| Evaluation by Competent Authorities | |
|---|---|
| | |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 8 February 2013 |
| **Materials and Methods** | As described by Applicant. |
| **Results and discussion** | As described by Applicant except:<br>X1 5.2 The RMS considers that the reduced motor activity observed at 1500 mg/kg is an adverse effect of treatment. However, this effect is considered to be a manifestation of general toxicity, and because no neuropathological changes were detected dinotefuran is not regarded as being acutely neurotoxic. |
| **Conclusion** | X2 5.3 The RMS concludes that the study NOAEL is 750 mg/kg, based on the observation of transient reduced motor activity at 1500 mg/kg. |
| **Reliability** | As described by Applicant. |
| **Acceptability** | Acceptable |
| **Remarks** | None |
| | **COMMENTS FROM ...** |
| **Date** | |
| **Materials and Methods** | |
| **Results and discussion** | |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **Remarks** | |

**Section A6.9-2** **Subchronic Neurotoxicity**
**Annex Point IIA6.9** **Rat**
**Oral, 13-week**

Official use only

| | | |
|---|---|---|
| | | **1 REFERENCE** |
| **1.1** | **Reference** | [redacted], 2001b, 13-week dietary neurotoxicity study with MTI-446 in rats, [redacted], unpublished report no. [redacted] 6648-148, September 18, 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<br>No applicable EU guideline<br>OECD guideline no. 424 (1997)<br>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)** | Not applicable |
| **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 | One/cage |
| 3.3.6 | Age/weight at study initiation | About 7 weeks old, weighing 207 -297 g for males and 146-205 g for females |
| 3.3.7 | Number of animals per group | 10/sex/group |

| Section A6.9-2<br>Annex Point IIA6.9 | | Subchronic Neurotoxicity<br>Rat<br>Oral, 13-week |
|---|---|---|
| 3.3.8 | Control animals | Yes |
| **3.4** | **Administration** | Oral by diet admixture |
| 3.4.1 | Exposure | Constant nominal concentrations in diet |
| 3.4.2 | Dose Levels | 0, 500, 5000 and 50000 ppm<br>Equivalent to overall mean achieved dose levels of:<br>33, 327 and 3413 mg/kg bw/day for males<br>40, 400 and 3806 mg/kg bw/day for females |
| 3.4.3 | Vehicle | Mixed with: Certified rodent diet #8728CM, Harlan Tekland, ad libitum |
| 3.4.4 | Concentration in vehicle | Not applicable |
| 3.4.5 | Total volume applied | Not applicable |
| 3.4.6 | Postexposure period | 13-weeks |
| 3.4.7 | Anticholinergic substances used | None |
| 3.4.8 | Controls | Plain diet |
| **3.5** | **Examinations** | |
| 3.5.1 | Body Weight | Body weights were recorded pre-dose, on day 1 and weekly thereafter. Body weights were also recorded on the days of FOB testing. |
| 3.5.2 | Signs of Toxicity | All animals were subjected to a battery of behavioral tests and observations (FOB) pre-dose and during weeks 2, 4, 8 and 13. 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. |

**Section A6.9-2** **Subchronic Neurotoxicity**
**Annex Point IIA6.9** **Rat**
**Oral, 13-week**

| | | | |
|---|---|---|---|
| 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** | The overall body weight gain of both sexes treated at 50000ppm was significantly ($p < 0.05$) reduced. The group mean body weights at 50000ppm were significantly lower than the controls from week 2 of the study (Table A6.9.2-1) and at termination were 20.8 and 18.9% lower than control values in males and females, respectively. The effect on body weight at 50000ppm was accompanied by reduced food consumption that was frequently significantly ($p < 0.05$) lower than control values (Table A6.9.2-1). The body weight gain and food consumption of both sexes at lower dose levels were unaffected by treatment with dinotefuran. |
| **4.2** | **Clinical signs of toxicity** | There were no deaths during the study and no treatment-related clinical signs at any dose level, |
| **4.3** | **Clinical Chemistry** | Not applicable |
| **4.4** | **Pathology** | There were no treatment-related gross lesions at necropsy at any dose level and no treatment-related microscopic lesions in the animals treated at 50000ppm in central and peripheral nervous tissues, skeletal muscle and other tissues examined |
| **4.5** | **Histopathology** | Histopathological alterations were infrequent, generally occurred in isolation and the distribution between the control and dinotefuran-treated group did not indicate an effect of treatment. |

**Section A6.9-2** **Subchronic Neurotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral, 13-week**

| | | | |
|---|---|---|---|
| **4.6** | **Other** | There were no treatment-related effects in either sex at any dose level at any of the assessment intervals on the 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. Similarly, there were no treatment-related effects in either sex at any dose level at any of the assessment intervals on the quantitative parameters, grip strength, speed of nociceptive reflex, foot-splay, body temperature and motor activity. Although minor, statistically significant ($p < 0.05$) differences between the groups were apparent for rearing activity (50000ppm females in week 2), number of urine pools (5000 and 50000ppm males in week 4), hindlimb grip strength (500ppm females in week 2), foot-splay (50000ppm females in week 8) and body temperature (50000ppm females in week 2), none was considered to be treatment-related because they were not dose-related, they was no consistency between testing intervals or they were small numerical differences. Motor activity in females at 50000ppm was significantly ($p < 0.05$) lower than the controls in week 2 during each 10-minute interval and during the entire 40-minute test period (Table A6.9.2-2). However, a relationship to treatment with dinotefuran is equivocal since a statistically significant effect was not apparent at other testing intervals. Furthermore, the transiently lower motor activity in week 2 is considered not to be an adverse effect and not to be indicative of neurotoxicity because it did not occur at subsequent testing intervals and a similar effect on locomotor activity in an open field did not occur at any dose level. All other quantitative assessments in dinotefuran-treated groups were comparable to, and not significantly ($p > 0.05$) different from, control values. | X1 |

**Section A6.9-2** **Subchronic Neurotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral, 13-week**

## 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 orally, by diet admixture, with dinotefuran at constant nominal concentrations of 0, 500, 5000 and 50000 ppm for 13 weeks, equivalent to overall mean achieved dose levels of 33, 327 and 3413 mg/kg bw/day for males and 40, 400 and 3806 mg/kg bw/day for females.

The animals were observed twice daily for morbidity/mortality and daily for clinical signs. Food consumption and body weights were recorded weekly, and a functional observation battery (FOB) of tests including a quantitative assessment of motor activity, was performed on all animals pre-dose, and during weeks 2, 4, 8 and 13. During week 14, the animals were deprived of food overnight and subjected to necropsy and *post mortem* examination of major organs and tissues. The tissues of all animals were perfusion-fixed *in situ* and 6 animals/sex from the groups treated at 0 or 50000ppm that were suitably perfusion-fixed were designated for neuropathological evaluation. The olfactory bulb, forebrain, caudate nucleus, hypothalamus/thalamus, midbrain, cerebellum, medulla, pituitary, 3 levels of spinal cord, eye, skeletal muscles and gross lesions from the 6 animal/sex in the groups treated at 0 or 50000ppm were processed to paraffin blocks and sections were stained with hematoxylin & eosin. Dorsal root and trigeminal ganglia, and all peripheral nerves from the same animals were embedded in epoxy resin, sectioned and stained with toluidine blue. The stained sections were examined microscopically.

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|---|---|---|---|
| **Section A6.9-2** | | **Subchronic Neurotoxicity** | |
| **Annex Point IIA6.9** | | **Rat** | |
| | | **Oral, 13-week** | |
| **5.2** | **Results and discussion** | There were no deaths during the study and no treatment-related clinical signs at any dose level, but the overall body weight gain of both sexes treated at 50000ppm was significantly reduced, resulting in reduced body weights at termination. The effect on body weight at 50000ppm was accompanied by reduced food consumption. The body weight gain and food consumption of both sexes at lower dose levels were unaffected by treatment with dinotefuran.<br><br>There were no treatment-related effects in either sex at any dose level at any assessment interval on FOB evaluations. Although minor differences between the groups were apparent for rearing activity (50000ppm females in week 2), number of urine pools (5000 and 50000ppm males in week 4), hindlimb grip strength (500ppm females in week 2), foot-splay (50000ppm females in week 8) and body temperature (50000ppm females in week 2), none was considered to be treatment-related because they were not dose-related, there was no consistency between testing intervals or they were small numerical differences. Motor activity in females at 50000ppm was lower than the controls in week 2 during each 10-minute interval and during the entire 40-minute test period. However, a relationship to treatment with dinotefuran is equivocal since a statistically significant effect was not apparent at other testing intervals. Furthermore, the transiently lower motor activity in week 2 is considered not to be an adverse effect and not to be indicative of neurotoxicity because it did not occur at subsequent testing intervals and a similar effect on locomotor activity in an open field did not occur at any dose level. There were no treatment-related gross lesions, microscopic lesions and neurohistological alterations. | X2 |
| **5.3** | **Conclusion** | | |
| 5.3.1 | NOEL | A no-observed-effect-level (NOEL) for neurotoxicity was established in both sexes as > 50000ppm, equivalent to dose levels of 3413 and 3806mg/kg bw/day in males and females, respectively, based on the absence of neurobehavioral and neuropathological effects at 50000ppm, the highest dose level employed. | |
| 5.3.2 | NOAEL | A no-observed-adverse-effect-level (NOAEL) for all effects was established in both sexes as 5000ppm, equivalent to dose levels of 327 and 400mg/kg bw/day in males and females, respectively, based on the occurrence of reduced body weight gain and food consumption at 50000ppm. | |
| 5.3.3 | Reliability | 1 | |
| 5.3.4 | Deficiencies | No | |

**Table A6.9.2-1: Group mean body weight and food consumption data at representative intervals**

| Week of study | Group mean body weight (g) in: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Males treated at (ppm): | | | | Females treated at (ppm): | | | |
| | 0 | 500 | 5000 | 50000 | 0 | 500 | 5000 | 50000 |
| 1 | 256 | 251 | 257 | 249 | 174 | 183 | 173 | 180 |
| 2 | 295 | 292 | 298 | 245* | 197 | 199 | 189 | 165* |
| 4 | 370 | 358 | 363 | 300* | 226 | 227 | 218 | 193* |
| 8 | 461 | 454 | 450 | 365* | 257 | 262 | 251 | 217* |
| 14 | 514 | 511 | 508 | 407* | 286 | 291 | 277 | 232* |
| Gain (weeks 1 - 14) | 258 | 260 | 251 | 159* | 112 | 107 | 103 | 52* |
| | Group mean food consumption (g/week) | | | | | | | |
| 1 | 174 | 177 | 182 | 132* | 138 | 141 | 124 | 87* |
| 2 | 182 | 176 | 181 | 156* | 131 | 134 | 125 | 114* |
| 4 | 191 | 184 | 185 | 174 | 132 | 138 | 130 | 107* |
| 8 | 182 | 187 | 183 | 173 | 128 | 137 | 128 | 107* |
| 13 | 175 | 186 | 183 | 156 | 126 | 134 | 137 | 99* |

* $p < 0.05$

**Table A6.9.2-2: Summary of quantitative motor activity data**

| Test interval | Group mean motor activity (counts/40min) in: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Males treated at (ppm): | | | | Females treated at (ppm): | | | |
| | 0 | 500 | 5000 | 50000 | 0 | 500 | 5000 | 50000 |
| Predose | 1115 | 1219 | 1098 | 1231 | 857 | 1177 | 1194 | 923 |
| 2 | 1179 | 1437 | 1643 | 1050 | 1280 | 1346 | 1742 | 705* |
| 4 | 1321 | 1561 | 1686 | 1394 | 1462 | 1358 | 1577 | 1049 |
| 8 | 1532 | 1462 | 1902 | 1630 | 1386 | 1502 | 1604 | 1165 |
| 13 | 1298 | 1436 | 1559 | 1789 | 1585 | 1678 | 1648 | 1234 |

* $p < 0.05$

| Evaluation by Competent Authorities | |
|---|---|
| | |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 8 February 2013 |
| **Materials and Methods** | As described by Applicant. |
| **Results and discussion** | As described by Applicant except:<br>X1 4.6 and X2 5.2 The RMS considers that the reduced motor activity observed in females at 50000 ppm at the week 2 assessment should conservatively be regarded as an adverse effect of treatment. However, this effect is considered to be a manifestation of general toxicity, and because no neuropathological changes were detected dinotefuran is not regarded as being acutely neurotoxic. |
| **Conclusion** | X3 5.3.2 The RMS agrees that the study NOAEL is 5000 ppm, but the basis of this conclusion is the observation of transient reduced motor activity at 50000 ppm as well as the reduced bodyweight gain and food consumption at this dose level. |
| **Reliability** | As described by Applicant. |
| **Acceptability** | Acceptable |
| **Remarks** | None |
| | **COMMENTS FROM ...** |
| **Date** | |
| **Materials and Methods** | |
| **Results and discussion** | |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **Remarks** | |

**Section A6.9-3** **Postnatal Developmental Neurotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral**

Official use only

| | | |
|---|---|---|
| | **1 REFERENCE** | |
| **1.1** | **Reference** | [redacted], 2006a, Transfer of [$^{14}$C]MTI-446 into milk of lactating rats after oral administration, [redacted], unpublished report no. A29136, August 9, 2006. |
| **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 guideline |
| **2.2** | **GLP** | Yes |
| **2.3** | **Deviations** | Yes<br>No applicable guideline |
| | **3 MATERIALS AND METHODS** | |
| **3.1** | **Test material** | As given in section 2 |
| 3.1.1 | Lot/Batch number | CP-2499 ([G-$^{14}$C]), 5500310 |
| 3.1.2 | Specification | |
| 3.1.2.1 | Description | [$^{14}$C-guanidine] dinotefuran ([G-$^{14}$C]), non-radiolabelled dinotefuran |
| 3.1.2.2 | Purity | [G-$^{14}$C]: radiochemical purity after purification; 96.0 %, specific activity 156 µCi/mg (5760 kBq/mg), Non-radiolabelled purity; 97.26% |
| 3.1.2.3 | Stability | [G-$^{14}$C]: radiochemical purity was checked at the time of administration, Non-radiolabelled expiration date; June 30, 2006 |
| **3.2** | **Reference Substance (positive control)** | None |
| **3.3** | **Test Animals** | |
| 3.3.1 | Species | Rat |
| 3.3.2 | Strain | Sprague Dawley, SPF-quality (outbred) |
| 3.3.3 | Source | [redacted] |
| 3.3.4 | Sex | Females |
| 3.3.5 | Rearing conditions | One/cage |
| 3.3.6 | Age/weight at study initiation | 8-10 weeks old at mating (dams), weighing about 260 g after delivery (dams) |
| 3.3.7 | Number of animals per group | 6 females/group |

| Section A6.9-3<br>Annex Point IIA6.9 | | Postnatal Developmental Neurotoxicity<br>Rat<br>Oral | |
|---|---|---|---|
| 3.3.8 | Control animals | No | |
| **3.4** | **Administration** | Oral by gavage | |
| 3.4.1 | Exposure | On days 2, 4, 8 and 12 of lactation | |
| 3.4.2 | Dose Levels | Two target dose levels: 50 mg/kg (corresponding to a diet concentration of 500-700 ppm), and 500 mg/kg (corresponding to a diet concentration of 5000-7000 ppm)<br>See Table A6.9.3-1 | |
| 3.4.3 | Vehicle | Water (MilliQ) | |
| 3.4.4 | Concentration in vehicle | Not applicable | |
| 3.4.5 | Total volume applied | Low dose (50 mg/kg): 37 mL<br>High dose (500 mg/kg): 129 mL | X |
| 3.4.6 | Postexposure period | 16 days | X |
| 3.4.7 | Anticholinergic substances used | None | |
| 3.4.8 | Controls | Not applicable | |
| **3.5** | **Examinations** | | |
| 3.5.1 | Body Weight | Individual body weights were recorded at the start of acclimatization, just before the administration on days 2, 4, 8 and 12 of lactation, and prior to sacrifice. The total weight of the litters was determined on days 2, 4, 8 and 12 of lactation. | |
| 3.5.2 | Signs of Toxicity | Not applicable, the study was designed to provide information on the transfer of [$^{14}$C]-dinotefuran and/or its radiolabelled metabolites into the milk of lactating rats to provide evidence of exposure of suckling pups to dinotefuran via the milk. | |
| 3.5.3 | Observation schedule | Clinical signs or unusual behavior of animals was visually checked each working day. | |

| | | | | |
|---|---|---|---|---|
| **Section A6.9-3**<br>**Annex Point IIA6.9** | | **Postnatal Developmental Neurotoxicity**<br>**Rat**<br>**Oral** | | |
| 3.5.4 | Sampling | Yes | | |
| | | Number of animals: | All dams | |
| | | Time points: | Three hours prior to milking, the dams were separated from the pups and milk injection into the mammary glands was stimulated by an intraperitoneal injection of oxytocin (4 IU/kg) about 5 minutes prior to milking. The milk specimen was obtained by an in-house built vacuum driven milking pump at the specified time point from 3 animals each, and then dams were placed back with their pups. After milking, 0.5 mL blood sample was withdrawn from the sublingual vein from each animal at the selected time point. Aliquots of blood were used for determination of total radioactivity in whole blood, and the remaining blood was centrifuged and separated to plasma. Volumes or weights of all specimens were measured. All adult and pup carcasses were stored deep frozen without analysis. | X |
| | | Parameters: | Radioactivity (RA) was measured by Liquid Scintillation Counting (LSC) equipped for computing quench-corrected disintegrations per minute (dpm). Aliquots of liquid specimens, diluted dose solution (0.1 mL), milk (0.05 g), and plasma (0.05 g), were added directry to the scintillant for the measurement of radioactivity. Aliquots of blood (about 0.05 g) were mixed with 1 mL tissue solubilizer, and then 0.5 mL isopropanol and 0.25 mL of 30% $H_2O_2$ were added and warmed to about 40 °C for at least 30 minutes. After equilibration to room temperature the scintillant was added prior to LSC. | |
| **3.6** | **Further remarks** | None | | |

**Section A6.9-3** **Postnatal Developmental Neurotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral**

## 4 RESULTS AND DISCUSSION

| | | |
|---|---|---|
| **4.1** | **Body Weight** | The weight gains of the lactating females and their litters were considered to be within the normal range. |
| **4.2** | **Clinical signs of toxicity** | Not applicable |
| **4.3** | **Sampling Low dose group** | After oral administration, the test substance was rapidly absorbed from GI into the systemic circulation. The concentrations in blood, plasma and milk 0.5 hours after administration were comparable on all sampling occasions from day 2 to day 12 of lactation. The mean values for whole blood and plasma were within the range 30.1 – 35.2 ppm dinotefuran equivalents, but the concentrations of milk, which were within the range 55.2 -62.9 ppm, were approximately 2-fold higher than whole blood/plasma concentrations. Within 1.5 hours after administration, the concentrations in blood and plasma had declined to about half the levels determined after 0.5 hours, and were within the range 13.9 – 17.5 ppm dinotefuran equivalents. The concentrations in milk were within the range 26.4 – 36.9 ppm dinotefuran equivalents, and remained approximately 2-fold higher than blood and plasma concentrations at all sampling occasions. The achieved concentrations in blood and plasma were very similar at all sampling time points during the observed lactation period, whereas the concentrations in milk showed slightly increasing values. See Table A6.9.3-2. |
| **4.4** | **Sampling High dose group** | After oral administration, the absorption and depletion profiles resembled those observed for the low dose level. However, the measured concentrations in blood and plasma were only approximately 4 times higher for a 10-fold increase in dose level. Thus, 2 hours after administration the concentrations in blood and plasma were within the range 104 - 144 ppm dinotefuran equivalents, and the concentrations in milk were 160- 199 ppm dinotefuran equivalents, on all sampling occasions from day 2 to day 12 of lactation. The concentrations in blood and plasma were very similar, and milk concentrations were 40 – 68% higher than the average concentration in blood and plasma on all sampling occasions.<br><br>Four hours after administration, the concentrations in blood and plasma remained very similar but had declined to 70 – 96 ppm dinotefuran equivalents. Milk concentrations had declined to 114 – 196 ppm, and were 50 – 161% higher than blood/plasma concentrations on all sampling occasions from day 2 to day 12 of lactation. The levels of RA in blood and plasma were not influenced by the period of lactation, but concentrations in milk at 4 hours tended to increase as lactation progressed.<br><br>However, at this time on lactation day 12, the measured value showed a high inter-individual variation and the correlation to the blood concentration was significantly different to all other time points. Therefore it was considered likely that the high concentration in milk was partially caused by contamination with urine, since at this time point the posterior-most teats were used for milk sampling. See Table A6.9.3-3. |

**Section A6.9-3** **Postnatal Developmental Neurotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral**

## 5 APPLICANT'S SUMMARY AND CONCLUSION

**5.1 Materials and methods**

Guidelines:

No applicable guideline.

Method:

The present study was conducted in support of a proposed assessment of the developmental neurotoxicity and the developmental immunotoxicity of dinotefuran in the rat. Therefore, it was designed to provide information on the transfer of [$^{14}$C]-dinotefuran and/or its radiolabelled metabolites into the milk of lactating rats to provide evidence of exposure of suckling pups to dinotefuran via the milk.

Two groups of 6 lactating rats were treated with oral doses of [$^{14}$C]-dinotefuran at two target dose levels, 50 mg/kg (corresponding to a diet concentration of 500-700 ppm), and 500 mg/kg (corresponding to a diet concentration of 5000-7000 ppm). Lactating dams were dosed orally, by gavage, on days 2, 4, 8 and 12 of lactation. The concentration of dinotefuran in maternal milk, whole blood and plasma was determined in 3 rats/group at each of two time points after each oral dose, 0.5 and 1.5 hours (50 mg/kg) and 2 and 4 hours (500 mg/kg).

**5.2 Results and discussion**

After oral administration of both the low and high dose levels, the test substance was rapidly absorbed from GI into the systemic circulation. Thereafter, dinotefuran and/or metabolites rapidly partitioned into maternal milk. For each dose level, the concentrations in whole blood and plasma were very similar, whereas concentrations in milk were consistently higher at all sampling intervals.

The determined concentrations in blood, plasma and milk of the high dose group were approximately 4 times higher than those achieved for the low dose, but for a 10-fold increase in dose level.

Elimination from systemic circulation and from maternal milk was also very rapid for both low and high doses of dinotefuran, but milk concentrations remained consistently higher than those of whole blood and plasma on all lactation days evaluated.

The extent of systemic absorption into whole blood or plasma was not influenced by the day of lactation within the range examined (LD2-LD12), but there was a trend towards increasing milk concentration as lactation progressed. However, at this time on lactation day 12, the measured value showed a high inter-individual variation and the correlation to the blood concentration was significantly different to all other time points. Therefore it was considered likely that the high concentration in milk was partially caused by contamination with urine, since at this time point the posterior-most teats were used for milk sampling.

**5.3 Conclusion**

The study provides evidence of neonatal exposure of suckling pups to dinotefuran and/or metabolites via the maternal milk.

5.3.1 Reliability

1

5.3.2 Deficiencies

Yes

No applicable guideline

**Table A6.9.3-1: Treatment schedule**

| Dose (mg/kg) | Treatment volume (mL/kg) | Dose regimen | No. of animals/ dose regimen | Time points | |
|---|---|---|---|---|---|
| | | | | hrs post-dose | No. of animals |
| 50 | 4 | Single p.o. Day 2 of lactation<br>Single p.o. Day 4 of lactation<br>Single p.o. Day 8 of lactation<br>Single p.o. Day 12 of lactation | 6 lactating rats | 0.5<br>1.5 | 3 lactating rats<br>3 lactating rats |
| 500 | 16 | Single p.o. Day 2 of lactation<br>Single p.o. Day 4 of lactation<br>Single p.o. Day 8 of lactation<br>Single p.o. Day 12 of lactation | 6 lactating rats | 2<br>4 | 3 lactating rats<br>3 lactating rats |

**Table A6.9.3-2: Concentration ot total radioactivity in blood, plasma and milk – low dose group**

| The day of dosing | Concentration of total Radioactivity [ppm dinotefuran equivalents] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 2 after delivery | | Day 4 after delivery | | Day 8 after delivery | | Day 12 after delivery | |
| Time points | 0.5 | 1.5 | 0.5 | 1.5 | 0.5 | 1.5 | 0.5 | 1.5 |
| Dose (mg/kg) | 51.7 ± 1.3 | 51.3 ± 0.8 | 49.8 ± 1.2 | 50.7 ± 1.3 | 48.1 ± 3.3 | 48.5 ± 0.6 | 50.5 ± 1.3 | 50.8 ± 2.2 |
| Blood | 33.1 ± 0.6 | 16.6 ± 4.9 | 30.5 ± 3.4 | 15.3 ± 0.6 | 30.1 ± 3.5 | 13.9 ± 2.4 | 31.2 ± 4.0 | 14.8 ± 2.9 |
| Plasma | 35.2 ± 0.7 | 17.5 ± 5.2 | 32.5 ± 3.5 | 16.1 ± 0.5 | 32.2 ± 3.6 | 14.8 ± 2.7 | 33.7 ± 4.3 | 15.8 ± 3.1 |
| Milk | 60.0 ± 2.7 | 26.4 ± 5.4 | 55.2 ± 11.1 | 27.8 ± 1.4 | 58.7 ± 6.6 | 30.7 ± 3.9 | 62.9 ± 19.0 | 36.9 ± 6.8 |

**Table A6.9.3-3: Concentration of total radioactivity in blood, plasma and milk – high dose group**

| The day of dosing | Concentration of total Radioactivity [ppm dinotefuran equivalents] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 2 after delivery | | Day 4 after delivery | | Day 8 after delivery | | Day 12 after delivery | |
| Time points | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 |
| Dose (mg/kg) | 491 ± 13 | 495 ± 3 | 509 ± 10 | 492 ± 9 | 492 ± 10 | 492 ± 6 | 496 ± 7 | 492 ± 4 |
| Blood | 136 ± 27 | 90 ± 21 | 104 ± 7 | 74 ± 1 | 108 ± 9 | 70 ± 16 | 106 ± 5 | 72 ± 15 |
| Plasma | 144 ± 30 | 96 ± 21 | 109 ± 6 | 77 ± 1 | 114 ± 8 | 75 ± 17 | 112 ± 4 | 77 ± 16 |
| Milk | 199 ± 53 | 141 ± 31 | 160 ± 12 | 114 ± 5 | 187 ± 16 | 136 ± 21 | 178 ± 22 | 196 ± 59 |

| Evaluation by Competent Authorities | |
|---|---|
| | |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 28/09/12 |
| **Materials and Methods** | As described by Applicant except,<br>Section 3.4.5 – The volumes of 37 and 129ml refer to the total volumes of stock solutions. The volume of each dose was 9 ml and 32 ml for the low and high dose groups, respectively.<br>Section 3.4.6 – There was no post-exposure period.<br>*DAVID: COMMENT relating to Section 3.5.1 has been deleted*<br>For information<br>Sections 3.5.4 - The specified time points for sampling were 0.5 & 1.5h for the low dose group and 2 & 4h for the high dose group. |
| **Results and discussion** | As described by Applicant.<br>N.B. The UK CA does not believe that it is possible to determine whether or not there is an increase in the concentration of radiolabel in the milk of lactating rats over the course of this study. |
| **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 A6.9-4** **Developmental Neurotoxicity and Immunotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral, dosage-range finding**

Official use only

| | | |
|---|---|---|
| | | **1 REFERENCE** |
| **1.1** | **Reference** | [redacted], 2009, Oral (Diet) Dosage-range finding developmental neurotoxicity and immunotoxicity study of MTI-446 (Dinotefuran) in Crl:CD (SD) rats, [redacted], unpublished report no. [redacted] 00001, January 16, 2009.<br>[redacted], 2009, Method validation to support analysis of MTI-446 in certified rodent diet dose formulations for Mitsui Chemicals, Inc., [redacted], unpublished report no. [redacted] 00003AV-08-6, January 30, 2009. |
| **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<br>No applicable guideline<br>The design of the study was discussed with, and agreed by, EPA |
| **2.2** | **GLP** | Yes |
| **2.3** | **Deviations** | No applicable guideline. |
| | | **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% (re-analysis by Charles River Laboratories; 99.5%) |
| 3.1.2.3 | Stability | Expiration date:31 December 2012 |
| **3.2** | **Reference Substance (positive control)** | None |
| **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 | Female |
| 3.3.5 | Rearing conditions | P generation: pre-mating: one/cage, mating: 1M + 1F, post-mating: 1F + litter/cage. F1 generation: after DL21: two or three/cage |
| 3.3.6 | Age/weight at | About 10 weeks old, weighing 219-242 g |

**Section A6.9-4** **Developmental Neurotoxicity and Immunotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral, dosage-range finding**

| | | |
|---|---|---|
| | study initiation | |
| 3.3.7 | Number of animals per group | 10 mated females/group |
| 3.3.8 | Control animals | Yes |
| **3.4** | **Administration** | Oral by diet admixture |
| 3.4.1 | Exposure | P generation females: continuous from Day 6 of gestation to Day 21 of lactation until sacrifice.<br>F1 generation rats were treated continuously after weaning until sacrifice.<br>Direct dosing of F1 generation pups during the lactation period (preweaning) was not performed since the transfer of dinotefuran into the milk of lactating females has been confirmed (Transfer of [$^{14}$C]-dinotefuran into milk of lactating rats after oral administration, A29136) |
| 3.4.2 | Dose Levels | The overall mean dose levels in the P females during gestation period, during lactation period, F1 generation males and F1 females were:<br>• 1000 ppm: 69.5, 141.2, 100.3 and 112.3 mg/kg/day<br>• 3000 ppm: 211.8, 423.9, 310.6 and 316.3 mg/kg/day<br>• 10000 ppm: 669.9, 1400.8, 1043.3 and 1119.7 mg/kg/day (respectively). |
| 3.4.3 | Vehicle | Basal diet: Certified rodent diet® (meal form) #5002 |
| 3.4.4 | Concentration in vehicle | Not applicable |
| 3.4.5 | Total volume applied | Not applicable |
| 3.4.6 | Postexposure period | 14 days or other |
| 3.4.7 | Anticholinergic substances used | None |
| 3.4.8 | Controls | Plain diet |
| **3.5** | **Examinations** | |
| 3.5.1 | P generation animals | The animals were observed twice daily for viability and for clinical signs and general appearance weekly at approximately the same time each week during the pre-exposure period and on DG 0. The rats were also examined for clinical observations, abortions, premature deliveries and deaths on DGs 6, 9, 12, 15, 18, 20 and 25 (rats that did not deliver a litter) and DLs 0, 4, 7, 13 and 21.Body weights were recorded weekly during pre-exposure period and on DGs 0, 6, 9, 12, 15, 18, 20 and 25 (if necessary) and DLs 0, 4, 7, 13 and 21. Food consumption were recorded on DGs 0, 6, 9, 12, 15, 18, 20 and 25 (if necessary) and DLs 0, 4, 7 and 13. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes, live litter size and pup viability at birth. Maternal behaviour was evaluated on DLs 0, 4, 7, 13 and 21, and variation from expected maternal behaviour were recorded on all other days of the postpartum period. |
| 3.5.2 | F1 generation | All pups in a litter were individually weighed on the day of birth, |

**Section A6.9-4** **Developmental Neurotoxicity and Immunotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral, dosage-range finding**

| | | |
|---|---|---|
| | pups | PNDs 4, 7, 11, 13, 17 and 21. Each litter was evaluated for viability at least twice daily. The number of pups in each litter and clinical observations were recorded once daily during the preweaning period. |
| 3.5.3 | F1 generation rats – Assays 1 and 2 | The immunologic evaluation test consisted of two phases; an Antibody-Forming Cell (AFC) phase and a non-AFC phase. The AFC phase consisted of the spleen IgM antibody response to the T-dependent Antigen, sRBC (primary response) and the rats assigned to Assay 1 were used. The non-AFC phase consisted of the following immunotoxicological assays; splenocyte phenotyping and Natural Killer (NK) Cell Assay and the rats assigned to Assay 2 were used.<br><br>Rats were observed for viability at least twice daily and clinical observations and general appearance were examined once weekly during the postweaning period. Body weights were recorded weekly during the postweaning period and prior to sacrifice. Food consumption were measured weekly during the postweaning period.<br>At the time of necropsy, spleen were harvested aseptically. Single-cell suspensions were prepared from each spleen by mashing the spleen, and centrifuged, and then resuspended in Earle's Balanced salt solution with HEPES for AFC test and in RPMI 1640 media supplemented with 10% FBS for non-AFC test. Viability of splenocytes was determined using propidium iodide (PI) and flow cytometer.<br>In assay 1 (AFC test), the primary IgM response to sheep red blood cells was measured using a modified haemolytic plaque assay of Jerne[1]. Spleen cell number, following lysis of RBC, was determined. The cells/spleen, AFC/$10^6$ spleen cells (specific activity), AFC/spleen (total spleen activity) and plaques were determined.<br><br>In assay 2 (non-AFC test), the spleen cell phenotypes were evaluated using the following antibodies; CD45RA for total B cells, CD5 for total T cells, CD4 for T helper cells, CD8 for cytotoxic T cells. NK cells were enumerated as cells that were NKP-P1A positive and CD8a negative. The samples were processed by flow cytometry for evaluation and enumeration. In the Natural Killer (NK) Cell assay, the NK cell was used as the effector cell and mouse lymphoma YAC-1 cells, introduced by inoculation of the Moloney Leukemia virus into a newborn A/Sn mouse, was used as the target cell. The mean ± SE percent cytotoxicity at each effector concentration was determined. |

[1] Jerne NK, Henry C, Nordin AA, Fun H, Koros MC, and Lefkovits I (1974): Plaque-forming cells; Methodology and theory, Trnspl. Rev., 18:130-191.

**Section A6.9-4** **Developmental Neurotoxicity and Immunotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral, dosage-range finding**

| | | | | |
|---|---|---|---|---|
| 3.5.4 | Necropsy | Yes | | |
| | | P generation female rats | Female rats were sacrificed by carbon dioxide asphyxiation and a gross necropsy of thoracic, abdominal and pelvic viscera was performed after completion of the 21-day postpartum period. The number and distribution of implantation sites was recorded. Female rats that did not deliver a litter were sacrificed on DG25 and examined for gross lesions. Uteri were examined to confirm the absence of implantation sites. | |
| | | F1 generation pups | Pups that died before initial examination of the litter for pup viability were evaluated for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sank were considered stillborn; pups with lungs that floated were considered liveborn and to have died shortly after birth. Pups found dead were examined for gross lesions and for the cause of death. On PND 4, all offspring selected for standardization were sacrificed by an intraperitoneal injection of sodium pentobarbital and necropsied. On PND 21, all F1 generation pups not selected for Assay 1 and 2 were sacrificed by carbon dioxide asphyxiation and examined for gross lesions. | |
| | | F1 generation rats – Assay 1 | Male and female rats were sacrificed by carbon dioxide asphyxiation 4 days following sensitization with the injection of sRBCs and completion of the exposure period (PWDs 36 to 40 for male rats and PWDs 43 to 47 for female rats). Rats were examined for gross lesions and spleens were harvested aseptically. Each excised spleen was placed in tube containing Earle's Balanced salt solution in HEPES medium and a "wet" weight was collected. | X1 |
| | | F1 generation rats – Assay 2 | Male and female rats were sacrificed by carbon dioxide asphyxiation following completion of the exposure period (PWDs 36 to 42 for male rats and PWDs 45 to 49 for female rats). Rats were examined for gross lesions and spleens were harvested aseptically. Each excised spleen was placed in tube containing Earle's Balanced salt solution in HEPES medium and a "wet" weight was collected. The female rat that died was examined for gross lesions and for the cause of death. | X2 |
| **3.6** | **Statistics** | Clinical observations and other proportion data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Continuous data were analysed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance, when appropriate. If the Analysis of Variance was significant, Dunnett's Test was used. If the Analysis of Variance was not appropriate, the Kruskal-Wallis Test was used, when less than or equal to 75% ties were present. In cases where the Kruskal-Wallis Test was significant, Dunn's Method of Multiple Comparisons was used. If there were greater than 75% ties, Fisher's Exact Test was used to analyze the data. Count data were evaluated using the procedures described above for the Kruskal-Wallis Test.<br>The data obtained the immunologic evaluation were first tested for | | |

homogeneity of variances using the Bartlett's Chi Square Test. Homogeneous data were evaluated by a parametric one-way analysis of variance. If significant differences occur, Dunnett's test was used. Non-homogeneous data were evaluated using a non-parametric analysis of variance. If significant differences occur, Gehan-Wilcoxon test was used when appropriate. The Jonckheere's Test was used to test for treatment level-related trends across the control and treatment groups.

## 4 RESULTS AND DISCUSSION

**4.1 P0 generation female rats**

All rats survived until scheduled sacrifice. There were no treatment-related clinical signs or gross lesions at necropsy. Body weights and body weights gains during the gestation and lactation periods were unaffected by exposure to the test substance at all dose levels. In absolute and relative feed consumption, all values were comparable among the 4 dosage groups and did not significantly differ with the exception of absolute and relative feed-consumption values for DGs 6 to 9, that were significantly reduced ($p \leq 0.01$) in the 10000 ppm group (about 10%). This reduction did not present and was not considered an adverse toxicological effect of the test substance.

Pregnancy occurred in 9, 9, 10 and 10 of the ten mated female rats in the 0, 1000, 3000 and 10000 ppm groups, respectively. All pregnant dams delivered litters. There were no effects of treatment on the numbers of dams delivering litters, the duration of gestation, numbers of implantation sites/litter, the gestation index, the numbers of dams with stillborn pups and of dams with all pups dying, litter sizes, viability and lactation indices, surviving pups per litter, sex ratio and live litter size at weighing.

**4.2 F1 generation pups**

Pup weights in the 10000 ppm group were significantly reduced ($p \leq 0.01$) on postpartum days 13, 17 and 21 (Table A6.9.4-1). These reductions were probably due to direct exposure of the pups to the test substance in the diet as well as exposure through the milk as pups start to eat feed approximately day 13 postpartum. No death and no treatment-related clinical signs and gross lesions at necropsy were observed at any dose level.

**4.3 F1 generation rats – immunological assays 1 and 2**

There were no treatment-related clinical signs, deaths or gross lesions at any dose level. However, one female rat in the 1000 ppm exposure group was found dead on day 25 postpartum, but death was considered not to be treatment-related. Body weights of male and female rats were significantly lower than control ($p \leq 0.05$ to $p \leq 0.01$) in the 1000 ppm and higher exposure groups on postnatal day 22. However, significantly lower body weights continued only in the 10000 ppm exposure group male rats through day 57 postpartum and in the female rats for days 29, 36, 57 and 64 postpartum (Table A6.9.4-2). Significantly lower body weights in male rats occurred in the 1000 ppm exposure group on postnatal day 29, but this reduction was not dosage dependent and not considered related to the test substance. Body weight gains of male and female rats were unaffected by exposures to the test substance at all dose levels. No significant differences occurred among the groups with the exception of days 57 to 64 postnatally in the 3000 and 10000 ppm female exposure groups, where a non-dosage dependent but significant reduction ($p \leq 0.01$) occurred. The general lack of significant reductions in body weight gains indicates that the lower body weights that occurred on postnatal

day 22 were due to the high dosage of the test substance consumed at or near weaning (Table A6_09_04-2). Absolute and relative feed consumption during the postweaning period were unaffected by exposure to the test substance as high as 10000 ppm.

For assay 1 (AFC phase), exposure to dinotefuran did not produce dosage-dependent decreases in the IgM antibody-forming cell responses to sheep red blood cells when evaluated as either specific activity or as total spleen activity for both male and female rats.

For assay 2 (non-AFC phase), terminal body weights in male rats and spleen weights (absolute and relative) in male and female rats did not differ significantly among the groups compared to the control group values. Terminal body weights in female rats were significantly reduced at 3000 ppm and 10000 ppm (Table A6.9.4-3). Overall, the phenotypic analysis of splenic subpopulations demonstrated a lack of effect at all dose levels on the number of Total B cell ($CD45^+$), Total T cell ($CD5^+$), Helper/DTH T cell ($CD4^+CD5^+$), Cytotoxic T cell ($CD8^+CD5^+$) and Natural Killer cell ($NKR\text{-}PIA^+CD8^-$). Although males at 10000 ppm showed significantly lower ($p \leq 0.01$) group mean absolute number of Natural Killer (NK) cells, this was due to a lower absolute number of spleen cells since the relative (percent) value for NK cells was comparable to the control value (Table A6.9.4-3). In Natural Killer Cell assay, there was no effect on NK activity after exposures as high as 10000 ppm in either male or female rats.

## 5 APPLICANT'S SUMMARY AND CONCLUSION

**5.1 Materials and methods**

Guideline:

No applicable guideline

The design of the study was discussed with, and agreed by, EPA.

Method:

The purposes of this study were to determine suitable dose levels of dinotefuran for use in a developmental neurotoxicity study, to evaluate specific aspects of the functional immunological status of F1 progeny exposed *in utero*, during lactation via maternal milk, and for five weeks following weaning, and to determine if there is sufficient evidence of an effect on functional immunological status of F1 progeny to warrant inclusion of immunological end-points in a subsequent formal developmental neurotoxicity/immunotoxicity study.

P generation female rats and F1 generation pups;
Groups of 10 mated female rats were treated orally with dinotefuran, by diet incorporation, from day 6 of gestation through day 20 of lactation until sacrifice at concentrations of 0, 1000, 3000 or 10000 ppm (equivalent to 69.5, 211.8 and 669.9 mg/kg/day). All P generation rats were sacrificed after completion of the 21-day postpartum period and a gross necropsy was performed. Viability, clinical observations, maternal body weight and gains, maternal behaviour, feed consumption, the number and distribution of corpora lutea, implantation sites, uterine contents, litter size, pup viability and body weights were evaluated.

F1 generation rats (selected for immunological evaluation);
Following weaning on PND 21, 20 F1 generation rats/sex/group were selected for immunological evaluation and directly exposed to dinotefuran in the diet at 0, 1000, 3000 or 10000 ppm for 5 weeks

**Section A6.9-4** **Developmental Neurotoxicity and Immunotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral, dosage-range finding**

| | | |
|---|---|---|
| | | (equivalent to 100.3, 310.6 and 1043.3 mg/kg/day). Two pups/sex/litter were selected for each of the two immunological assays, an antibody-forming cell (AFC) assay and splenocyte phenotyping with a natural killer (NK) cell assay. The following parameters were evaluated; viability, clinical observations, body weight and gains, behaviour, feed consumption, spleen weights, immunological evaluations (spleen IgM AFC response, spleen cell phenotyping, NK cell activity) and necropsy observations. |
| **5.2** | **Results and discussion** | P generation female rats and F1 generation pups;<br>No treatment-related effects were observed in maternal rats at any dose level. Pup weights in the 10000 ppm group were significantly reduced for postpartum days 13, 17 and 21 at which time direct exposure of the pups to the test substance in the diet occurs as well as exposure through the milk. No deaths, treatment-related clinical signs or gross lesions were observed at any dose level.<br><br>F1 generation rats (selected for immunological evaluation);<br>Body weights of male and female rats at 10000 ppm were reduced during the study period, due to lower weight at weaning, but body weight gains were unaffected by treatment.<br><br>There was no effect at any dose level on AFC-forming activity in response to sheep RBC antigen when evaluated either as specific activity or as total spleen activity in either male or female rats. Overall, there was no effect at any dose level on the distribution and number of splenocyte phenotypes (total B cells ($CD45^+$), total T cells ($CD5^+$), Helper/DTH T cells ($CD4^+CD5^+$), Cytotoxic T cells ($CD8^+CD5^+$) and Natural Killer cells ($NKR\text{-}PIA^+CD8^-$)). Although males at 10000 ppm showed lower numbers of Natural Killer (NK) cells, this was due to a lower absolute number of spleen cells since the relative (percent) value for NK cells was comparable to the control value. In the Natural Killer (NK) Cell assay, there was no effect on NK activity after exposures as high as 10000 ppm in either male or female rats. |
| **5.3** | **Conclusion** | In conclusion, a no-observed-adverse effect-level (NOAEL) for P generation maternal toxicity was established as 10000ppm, equivalent to an average maternal dose level of 1035 mg/kg bw/day, based on the absence of treatment-related effects at 10000ppm, the highest dose level employed.<br><br>An NOAEL for general toxicity following exposure to dinotefuran in the F1 generation was 3000ppm (310.6/316.3 mg/kg/day in males and females), based on a reduction in weaning body weight at 10000 ppm. Since lower weaning body weights at 1000 or 3000 ppm were transient, they were considered not to be adverse.<br><br>The NOAEL for the functional assessment of the immune system was 10000 ppm (1043.3/1119.7 mg/kg/day in males and females), the highest dose tested. There were no effects on innate and humoral components of the immune system for F1 generation male and female rats up to 10000 ppm. |
| 5.3.1 | NOAEL<br>P generation maternal toxicity | 10000ppm, equivalent to an average maternal dose level of 1035 mg/kg bw/day. |
| 5.3.2 | NOAEL<br>General toxicity in | 3000ppm (310.6/316.3 mg/kg/day in males and females) |

**Section A6.9-4** **Developmental Neurotoxicity and Immunotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral, dosage-range finding**

| | | |
|---|---|---|
| | F1 generation | |
| 5.3.3 | NOAEL<br>Functional assessment of immune system | 10000 ppm (1043.3/1119.7 mg/kg/day in males and females) |
| 5.3.4 | Reliability | 1 |
| 5.3.5 | Deficiencies | No applicable guideline |

**Table A6.9.4-1: Summary of mean pup weights/litter**

| Parameter | Lactation day | Litter treated at (ppm): | | | |
|---|---|---|---|---|---|
| | | 0 | 1000 | 3000 | 10000 |
| Mean body weight / litter (g) | Day 0 | 6.7 | 6.2 | 6.5 | 6.6 |
| | Day 4 (pre-cull) | 10.6 | 9.9 | 10.5 | 10.3 |
| | Days 4 (post-cull) | 10.7 | 10.0 | 10.6 | 10.4 |
| | Days 7 | 15.8 | 14.9 | 15.6 | 15.1 |
| | Days 11 | 22.6 | 21.1 | 21.3 | 20.7 |
| | Days 13 | 26.0 | 23.9 | 23.9 | 22.6** |
| | Day 17 | 33.0 | 30.0* | 30.6 | 28.1** |
| | Day 21 | 46.7 | 41.5* | 42.3 | 38.2** |

* $p \leq 0.05$;
** $p \leq 0.01$

**Table A6.9.4-2: Summary of mean body weights and body weight changes – F1 generation rats**

| Parameter | Days Postpartum | Males treated at (ppm): | | | | Females treated at (ppm): | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1000 | 3000 | 10000 | 0 | 1000 | 3000 | 10000 |
| Mean body weight (g) | Day 22 | 53.4 | 47.7** | 48.8* | 41.8** | 51.8 | 44.4** | 46.8* | 42.1** |
| | Day 29 | 96.3 | 90.0* | 93.6 | 83.3** | 88.9 | 82.8 | 86.2 | 79.2** |
| | Day 36 | 152.4 | 146.2 | 151.6 | 135.8** | 131.7 | 124.4 | 128.4 | 119.6** |
| | Dat 43 | 214.0 | 204.8 | 212.1 | 193.4** | 163.8 | 156.7 | 159.2 | 151.6 |
| | Day 50 | 274.0 | 266.7 | 269.6 | 250.5** | 189.4 | 184.8 | 184.6 | 176.1 |
| | Day 57 | 332.0 | 327.1 | 330.0 | 308.1** | 214.2 | 206.5 | 208.8 | 198.6* |
| | Day 64 | - | - | - | - | 235.8 | 225.4 | 223.4 | 214.3** |
| Weight changes (g) | Days 22 - 29 | 42.8 | 42.3 | 44.8 | 41.5 | 37.2 | 37.6 | 39.3 | 37.0 |
| | Days 29 - 36 | 56.2 | 56.2 | 58.0 | 52.5 | 42.8 | 41.6 | 42.2 | 40.4 |
| | Days 36 - 43 | 61.6 | 58.6 | 60.5 | 57.6 | 32.0 | 32.3 | 30.8 | 32.0 |
| | Days 43 - 50 | 59.9 | 62.0 | 57.4 | 57.0 | 25.7 | 28.1 | 25.4 | 24.6 |
| | Days 50 - 57 | 58.1 | 60.4 | 60.4 | 57.6 | 24.8 | 21.7 | 24.2 | 22.4 |
| | Days 57 - 64 | - | - | - | - | 21.6 | 18.9 | 14.6** | 15.8** |
| | Days 22 - 57 | 278.6 | 279.4 | 281.2 | 266.3 | - | - | - | - |
| | Days 22 - 64 | - | - | - | - | 184.0 | 180.3 | 176.6 | 172.2 |

* $p \leq 0.05$;
** $p \leq 0.01$

**Table A6.9.4-3: Summary of immunological evaluation – F1 generation rats**

| Parameter | | Males treated at (ppm): | | | | Females treated at (ppm): | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1000 | 3000 | 10000 | 0 | 1000 | 3000 | 10000 |
| Assay 1 | | | | | | | | | |
| Spleen weight (mg) | | 619 | 846** | 803* | 776 | 521 | 546 | 549 | 557 |
| Assay 2 | | | | | | | | | |
| Terminal body weight (g) | | 369.2 | 369.7 | 373.7 | 341.1 | 254.2 | 246.1 | 234.7* | 218.0** |
| Spleen cells (x107) | | 88.04 | 88.58 | 74.38 | 69.66* | 57.27 | 62.80 | 59.57 | 55.67 |
| Total B cell | Absolute | 485.2 | 503.2 | 433.6 | 386.3 | 310.6 | 323.8 | 348.5 | 316.0 |
| Total T cell | Absolute | 251.4 | 246.0 | 195.9 | 209.5 | 167.6 | 203.7 | 162.9 | 163.1 |
| Helper/DTH T cell | Absolute | 139.9 | 131.0 | 112.2 | 124.5 | 99.4 | 117.9 | 97.0 | 92.3 |
| Cytotoxic T cell | Absolute | 109.8 | 111.2 | 83.3 | 83.8 | 74.8 | 90.8 | 68.8 | 72.8 |
| NK cell | Absolute | 140.3 | 142.4 | 110.9 | 109.8* | 97.8 | 114.6 | 90.7 | 95.8 |

* $p \leq 0.05$;
** $p \leq 0.01$

| Evaluation by Competent Authorities | |
|---|---|
| | |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 8 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 A6.9-5 Developmental Neurotoxicity
## Annex Point IIA6.9 Rat
## Oral

Official use only

### 1 REFERENCE

| | | |
|---|---|---|
| **1.1** | **Reference** | [redacted], 2010, Oral (Diet) developmental neurotoxicity study of MTI-446 (Dinotefuran) in Crl:CD (SD) rats, [redacted], unpublished report no. [redacted] 00002, October 19, 2010. |
| **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<br>OPPTS guideline 870.6300<br>OECD 426 |
| **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% (re-analysis by [redacted]; 99.5%) |
| 3.1.2.3 | Stability | Expiration date:31 December 2012 |
| **3.2** | **Reference Substance (positive control)** | None |

## Section A6.9-5 Developmental Neurotoxicity
**Annex Point IIA6.9** **Rat**
**Oral**

| | | |
|---|---|---|
| **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 | Females |
| 3.3.5 | Rearing conditions | P generation: pre-mating: one/cage, mating: 1M + 1F, post-mating: 1F + litter/cage. F1 generation: after DL21: two or three/cage |
| 3.3.6 | Age/weight at study initiation | Approximately 10 weeks old, weighing 240 - 294 g (day 6 of gestation) |
| 3.3.7 | Number of animals per group | 25 mated female rats per group |
| 3.3.8 | Control animals | Yes |
| **3.4** | **Administration** | Oral by admixture to the diet |
| 3.4.1 | Exposure | From day 6 of gestation through day 21 of lactation until sacrifice and necropsy. |
| 3.4.2 | Dose Levels | 0, 1000, 3000 or 10000 ppm<br>The overall mean dose levels in the P females were:<br>79.4, 237.4 and 784.1 mg/kg/day during gestation and;<br>158.0, 500.7 and 1642.9 mg/kg/day during lactation. |
| 3.4.3 | Vehicle | Certified rodent diet® (meal form) #5002, PMI® Nutrition International, *ad libitum* . |
| 3.4.4 | Concentration in vehicle | Not applicable |
| 3.4.5 | Total volume applied | Not applicable |
| 3.4.6 | Postexposure period | 21 days |
| 3.4.7 | Anticholinergic substances used | None |
| 3.4.8 | Controls | Plain diet |
| **3.5** | **Examinations** | |
| 3.5.1 | Body Weight | P generation animals: Recorded weekly during the pre-exposure period, on DG (day of gestation) 0 and daily thereafter.<br>F1 generation pups: Recorded on DL (day of lactation) 0, 4, 7, 11, 13, 17 and 21, and weekly during the post-weaning period and prior to sacrifice for pups selected for further study (subsets 1 – 5). |
| 3.5.2 | Food consumption | P generation animals: Recorded on DG 0, and daily during all other periods.<br>F1 generation pups: Measured weekly during the post-weaning period. |

| Section A6.9-5<br>Annex Point IIA6.9 | | Developmental Neurotoxicity<br>Rat<br>Oral | |
|---|---|---|---|
| 3.5.3 | Viability | P generation animals: Weekly at approximately the same time each week during the pre-exposure period and on DG 0.<br>F1 generation pups: At least twice daily | |
| 3.5.4 | Clinical observations | P generation animals: Daily starting on DG6 by an observer unaware of dosage group. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes, live litter size and pup viability at birth.<br>F1 generation pups: Recorded once daily during the pre-weaning period.<br>Additionally, pups in subset 4 (see below) were examined outside of the home cage for detailed clinical observations on DL 4 and 11, perfomed by an observer unaware of the dosage group assignment of the pups. These observations included (but were not limited to): general appearance (skin, fur, changes in eyes, eyeballs and mucous membranes), body position and posture (e.g., hunchback posture), autonomic nervous system function (lacrimation, piloerection, pupil diameter, respiration, excretion), motor coordination, ambulatory abnormalities, reaction to being handled and to environmental stimulation, nervous system (tremor, convulsion, muscular contractions), changes in exploratory behavior, ordinary behavior (changes in grooming, headshaking, gyration), abnormal behavior (autophagia, backward motion, abnormal vocalization) and aggression.<br>Subset 4 (up to 25 rats/sex/group): evaluated for detailed clinical observations outside the home cage on DL 4 and 11, and the day of preputial separation or vaginal patency, Ten rats/sex/group were killed on ca. day 69 for brain weight/gross dimensions recording and microscopic brain measurements (all groups) and neurohistopathology examinations (0 and 10000 ppm only). | |
| 3.5.5 | Signs of Toxicity | F1 generation pups: Subset 2 (up to 25 rats/sex/group): evaluated for day of preputial separation or vaginal patency, and passive avoidance response as a measure of learning, short and long-term memory and hyperactivity on ca. day 22. Water maze testing, as a measure of coordination, swimming ability, learning and memory on ca. day 60.<br>Subset 3 ((up to 24 rats/sex/group): evaluated for day of preputial separation or vaginal patency, and motor activity on days 13, 17, 21 and ca. 60. The acoustic startle habituation response was measured on days 22 and ca. 59. | |

**Section A6.9-5** **Developmental Neurotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral**

3.5.6 Pathology

P generation animals: Parental generation females delivering a litter were sacrificed by carbon dioxide asphyxiation and a gross necropsy of thoracic, abdominal and pelvic viscera was performed after completion of the 21-day postpartum period. The number and distribution of implantation sites was recorded. Female rats that did not deliver a litter were sacrificed on presumed DG25 and examined for gross lesions. Uteri were examined to confirm the absence of implantation sites. Females delivering a litter but not selected for further evaluation were subjected to a gross necropsy and and examination of thoracic, abdominal and pelvic viscera was performed after completion of the 21-day postpartum period. The number and distribution of implantation sites was recorded.

F1 generation pups:
Pups that died before initial examination of the litter for pup viability were evaluated for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sank were considered stillborn; pups with lungs that floated were considered liveborn and to have died shortly after birth. Pups found dead were examined for gross lesions and for the cause of death. On day 4, all offspring selected for culling were sacrificed by an intraperitoneal injection of sodium pentobarbital and necropsied.

Pups that died before scheduled termination and not selected for further study were examined for gross lesions and the cause of death as soon as possible after the observation was made. Pups found dead on day 1 to 4 were preserved in Bouin's solution for possible future evaluation.
All offspring not selected for continued evaluation were necropsied prior to weaning and gross lesions were retained in Bouin's solution.

3.5.7 Histopathology

Subset 1 (up to 25 rats/sex/group): 10 rats/sex/group killed on day 21 for brain weight/gross dimensions recording and microscopic brain measurements (all treated groups) and neurohistopathological examination (0 and 10000 ppm only). Pups not selected for neurohistopathology were sacrificed and evaluated for gross lesions.

Gross lesions were subjected to histological evaluation.

**3.6 Statistics**

Adult data were evaluated with the individual rat as the unit measured. Litter values were used in evaluation of pup data, as appropriate.

Clinical observations and other proportion data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Continuous data were analysed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance, when appropriate. If the Analysis of Variance was significant, Dunnett's Test was used. If the Analysis of Variance was not appropriate, the Kruskal-Wallis Test was used, when less than or equal to 75% ties were present. In cases where the Kruskal-Wallis Test was significant, Dunn's Method of Multiple Comparisons was used. If there were greater than 75% ties, Fisher's Exact Test was used to analyze the data. Count data were evaluated using the procedures described above for the Kruskal-Wallis Test.

## 4 RESULTS AND DISCUSSION

**4.1 Body Weight**

P generation animals:

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**Oral**

| | | |
|---|---|---|
| | | Body weight gains for days 6 to 9 of gestation and days 6 to 20 of gestation were significantly reduced in the 10000 ppm exposure group compared to the control group values (Table A6.9.5-1). Mean body weight gain at 10000 ppm was reduced by 11.2% from GD 6 – GD 20, but mean body weights were generally comparable among the dosage groups. Body weight gains during the lactation period did not differ significantly among the exposure groups. Treatment at up to 3000 ppm during gestation and lactation did not affect body weight gains or body weights.<br><br>F1 generation pups:<br><br>Group mean body weights and body weight gains were unaffected by maternal treatment at all dose levels. A single significant increase ($p \leq 0.01$) in body weight gain of subset 4 male rats at 10000 ppm occurred on days 28 to 35 but was considered unrelated to maternal treatment because the increase did not persist and a similar increase was not evident in the other subsets evaluated. |
| **4.2** | **Food consumption** | P generation animals:<br><br>Absolute and relative feed consumption values were generally comparable among the treated and control groups throughout gestation and lactation and none of the treated group values differed from the control group<br><br>F1 generation pups:<br><br>Absolute and relative feed consumption were not affected by maternal treatment at any dose level. Statistically significant differences that occurred were considered not to be treatment-related because the differences were not dosage-dependent and/or did not persist. |
| **4.3** | **Viability** | P generation animals:<br><br>All P generation female rats survived to scheduled sacrifice and there were no treatment-related adverse clinical observations. Pregnancy incidences were 92.0, 100.0, 92.0 and 96.0% at 0, 1000, 3000 and 10000 ppm, respectively, and all pregnant dams delivered litters. All natural delivery observations and litter parameters (duration of gestation, implantation sites/litter, dams with stillborn pups, dams with no liveborn pups, and the gestation index, lactation index, pup survival, pup weights and sex ratio) were comparable in all treated and control groups (Table A6.9.5-2). Although a statistically significantly ($p < 0.01$) lower viability index and significantly ($p < 0.01$) higher pup mortality from DL1 – 4 occurred at 3000 ppm, they were considered unrelated to treatment since the values at 10000 ppm were comparable to the control values. No clinical or necropsy observations in the F1 generation pups were attributed to maternal exposure to the test substance at any dose level.<br><br>F1 generation pups:<br><br>There was no treatment-related mortality at any dose level in F1 generation offspring retained beyond weaning for further investigation. Two females at 10000 ppm were found dead and two control females were missing or killed due to poor clinical condition. All other animals survived to scheduled sacrifice. |
| **4.4** | **Clinical** | P generation animals: |

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**Oral**

| | | |
|---|---|---|
| | **observations** | Detailed clinical observations revealed no treatment-related effects at any dose level.<br>F1 generation pups:<br>All clinical observations, including the detailed clinical observations outside the home cage on days 4 and 11, in the F1 offspring from all treated groups were considered unrelated to treatment and evaluation of autonomic functions did not reveal any treatment-related effects. Statistically significant differences between the treated and control groups were confined to a higher ($p < 0.01$) incidence of umbilical hernia in females at 3000 ppm and a higher ($p < 0.01$) incidence of red or black-coloured tail/tip of tail. |
| **4.5** | **Signs of toxicity** | F1 generation pups:<br>Motor activity evaluated in subset 3 on days 13, 17, 21 and 60 ± 2 was not affected by maternal treatment at any dose level. All group mean values for the individual 10-minute blocks and totals (1 hour) for the number of movements and time spent in movement were comparable between the treated and control groups, and there were no statistically significant differences.<br>Acoustic startle habituation evaluated in subset 3 on days 22 and 60 ± 2 was not affected by maternal treatment at any dose level. No statistically significant differences occurred among the groups for either the pattern of response magnitudes or the average response magnitude. Maternal treatment at all dose levels did not affect learning and memory as evaluated in a passive avoidance paradigm in subset 2 at ca. 22 days of age and again one week later, or in a water maze test at ca. 60 days of age and again one week later.<br>In the passive avoidance test, the mean number of trials to response, the latency of response and the numbers of animals failing to learn were comparable in all treated and control groups and none of the values for the treated groups was significantly different from the control values.<br>In the water maze performance assessment, the number of trials to achieve criterion, errors/trial, the numbers failing to learn and the latency periods were comparable in all treated and control groups and none of the values for the treated groups was significantly different from the control values. |
| **4.6** | **Pathology** | P generation animals:<br>No maternal gross lesions related to treatment were evident at necropsy.<br>F1 generation pups:<br>There were no treatment-related necropsy observations at any dose level.<br>There was no effect of treatment at any dose level on brain weight and cerebral and cerebellar lengths evaluated in subsets 1and 4 at 21 and 69 days of age, respectively. All group mean values were comparable to, and not significantly ($p>0.05$) different from, control values, with the exception of 1000 ppm males on day 21 post partum that showed significantly ($p<0.05$) lower mean brain weight than controls. Mean brain weights in males at 3000 or 10000 ppm were comparable to the control value.<br>Maternal treatment at all dose levels did not affect the day of vaginal patency or preputial separation in subsets 2, 3 and 4. The mean day of occurrence for each sex were comparable between the treated and control |

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groups and there were no statistically significant differences. For each sex, group mean body weights at these events were comparable in all treated and control groups.

**4.7 Histopathology**

F1 generation pups:

There were no microscopic changes in the gross lesions evaluated histologically that were considered to have been the result of test substance administration. The changes observed were typical of those that occur spontaneously or as incidental findings in rats of this age and strain.

**4.8 Statistics**

No statistically significant inter-group differences were noted at any dose level for brain weights or for gross cerebral and cerebellar measures in 22 day old (subset 1) and adult (subset 4) rats. In addition, none of the microscopic brain measurements (frontal cortex, parietal cortex, striatum, corpus callosum, hippocampus and cerebellum) were significantly different ($p > 0.05$) from the controls at any dose level for either 22 day old or adult rats. No treatment-related microscopic lesions were present in any of the tissues examined in the central and peripheral nervous systems in 22 day old and adult offspring at 10000 ppm.

## 5 APPLICANT'S SUMMARY AND CONCLUSION

**5.1 Materials and methods**

Guidelines:

OPPTS guideline 870.6300, OECD 426

No relevant deviations from test guidelines.

Method:

The purpose of this study was to provide information for use in evaluating the potential for functional or histopathological neurotoxic effects in offspring after exposure to MTI-446 (Dinotefuran) *in utero* and/or via maternal milk during the lactation period. The study was performed in compliance with the requirements of OPPTS 870.6300 and OECD 426 test guidelines. This study summary has been prepared from a QA-audited draft report.

Four groups of 25 mated Crl:CD(SD) female rats were treated orally, in the diet, with Dinotefuran at concentrations of 0, 1000, 3000 or 10000 ppm from day 6 of gestation through day 21 of lactation until sacrifice and necropsy. The overall mean dose levels in the P females were 79.4, 237.4 and 784.1 mg/kg/day during gestation and 158.0, 500.7 and 1642.9 mg/kg/day during lactation.

Examinations performed on parental females comprised viability, clinical observations, detailed clinical observations, body weight and body weight changes, maternal behaviour, food consumption, necropsy observations, the number and distribution of corpora lutea, implantation sites, uterine contents, litter size and pup viability. On day 4 of lactation, litters were culled to 5 pup/sex, where possible. During lactation, offspring clinical observations were recorded daily and body weights recorded every 3 – 4 days.

Additionally, pups in subset 4 (see below) were examined outside of the home cage for detailed clinical observations on DL 4 and 11, perfomed by an observer unaware of the dosage group assignment of the pups.

Further evaluations were performed on subsets of up to 25

**Section A6.9-5** **Developmental Neurotoxicity**
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animals/sex/group selected for further study:

Ten rats/sex/group killed on day 21 for brain weight/gross dimensions recording and microscopic brain measurements (all treated groups) and neurohistopathological examination (0 and 10000 ppm only). Pups not selected for neurohistopathology were sacrificed and evaluated for gross lesions. The day of preputial separation or vaginal patency was recorded. The passive avoidance response as a measure of learning, short and long-term memory and hyperactivity was evaluated on ca. day 22, and water maze testing, as a measure of coordination, swimming ability, learning and memory, was evaluated on ca. day 60. A quantitative assessment of motor activity was performed on days 13, 17, 21 and ca. 60, and the acoustic startle habituation response was measured on days 22 and ca. 59. Detailed clinical observations outside the home cage were recorded on DL 4 and 11. Ten rats/sex/group were killed on ca. day 69 for brain weight/gross dimensions recording and microscopic brain measurements (all groups) and neurohistopathology examinations (0 and 10000 ppm only).

All offspring culled on DL 4, those dying during the study, and those retained for further study were subjected to necropsy, preservation of gross lesions and histopathological evaluation.

**5.2 Results and discussion**

There was no effect of treatment at any dose level on the parental females at all dose levels, with the exception of an 11.2% decrease in maternal weight gain from day 6 to day 20 of gestation at 10000 ppm. The body weight gain during gestation at lower dose levels was unaffected by treatment. There was no effect of treatment at any dose level on reproductive capacity and litter parameters. Pup survival, clinical condition, autonomic functions, and weight gain during lactation were unaffected by treatment.

Survival, food consumption and weight gain beyond weaning of offspring selected for further study were unaffected by treatment at all dose levels. There were no treatment-related gross lesions at necropsy. There was no effect of treatment at any dose level on preputial separation / vaginal patency, motor activity on days 13, 17, 21 and 60 ± 2, the acoustic startle inhibition response on days 22 and 60 ± 2, learning and memory as evaluated in a passive avoidance paradigm at ca. 22 days of age, or in a water maze test at ca. 60 days of age.
There were no microscopic changes in the gross lesions evaluated histologically in F1 offspring that were considered to have been the result of test substance administration.

There was no effect of treatment at any dose level on brain weight and cerebral and cerebellar lengths evaluated at 21 and 69 days of age. The microscopic brain measurements (frontal cortex, parietal cortex, striatum, corpus callosum, hippocampus and cerebellum) were comparable to control values at all dose levelf for both 22 day old and adult rats. No treatment-related microscopic lesions were present in any of the tissues examined in the central and peripheral nervous systems in 22 day old and adult offspring at 10000 ppm.

It was concluded that the no-observable-effect-level (NOEL) and no-observed-adverse-effect level (NOAEL) was 10000 ppm for both

**Section A6.9-5** **Developmental Neurotoxicity**

**Annex Point IIA6.9** **Rat**

**Oral**

| | | |
|---|---|---|
| | | functional and histopathological developmental neurotoxicity in F1 generation rats whose mothers were exposed to Dinotefuran. This dietary concentration was the highest dose tested and provided an average maternal dose level of 784 mg/kg/day during gestation and 1643 mg/kg/day during lactation.<br>An NOAEL for all toxicological effects was established as 3000 ppm (equivalent to an average maternal dose level of 237 mg/kg/day during gestation and 501 mg/kg/day during lactation) based on the occurrence of reduced maternal body weight gain during gestation at 10000 ppm. |
| **5.3** | **Conclusion** | |
| 5.3.1 | LOAEL | Not determined |
| 5.3.2 | NO(A)EL | 10000 ppm (for both functional and histopathological developmental neurotoxicity in F1 generation rats whose mothers were exposed to dinotefuran) |
| 5.3.3 | NOAEL | 3000 ppm (for all toxicological effects) |
| 5.3.4 | Reliability | 1 |
| 5.3.5 | Deficiencies | No |

**Table A6.9.5-1: Group mean body weight gains during gestation and lactation – F0 generation**

| Days of gestation (DG) or lactation (DL) | Group mean body weight gain (g) at (ppm): | | | |
|---|---|---|---|---|
| | 0 | 1000 | 3000 | 10000 |
| DG 0 – 6 | 34.9 | 34.5 | 37.6 | 36.0 |
| DG 6 – 9 | 14.2 | 14.8 | 12.0 | 7.7** |
| DG 9 – 12 | 17.5 | 17.4 | 18.3 | 17.2 |
| DG 12 – 15 | 20.9 | 17.7 | 21.4 | 19.5 |
| DG15 -18 | 37.6 | 36.6 | 36.4 | 34.1 |
| DG 18 – 20 | 30.1 | 30.0 | 28.3 | 28.3 |
| DG 6 – 20 | 120.3 | 116.4 | 116.4 | 106.8** |
| DL 0 - 21 | 31.8 | 28.8 | 34.2 | 33.0 |

** $p < 0.01$

**Table A6.9.5-2: Group mean litter data – F0 generation**

| Parameter | Group mean body weight gain (g) at (ppm): | | | |
|---|---|---|---|---|
| | 0 | 1000 | 3000 | 10000 |
| No. assigned to natural delivery | 25 | 25 | 25 | 25 |
| Pregnant | 23 | 25 | 23 | 24 |
| Delivering a litter | 23 | 25 | 23 | 24 |
| Duration of gestation (days) | 22.6 | 22.7 | 22.5 | 22.5 |
| Implantation sites/litter | 14.9 | 14.5 | 15.0 | 14.5 |
| Dams with stillborn pups | 1 | 3 | 3 | 2 |
| Litters with 1 or more liveborn pups | 23 | 25 | 23 | 24 |
| Mean liveborn pups/litter | 14.3 | 13.7 | 13.8 | 13.3 |
| Mean stillborn pups/litter | 0.0 | 0.1 | 0.1 | 0.1 |
| Mean litter size on LD0 | 14.3 | 13.7 | 13.7 | 13.3 |
| Viability index (%) | 99.4 | 98.5 | 95.9** | 99.1 |
| Lactation index (%) | 100 | 100 | 100 | 99.0 |
| Surviving pups/litter on LD21 | 10.0 | 10.0 | 9.5 | 9.9 |
| Sex ratio on LD21 (% males) | 48.1 | 50.0 | 49.0 | 49.5 |
| Mean pup weight/litter at LD0 (g) | 6.6 | 6.6 | 6.5 | 6.6 |
| Mean pup weight/litter at LD21 (g) | 50.2 | 48.6 | 51.3 | 50.2 |

** $p < 0.01$

| Evaluation by Competent Authorities | |
|---|---|
| | |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | *15 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** | *Accepable* |
| **Remarks** | *None* |
| | **COMMENTS FROM ...** |
| **Date** | |
| **Materials and Methods** | |
| **Results and discussion** | |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **Remarks** | |

## Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

**Annex Point IIA7.6.2.1**

| | | | Official use only |
|---|---|---|---|
| | | **1 REFERENCE** | |
| **1.1** | **Reference** | Sydney, P., 1998, MTI-446: Determination of hydrolysis as a function of pH. Huntingdon Life Sciences, unpublished report no. 95/MTO098/1216 (MRID 45640101), July 29, 1998.<br>Sydney, P., 2000, Report amendment 1: Determination of hydrolysis as a function of pH. Huntingdon Life Sciences, unpublished report no. 95/MTO098/1216, April 17, 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<br>OECD Guideline 111<br>Hydrolysis test C7 (Annex to 92/69 EEC) | |
| **2.2** | **GLP** | Yes | |
| **2.3** | **Deviations** | No | **X** |
| | | **3 MATERIALS AND METHODS** | |
| **3.1** | **Test material** | MTI-446 (common name: dinotefuran) | |
| 3.1.1 | Lot/Batch number | Lot number OT-9536 | |
| 3.1.2 | Specification | | |
| 3.1.2.1 | Purity | 99.5% | |
| 3.1.2.2 | Further relevant properties | Solid | |
| **3.2** | **Reference substance** | Dinotefuran (same as test material)<br>1-methyl-3-(tetrahydro-3-furylmethyl) urea (Metabolite UF ) | |
| **3.3** | **Test solution** | Buffer solutions were created using chemicals described in Table A7.1.1.1.1-1 in order to produce solutions of pH 4.0, 7.0, 9.0, 11.0 and 13.0. The test solutions were adjusted to within ± 0.05 pH of the desired value before being purged with nitrogen. | |
| **3.4** | **Testing procedure** | | |
| 3.4.1 | Test system | The solutions in Pyrex bottles were sterilised by autoclaving. To 100ml aliquots of each buffer solution (pre-equilibrated to 50°C prior to use in the test) were added approximately 200mg of dinotefuran to give a nominal concentration of 2g/l. The solutions were adjusted to within 0.05pH units of the test pH value and purged with nitrogen. Table A7.1.1.1.1-2 summarises details. | |
| 3.4.2 | Temperature | 50°C. | **X** |

## Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

**Annex Point IIA7.6.2.1**

3.4.3 pH

The initial and the final pH for each experiment.

| Nominal pH | Initial pH | Final pH |
|---|---|---|
| 4 | 3.99 | 4.05 |
| 7 | 7.00 | 7.03 |
| 9 | 9.03 | 9.05 |
| 11 | 11.04 | 10.72 |
| 13 | 12.99 | 12.04 |

3.4.4 Duration of the test

At pH 4, 7, 9: 170 hours

At pH 11: 120 hours

At pH 13: 96 hours

3.4.5 Number of replicates

2

3.4.6 Sampling

Samples were analysed after incubation for 0.25, 2.4, 24, 95/96, 118/120 and 170 hours.

3.4.7 Analytical methods

The analysis was by reversed phase HPLC. Dinotefuran was detected by UV absorption at 270nm. Duplicate aliquots (1ml) were each diluted to 20ml with HPLC mobile phase. The injection volume was 20µl. The column was YMC-pack ODS-AQ (25cm x 4.6mm internal diameter). The mobile phase was water: methanol (4:1v/v) and the flow rate was 1ml/min.

The hydrolysis product (UF) was identified by LC-MS/MS with a Finnigan MAT instrument with positive ion detection. Chromatography was with ODS-2 column and water: methanol (85:15 v/v) at 0.7ml/min. The retention time for UF was approximately 5 minutes.

**3.5 Preliminary test**

Yes

The preliminary test was the only test reported in the study as less than 10% degradation was observed at pH 4, 7 and 9 in the preliminary test.

**4 RESULTS**

**4.1 Concentration and hydrolysis values**

The results are summarised in Table A7.1.1.1.1-3. There was no significant change in the concentration of dinotefuran when incubated at pH 4, pH 7 or pH 9 at 50°C. Less than 10% degradation was observed.

**4.2 Hydrolysis rate constant ($k_h$)**

As there was no degradation at lower pH values, no rate constants were calculated. It was possible to calculate rate constants at pH 11 and 13.

: $\ln C_t$ (pH 11 and 50°C) $= 0.710 - 1.55 \times 10^{-2} t$,

: $\ln C_t$ (pH 13 and 50°C) $= 0.751 - 1.65 \times 10^{-1} t$

**4.3 Dissipation time**

There was less than 10% degradation of dinotefuran over 170 hours when incubated at pH 4, pH7 or pH9 at 50°C. The half- life at pH 11 was 45 hours. The half-life at pH 13 was 4.2 hours.

**4.4 Concentration – time data**

The results are summarised in Table A7.1.1.1.1-3

## Section A7.1.1.1.1 / Annex Point IIA7.6.2.1 — Hydrolysis as a function of pH and identification of breakdown products

| | | |
|---|---|---|
| **4.5** | **Specification of the transformation products** | There were no significant transformation products at the required pH values. The only transformation product observed in any significant amounts was found at pH 11 and 13 and identified as 1-methyl-3-(tetrahydro-3-furylmethyl)urea [UF] by co-chromatography and LC-MS/MS with reference standard. |
| | | **5 APPLICANT'S SUMMARY AND CONCLUSION** |
| **5.1** | **Materials and methods** | Guidelines:<br>OECD Guideline 111; Hydrolysis test C7 (Annex to 92/69 EEC)<br>No relevant deviations from test guidelines.<br>Method:<br>Only the preliminary test was performed as there was little degradation at pH 4, 7 and 9 at 50 °C. Two additional pH values (pH 11 and 13) were studied. There were no other deviations from the test guideline. |
| **5.2** | **Results and discussion** | Dinotefuran was hydrolytically stable at pH 4,7 and 9 at 50°C |
| 5.2.1 | $k_H$ | Not applicable |
| 5.2.2 | $DT_{50}$ | Not applicable |
| 5.2.3 | $r^2$ | Not applicable |
| **5.3** | **Conclusion** | Dinotefuran can be considered as stable to hydrolysis under environmentally relevant conditions with a half life greater than one year as it was stable at pH 4, 7 and 9 at 50 °C. At pH 11 and 13 and 50°C, hydrolysis to 1-methyl-3-(tetrahydro-3-furylmethyl) urea [UF] was observed with half lives of 45 hours and 4.2 hours respectively. Although not required by the guideline, degradate UF was identified by LC-MS/MS. |
| 5.3.1 | Reliability | 1 |
| 5.3.2 | Deficiencies | No |

**Table A7.1.1.1.1-1: Type and composition of buffer solutions (specify kind of water if necessary)**

| pH | Type of buffer (final molarity) | Composition |
|---|---|---|
| 4 | Not stated | Disodium hydrogen orthophosphate dodecahydrate (13.8g) and citric acid monohydrate (6.45g) were dissolved in purified water (950ml) and the pH adjusted to 4.0 with 1M hydrochloric acid. The volume was adjusted to 1000 ml with purified water. |
| 7 | Not stated | Potassium dihydrogen orthophosphate (3.4g) was dissolved in purified water (950ml). 1M sodium hydroxide (15ml) was added and the pH adjusted to 7.0 with 1M hydrochloric acid. The volume was adjusted to 1000ml with purified water. |
| 9 | Not stated | Disodium tetraborate decahydrate (16.6g) and potassium dihydrogen orthophosphate (1.80g) were dissolved in purified water (950ml) and the pH adjusted to 9.0 with 1M hydrochloric acid. The volume was adjusted to 1000ml with purified water. |
| 11 | Not stated | Glycine (7.54g) and sodium chloride (5.84g) were dissolved in 1000 ml of purified water (Solution A). Sodium hydroxide (2.04g) was dissolved in 500ml of purified water (Solution B). Portions of Solution A and Solution B (51ml and 49ml respectively) were mixed thoroughly. |
| 13 | Not stated | Glycine (7.54g) and sodium chloride (5.84g) were dissolved in 1000ml of purified water (Solution A). Sodium hydroxide (2.04g) was dissolved in 500ml of purified water (Solution B). Portions of Solution A and Solution B (7.5 ml and 92.5 ml respectively) were mixed thoroughly. |

**Table A7.1.1.1.1-2: Description of test system**

| | |
|---|---|
| **Glassware** | Pyrex |
| **Other equipment** | Water bath, pH meter |
| **Method of sterilization** | High temperature sterilisation by autoclave |

**Table A7.1.1.1.1-3: Mean concentration (g/l) of dinotefuran (parent compound) with time at various pH values**

| | Hours of incubation | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **2.4** | **24** | **95 / 96** | **118 / 120** | **170** |
| **pH 4** | 1.85 | 1.85 | 1.87 | 1.83 | 1.84 | 1.83 |
| **pH 7** | 1.91 | 1.89 | 1.91 | - | 1.88 | 1.84 |
| **pH 9** | 2.05 | 2.07 | 2.01 | - | 1.93 | 1.84 |
| **pH 11** | 2.10 | 1.97 | 1.34 | 0.46 | 0.32 | - |
| **pH 13** | 2.05 | 1.48 | 0.04 | - | - | - |

| Evaluation by Competent Authorities | |
|---|---|
| | |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 9 July 2012 |
| **Materials and Methods** | The Applicant's version is considered to be acceptable, noting the following :-<br>**3.4.2** : Temperature maintained at 50 °C in a thermostatically controlled incubator. |
| **Results and discussion** | The Applicant's version is considered to be acceptable. |
| **Conclusion** | The Applicant's version is considered to be acceptable. |
| **Reliability** | Accepted as being 1 |
| **Acceptability** | The Applicant's version is considered to be acceptable, noting the following :-<br>**2.3** : There is deviation to the guideline in that additional alkaline pH's (namely 11 and 13) were also tested but this is not detrimental to study reliability. |
| **Remarks** | It is clear that the compound is hydrolytically stable at environmentally relevant pH even when temperatures have been increased to 50 °C. Further testing indicates the potential for abiotic degradation at high alkaline pH and when temperature has been significantly elevated above environmental conditions.<br>Based upon equation 25 of the TGD on risk assessment, any $DT_{50}$ value derived in the study would need to be increased by a factor of 20.91 to account for the difference between 50 °C (test temperature) and 12 °C (average EU outdoor temperature). By inference, no significant degradation after 170 hr at 50 °C could be considered representative of hydrolytic stability after 3554 hr (148 d) at 12 °C. |
| | **COMMENTS FROM ...** |
| **Date** | |
| **Materials and Methods** | |
| **Results and discussion** | |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **Remarks** | |

**Section A7.1.1.1.2**
**Annex Point IIA7.6.2.2**

# Phototransformation in water including identity of transformation products

**Official use only**

## 1 REFERENCE

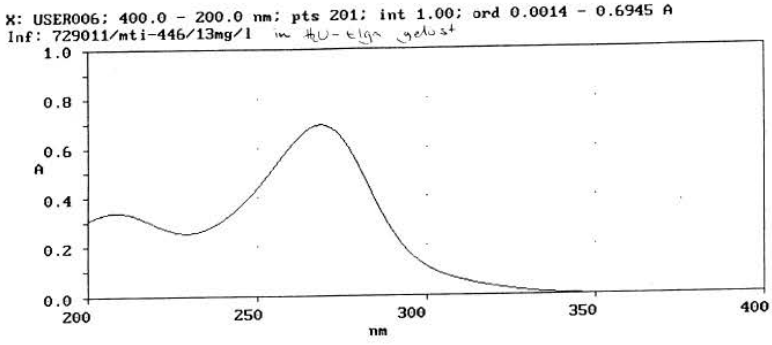
| | | |
|---|---|---|
| **1.1** | **Reference** | Van der Gaauw, A., 2002, Aqueous Photolysis of $^{14}$C-MTI-446 under Laboratory Conditions and Determination of Quantum Yield. RCC Ltd., unpublished report no. 729011, MRID 45640105, February 7, 2002. |
| **1.2** | **Data protection** | Yes |
| 1.2.1 | 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<br>Directives 95/36/EEC and 94/37/EEC;<br>SETAC (1995);<br>US EPA OPPTS 835.2210 |
| **2.2** | **GLP** | Yes |
| **2.3** | **Deviations** | No |

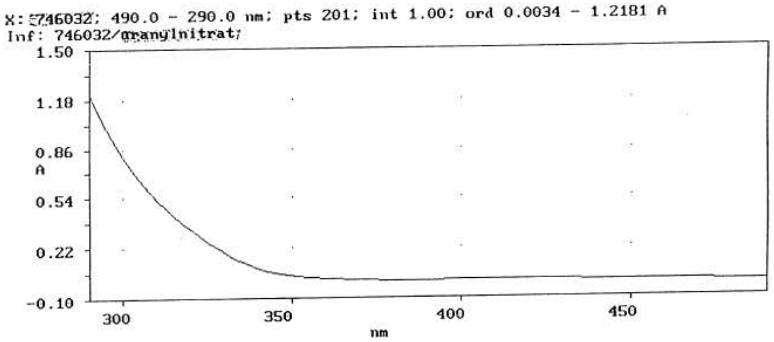
## 3 MATERIALS AND METHODS

| | | |
|---|---|---|
| **3.1** | **Test material** | $^{14}$C dinotefuran |
| 3.1.1 | Lot/Batch number | Batch no VB 9303 (G-label) and VB 9301 (F-label) mixed in equal amounts |
| 3.1.2 | Specification | Not applicable |
| 3.1.2.1 | Purity | 98.6% (radiochemical purity) |
| 3.1.2.2 | Radiolabelling | G-label (guanidine side chain label) plus F-label (furanyl ring label) |
| 3.1.2.3 | UV/VIS absorption spectra and absorbance value | Absorption spectra of dinotefuran (13.0 mg/L in purified water : first graph) and of actinometer solution (diluted 10 times : second graph): |



**Section A7.1.1.1.2**
**Annex Point IIA7.6.2.2**

## Phototransformation in water including identity of transformation products




Max absorbance at 270nm

3.1.3 Further relevant properties

Solubility in water: 39.83 g/L at 20°C.

**3.2 Reference substances**

1-methyl-2-nitroguanidine (MNG) : Lot no FCT01

Nitroguanidine (NG) : Lot no FGA01

1-methyl-3-(tetrahydro-3-furylmethyl)urea (UF) : Lot no OFU-1291

1-methyl-3-(tetrahydro-3-furylmethyl)guanidinium dihydrogen phosphate (DN phosphate) : Lot no OFU-1290

2-nitro-1-(tetrahydro-3-furylmethyl)guanidine (FNG) : Lot no OFU-1224

6-hydroxy-5-(2-hydroxyethyl)-1-methyl-1,3-diazinane-2-ylidene-N-nitroamine (PHP) : Lot no OFU-1299

1-[4-hydroxy-2-(hydroxymethyl)butyl]-3-methyl-2-nitroguanidine (446-DO) : Lot no OFU-1297

3-(methylamino)-9-oxa-2-aza-4-azoniabicyclo[4.3.0]non-3-ene hydrogen succinate (BCDN Succinate) : Lot no 1518TO

1-methylguanidinium chloride (MG Hydrochloride) : Lot no FHE02

Guanidinium chloride : Lot no GF01

**3.3 Test solution**

The pH7 phosphate buffer solution (Baker buffer No 5656) was diluted to 0.02mol/L with ultra purified deionised water. The buffer solution and water were sterilised by autoclaving and tests for sterility were performed at the start and end of incubation.

**3.4 Testing procedure**

3.4.1 Test system

The test substance was prepared in the buffer solution at a concentration of 0.720mg/L. This was achieved by mixing equal aliquots of the two radiolabels, removing the acetone, adding the sterilized buffer solution followed by ultrasonication to re-dissolve. Aliquots (80ml) of the solution were continuously illuminated in separate 100ml glass vessels (diameter 6.0cm) covered with quartz glass plates which were screwed onto the top of the vessels. The samples were cooled to 23.4°C and stirred continuously with magnetic stirrers. A control buffer solution was treated in the same way as the irradiated solutions, except that it was kept in the dark. Filtered humidified air was drawn through the incubation vessels at about 10ml/min. Any radioactive $CO_2$ or other volatiles in the purged air were captured in traps of 2M NaOH, followed by ethylene glycol.

3.4.2 Properties of light source

Xenon arc lamp 1.8KW (Suntest CPS apparatus, manufactured by Heraeus, Germany) with controllable irradiance between 400 – 765 $W/m^2$.

Light of wavelength less than 290nm was filtered out.

**Section A7.1.1.1.2**
**Annex Point IIA7.6.2.2**

## Phototransformation in water including identity of transformation products

| | | |
|---|---|---|
| 3.4.3 | Determination of irradiance | Incident light intensity was measured with an actinometric solution composed of uranyl nitrate (0.02 mol/l) and oxalic acid (0.1 mol/l). Oxalic acid was determined before and after irradiation for 20 minutes by titration with an acidic solution of $KMnO_4$ (0.02 mol/l) |
| 3.4.4 | Temperature | Mean value of 23.4 °C (+/-0.1 °C) for the irradiated samples and mean of 23.6 °C (+/-0.5 °C) for the dark controls. |
| 3.4.5 | pH | The pH values determined throughout the experiment remained constant between a range of 7.05 - 7.18. |
| 3.4.6 | Duration of the test | A continuous irradiation period of 13.8 days was maintained along with a corresponding sample maintained in the dark under the same conditions. |
| 3.4.7 | Number of replicates | 2 |
| 3.4.8 | Sampling | Aliquots of between 3.0 and 4.8ml were taken at 0, 0.1, 0.2, 0.4, 0.9, 1.8, 4.9, 6.9, 11.0 and 13.8 days of incubation. The dark controls were sampled in the same way at 0, 6.9 and 13.8 days of incubation. Trapping solutions were changed and analysed at each sampling interval. |
| 3.4.9 | Analytical methods | Total radioactivity was measured at each sampling interval by liquid scintillation counting of duplicate 200µl aliquots. Quantification of photo-degradation products was achieved by reversed phase (C18) high performance liquid chromatography (HPLC) with radio detection. Thin layer chromatography (TLC) with radio and UV detection was used for confirmation. |
| **3.5** | **Transformation products** | Up to 18 products of photo-degradation were detected of which six were characterized by co-chromatography with reference compounds. Dinotefuran was shown by radiochromatography to be stable in the samples kept in the dark. |
| 3.5.1 | Method of analysis for transformation products | A second HPLC system was required to separate all the transformation products. |

### 4 RESULTS

| | | |
|---|---|---|
| **4.1** | **Screening test** | Not performed |
| **4.2** | **Actinometer data** | The data from the actinometer which was used to calculated the quantum yield is summarised in Table A7.1.1.1.2-2 |
| **4.3** | **Controls** | The dark controls were at the same concentration, temperature and pH as the irradiated samples. At all sampling points, over 97% of the applied radioactivity was recovered as dinotefuran. |
| **4.4** | **Photolysis data** | |
| 4.4.1 | Concentration values | The mean initial measured concentration was 0.720 mg/l which declined 0.016 mg/l after 6.9 days. All the mean values are given in Table A7.1.1.1.2-1 |
| 4.4.2 | Mass balance | The total radioactivity recovered from the test samples ranged from 95.5% to 105.0% of the applied radioactivity. The corresponding values for the dark controls were 101.8% to 103.8%. |