufacture or authorised uses of dinotefuran in Japan and in the United States.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*Note that it is not possible to evaluate the statement that there have been no reports of adverse health effects associated with the manufacture or authorised uses of dinotefuran in Japan and in the United States.*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section IIIA 6.12.4 Annex Point IIA, VI.6.12	**Epidemiological studies on the general population, if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Dinotefuran is a new substance in the EU. The registration for use of dinotefuran in Japan and in the United States is recent (April 2002 and September 2004, respectively) and it has not been used commercially in Europe. There have been no epidemiological studies conducted with this compound. There have been no reports of adverse health effects associated with the manufacture or authorised uses of dinotefuran in Japan and in the United States.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*Note that it is not possible to evaluate the statement that there have been no reports of adverse health effects associated with the manufacture or authorised uses of dinotefuran in Japan and in the United States.*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section IIIA 6.12.5 Annex Point IIA, VI.6.12	**Diagnosis of poisoning including specific signs of poisoning and clinical tests, if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Dinotefuran is a new substance in the EU. Given the low acute oral, dermal and respiratory toxicity, it would not be expected that accidental over-exposure would lead to serious illness. Therefore, no specific clinical tests are applicable.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable. The RMS agrees that given the low acute oral, dermal and respiratory toxicity,it is reasonable to assume that accidental over-exposure would not lead to serious illness.*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section IIIA 6.12.6 Annex Point IIA, VI.6.12	**Sensitisation/allergenicity observations, if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Dinotefuran is a new substance in the EU. There are no specific antidotes or therapeutic regimes. No specific human effects of dinotefuran are known. Therefore, no specific intervention is indicated except to prevent further exposure. Therapeutic efforts should be directed toward alleviation of any symptoms of illness.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section IIIA 6.12.7 Annex Point IIA, VI.6.12	**Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Dinotefuran is a new substance in the EU. There is no specific antidote. Treat symptomatically. a) Inhalation. Move to fresh air. Provide oxygen or artificial respiration if needed. Consult a physician after significant exposure. b) Skin contact. Wash off immediately with soap and plenty of water. c) Eye contact: Rinse eye immediately with plenty of water. Also rinse under the eyelids. Keep eye wide open while rinsing. If eye irritation persists, consult a specialist. d) Ingestion. Call a physician immediately. Drink 1 or 2 glasses of water. Do not induce vomiting without medical advice. Never give anything by mouth to an unconscious person. Note to physician: Efforts should be directed toward alleviation of any symptoms of illness and to prevent further absorption of dinotefuran.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section IIIA 6.12.8 Annex Point IIA, VI.6.12	**Prognosis following poisoning**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Dinotefuran is a new substance in the EU. There are no specific known human effects of dinotefuran. Given the low acute oral, dermal, and respiratory toxicity of dinotefuran, it would not be expected that accidental over-exposure by oral, dermal and inhalation routes would lead to serious illness. Effects of human exposure to dinotefuran should be transitory and resolved 24 hours after exposure. No specific human symptoms of dinotefuran toxicity are known. Effects of human exposure to dinotefuran should be transitory and resolved 24 hours after exposure. The time between over-exposure and commencement of treatment should be as short as possible but is not expected to be crucial for the final health status.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*The Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.13 **Annex Point IIIAVI.2** **IUCLID**	**Toxic effects on livestock and pets**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Dinotefuran is not intended to be used in spaces in which animals are housed, kept or transported nor where exposure is possible via drinking water or feedstuffs. Therefore further studies are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.14 **Annex Point III-XI.2** **IUCLID**	**Other test(s) related to the exposure of humans**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Human exposure to degradation products, by-products and reaction products generated from dinotefuran, other than mammalian metabolites is not considered to be significant through normal use of the biocidal product. Therefore other tests are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.15.1 Annex Point IIIA XI.1.1, 1.3, 1.6	**Identification of the residues (identity and concentrations), degradation and reaction products and of metabolites of the active substance in contaminated foods or feedingstuffs**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Dinotefuran is not intended to be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feedstuff for livestock is prepared, consumed or stored. Therefore, further studies on the identification of the residues, degradation and reaction products and of metabolites of the active substance in foods or feedstuffs are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		