Section A6.15.2 **Annex Point** **IUCLID**	**Behaviour of the residues of the active substance, its degradation and reaction products and where relevant, its metabolites on the treated or contaminated food or feedingstuffs including the kinetics of disappearance**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Dinotefuran is not intended to be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feedstuff for livestock is prepared, consumed or stored. Therefore, further studies relating to the behaviour of residues on food or feedstuffs are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.15.3 **Annex Point IIIA XI.1.4** **IUCLID**	**Estimation of potential or actual exposure of the active substance to humans through diet and other means**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Dinotefuran is not intended to be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feedstuff for livestock is prepared, consumed or stored. Therefore, further studies relating to the estimation or potential or actual exposure to humans through the diet and other means are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.15.4 Annex Point IIIA XI.1.7 IUCLID	**Proposed acceptable residues and the justification of their acceptability**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Dinotefuran is not intended to be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feedstuff for livestock is prepared, consumed or stored. Therefore, further studies relating to proposed acceptable residues and the justification of their acceptability are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.15.5 Annex Point IIIA XI.1.8 IUCLID	Any other available information that is relevant	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Dinotefuran is not intended to be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feedstuff for livestock is prepared, consumed or stored.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.15.6 **Annex Point IIIA XI.1.9** **IUCLID**	**Summary and evaluation of data submitted under point 6.15**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	No data has been submitted under Section A6.15, therefore no summary and evaluation is required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.16 **Annex Point IIIA VI.3.5, XI.2** **IUCLID**	**Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary may be required**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Further test related to the exposure of dinotefuran to humans in its proposed biocidal product are not considered necessary based on evaluation of the data submitted.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.17 **Annex Point IIIA, VI.6** **IUCLID**	**If the active substance is to be used in products for action against plants then tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals shall be required**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Dinotefuran is not intended to be used in products for action against plants therefore tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Non-submission is justified*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.18 Annex Point IIA; IIIA	Summary of mammalian toxicology and conclusions	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	This section is covered by Dossier Document IIA: Effects and Exposure.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*6 March 2013*	
Evaluation of applicant's justification	*The RMS agrees that the requirement to provide a summary of the mammalian toxicology and conclusions is covered by information provided in the Document II-A*	
Conclusion	*See above*	
Remarks	*None*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.2-1 **Metabolism studies in mammals**

Annex Point IIA6.2 **Rat**

Oral

Official use only

1 REFERENCE

1.1 Reference

[redacted] 2000a, Metabolism of [^{14}C]-MTI-446 in Rats, [redacted], unpublished report no. [redacted] 6648-136, January 27, 2000.

[redacted] 2000b, First amendment to report - Metabolism of [^{14}C]-MTI-446 in Rats, [redacted], unpublished report no. [redacted] 6648-13, March 28, 2000.

[redacted], 2001, Second amendment to report - Metabolism of [^{14}C]-MTI-446 in Rats, [redacted], unpublished report no. [redacted] 6648-136, November 5, 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

EPA-FIFRA, Subdivision F, § 85-1

JMAFF 59 NohSan no. 4200

2.2 GLP Yes

2.3 Deviations No

3 MATERIALS AND METHODS

3.1 Test material

1. Unlabelled dinotefuran
2. [^{14}C-tetrahydrofuran] dinotefuran ([F-^{14}C])
3. [^{14}C-guanidine] dinotefuran ([G-^{14}C])

3.1.1 Lot/Batch number

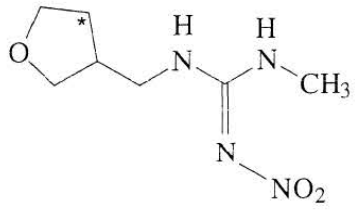
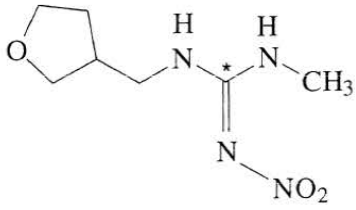
1. 22-00210 and OFU-1265
2. 5091-20 and 5109-34
3. 5091-30 and 5109-28

3.1.2 Specification

Section A6.2-1 Metabolism studies in mammals

Annex Point IIA6.2 Rat

Oral

3.1.2.1	Description	1. White solid 2. Brown powder 3. Brown powder
3.1.2.2	Purity	1. 98.92 % (Batch no. 22-00210) and 99.47% (Batch no. OFU-1265) 2. >98% and 100% 3. >99% and 100% Specific activity: 28.9 and 27.5 µCi/mg ([F-^{14}C]), 42.9 and 39.2 µCi/mg ([G-^{14}C])
3.1.2.3	Stability	Radiolabelled dinotefuran was purified prior to preparation.
3.1.2.4	Radiolabelling	F-^{14}C  G-^{14}C  * indicates position of radiolabel

3.2 Test Animals

3.2.1	Species	Rat
3.2.2	Strain	Crl:CD(SD) BR (male and female), Hla(SD)CVF (male and female bile duct-cannulated rats)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	>4 weeks old, weighing 76 - 282g or 228 - 329g (time-mated female rats) at arrival
3.2.6	Number of animals per group	Other, see Table A6.2.1-1
3.2.7	Control animals	Yes (oral administration)

3.3 Administration/ Exposure

3.3.1	Duration of treatment	Single intravenous or oral dose, or after multiple daily oral doses for 7 or 15 days
3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	Up to 168 hours

Section A6.2-1 **Metabolism studies in mammals**

Annex Point IIA6.2 **Rat**

Oral

3.3.4	Sampling time	Other, see Table A6.2.1-1
3.3.5	Oral or intravenous	
3.3.5.1	Type	Administered orally by gavage or intravenously
3.3.5.2	Concentration	0, 50, 1000 mg/kg
3.3.5.3	Vehicle	0.5% carboxymethyl cellulose (0.5% CMC) for oral administration, or 0.9% saline for intravenous administration
3.3.5.4	Concentration in vehicle	Not applicable
3.3.5.5	Total volume applied	10mL/kg
3.3.5.6	Controls	Vehicle (0.5% CMC) only, oral
3.4	**Study elements**	Preliminary study (PRE): groups P-1 and P-2 Absorption and excretion (AE): groups A, B, C, C-1 and D Pharmacokinetic (PK): groups E, F, F-1 and G Tissue distribution (TD): groups H, I, I-1 and J Biliary excretion (BE): groups K and L Placental transfer (PT): group M Milk transfer (MT): group N Qualitative whole-body autoradiography (WBA): groups O and Q Vehicle Control: group R
3.5	**Samples and observations**	Types and timing of observations and sampling regime for each study element: see Table A6.2.1-1. -PRE and vehicle control: preliminary study and vehicle control animals were housed in individual metabolism cages for the separate collection of urine and feces, and expired air for preliminary study animals. Whole blood/ plasma was collected at termination. After final excreta collection, carcasses retained. -AE: animals were housed in individual metabolism cages for the separate collection of urine and feces. Whole blood/ plasma was collected at termination. After final excreta collection, major organs and tissues, and carcasses were collected. - PK: whole blood was collected via a jugular vein into heparinized tubes and plasma was harvested at each time point. The cellular fraction was discarded. After final collection, carcasses discarded. - TD: whole blood and plasma from all animals was collected at termination. The major tissues and organs, and carcasses were collected from 3 animals at each sacrifice, and then excised, rinsed with saline, blotted dry, weighed, and placed on ice. - BE: bile samples were collected in containers surround by ice. Urine and faeces were collected separately. Whole blood/plasma, major organs and tissues, and carcasses were collected at termination. -PT: 3 animals/time point were sacrificed via exsanguination. Two whole foetuses/animal, selected tissues from 2 further foetuses/animal (placenta, amniotic fluid, blood, brain, heart, liver, lungs, kidneys, carcass), maternal tissues were collected. Tissues were excised, rinsed with saline, blotted dry, weighed, and place on ice.

Section A6.2-1 **Metabolism studies in mammals**
Annex Point IIA6.2 **Rat**
Oral

- MT: milk were collected from 3 animals/time point. Pups were removed from the mothers approximately 4 hours before collection of milk. Animals received a subcutaneous injection of oxytocin before milking to stimulate lactation, and then anesthetized immediately before the start of milk collection and milk (approximately 1 mL) was collected using a specially constructed milking machine. Whole blood and plasma was collected after milk collection. Carcasses discarded.

- WBA: 1 animal/sex/time point was sacrificed. All samples, except blood, were stored at -20°C. Blood was stored at approximately 5 °C until radioanalysis. Plasma from remaining blood was taken by centrifugation.

Cage rinse, wash and wipe in the groups P-1, P-2, A, B, C, C-1, D, K and L were performed and analysed.

All samples collected were weighed.

All tissues and fluids collected were homogenised unless the entire sample was used, sampled, and then analysed, in duplicate if sample size permitted. Specific tissues were digested in NaOH before analysis. Sample weights added to the appropriate scintillant were 0.5ml trapping solution (CO_2), 0.03 or 0.1g (urine), whole sample (faeces, and plasma from groups E, F, F-1 and G), 0.2g (whole blood), 0.2g (plasma from all other groups), 0.02g (bile), 0.1g (milk), 0.5g (cage wash / rinse / wipe), and 0.2 - 0.5g (tissues). Scintillation counts were automatically corrected for counting efficiency.

3.6 Analytics

Radioanalysis procedures were validated in triplicate on fortified control samples of 11 representative tissues and fluids. All sample combustion was performed using Packard model 306 or 307 or Harvey model OX-300 sample oxidisers and analysed using Packard model 1500 or 1900TR liquid scintillation counters for at least 5 minutes or 10^5 accumulated counts.

Pharmacokinetic parameters were calculated by data-fitting to a mono- or bi-exponential equation using Pharma-NCA 1.4b software (Simed, France) which was also used to generate the best model-fitting criteria.

Frozen carcasses for whole body autoradiography were embedded in 2% and 10% carboxymethylcellulose, frozen at -70°C and sectioned at approximately 40µm. Sections from 6 levels in the sagittal plane, including all major organs, tissues and fluids, were collected. Single section sets from each animal were exposed to Molecular Dynamics PhosphorImager screens for approximately 4 days and scanned using a Molecular Dynamics 445 SI PhosphorImager. Autoradiographs were visually analysed to estimate distribution trends of total radioactivity for selected tissues and organs. Additionally, tissues were measured by image analysis.

Pooled tissue, fluid and excreta samples for metabolite identification were analysed for radioactivity and homogeneity either by direct LSC or by combustion and LSC. With the exception of urine and bile samples, pooled samples were sequentially extracted and samples containing large amounts of radioactivity were further processed by solid phase extraction (SPE). Total radioactive content of each fraction was determined by LSC. Samples with sufficient radioactivity for further analysis were subjected to HPLC to determine metabolite profiles by comparison with reference standards. Urine was also analysed by 2-dimensional TLC. Urine and faecal samples were used for isolation / fractionation of radioactive metabolites by sequential

Section A6.2-1 **Metabolism studies in mammals**
Annex Point IIA6.2 **Rat**
Oral

SPE. Selected column eluates were subjected to analysis by LC-MS or LC-MS-MS to obtain spectral data from the isolated metabolites.

4 RESULTS AND DISCUSSION

4.1 Toxic effects and clinical signs

All animals appeared clinically healthy throughout the study, with the exception of following animals.

One animal in group E appeared pale and hypoactive between the 1- and 4-hour blood collections and recovered by the 24-hour collection. In an animal in Group F, there were difficulties in obtaining the 4-hour blood sample, and then the animal died shortly after the collection. The animals in group G appeared lethargic approximately 45 minutes to 1 hour postdose. Animals had recovered by 4 hours postdose.

4.2 Preliminary study (PRE)

See Table A6.2.1-2.

Treatment of the two preliminary groups with F-labelled and G-labelled dinotefuran demonstrated that the distribution of radioactivity between urine, faeces and carcass was similar, and that radioactivity in expired air amounted to 0.01 - 0.05% of administered dose. Therefore, the definitive study was performed with an approximate 1:1 ratio of both radiolabelled forms. Expired air was not collected.

4.3 Absorption and excretion (AE)

See Table A6.2.1-3.

The total mean recovery of radioactivity ranged from 92.7 to 103% of administered dose, with 87.7 to 99.8% recovered in urine, 1.06 to 3.16% recovered in faeces, 0.62 to 6.42% recovered from the cages. The similar, and extensive, recoveries in urine following oral and intravenous administration indicate almost complete absorption of [^{14}C]-dinotefuran from the gastrointestinal tract. Radioactivity was eliminated rapidly with 84.3 to 98.9% of administered single doses excreted in urine within 24 hours. The absorption and route and rate of elimination were not influenced by sex, dose level or dose regimen.

4.4 Pharmacokinetics (PK)

See Table A6.2.1-4.

The mean maximum plasma concentration of [^{14}C]-dinotefuran ranged from 40.8 to 47.4ppm at 0.25 to 0.625 hours after administration of single or repeated low oral doses. A single high oral dose produced C_{max} values of 566 and 471ppm in males and females, respectively, at approximately 2 hours after administration. The elimination half-life ranged from 3.64 to 16.1 hours for single and repeated low oral doses. A single high oral dose produced $T_{1/2}$ values of 13.8 and 15.2 hours in males and females, respectively. AUC values following single and repeated low oral doses were in the range 69.0 to 110ppm.hr, compared with values of 2660 and 2360 ppm.hr in males and females respectively after a single high oral dose. Since the ratios of dose to AUC were comparable for low and high dose levels, absorption and pharmacokinetic characteristics of [^{14}C]- dinotefuran are considered to be linear within the dose range 50 - 1000mg/kg.

Section A6.2-1		**Metabolism studies in mammals**
Annex Point IIA6.2		**Rat**
		Oral
4.5	**Tissue distribution (TD)**	See Table A6.2.1-5. Radioactivity was widely distributed in all tissues examined 0.5 hours after a single oral dose of 50mg/kg. At this time, only the concentrations in the kidneys (79.4ppm), stomach (67.3ppm) and urinary bladder (45.8ppm) were higher than in plasma (40.6ppm) for male. Tissue concentrations declined quickly and at 168 hours after dosing all tissues, with the exception of male skin (0.05ppm), kidneys (0.01ppm) and mammary gland (0.02ppm), were below the limits of detection (0.001ppm). All tissues were below the limits of detection 168 hours after a single intravenous dose. With the exceptions of male and female skin (0.007 and 0.014ppm), female bone (0.004ppm), female intestinal tract (0.003ppm) and female mammary gland (0.018ppm), all tissues were below the limit of detection 168 hours after 15 oral doses of 50mg/kg/day). Radioactivity was widely distributed in most tissues examined following 7 daily oral doses of 50mg/kg and after a single oral dose of 1000mg/kg. In these groups, low concentrations occurred in plasma (0.002 - 0.028ppm) and the highest concentrations occurred in female mammary gland (0.324 - 0.703ppm) and in the skin (0.193 - 0.692ppm). The results indicate that the disposition of radioactivity is similar following single or multiple dosing regimens and after low or high doses.
4.6	**Biliary excretion (BE)**	See Table A6.2.1-6. A small amount of radioactivity (0.58 - 0.88% dose) was detected in bile samples after single doses of either 50 or 1000mg/kg, indicating very limited enterohepatic re-circulation of radioactivity.
4.7	**Placental transfer (PT)**	See Table A6.2.1-7. Radioactivity was rapidly transferred to foetuses and rapidly distributed to the foetal tissues. Maximum foetal concentrations occurred in all tissues examined within 0.5 hours of maternal treatment. Subsequently, radioactivity declined rapidly to low levels within 4 hours. Similar concentrations occurred in maternal and foetal blood suggesting a rapid equilibration and similar tissue distribution in maternal and foetal tissues.
4.8	**Milk transfer (MT)**	See Table A6.2.1-8. Radioactivity was rapidly transferred from maternal blood to the milk of lactating animals at day 12 *post partum*. C_{max} values for maternal plasma and milk were 29.3 and 34.8µg equivalents/g, respectively, 0.5 hours post dose. Concentrations in milk declined rapidly to 6.51µg equivalents/g after 4 hours. Calculation of pharmacokinetic parameters gave an elimination $T_{1/2}$ of 1.39 hours in milk, indicating that within 14 hours of administration the expected concentration of radioactivity would be lower than the limit of detection (0.002ppm).

Section A6.2-1		**Metabolism studies in mammals**	
Annex Point IIA6.2		**Rat**	
		Oral	
4.9	**Qualitative whole-body autoradiography (WBA)**	Whole body autoradiography after single oral doses of 50 or 1000mg/kg were consistent with the results obtained for the tissue distribution groups mentioned previously. Tissue radioactivity derived from [^{14}C]-dinotefuran was widely distributed and highest at the first sampling interval, 0.5 hours and 1.5 hours, for 50 and 1000mg/kg, respectively. Thereafter, levels of radioactivity were declining 1.5 hours after administration of 50mg/kg and 8 hours after administration of 1000mg/kg. Elimination was almost complete after 24 hours (50mg/kg) and 72 hours after 1000mg/kg, no detectable radioactivity was apparent in either sex. The highest levels of radioactivity were generally found in the urine, followed by the gastrointestinal contents. Low levels of radioactivity were detected in the brain and gonads of both sexes after 50 or 1000mg/kg. There were no apparent sex-related or dose-related differences in the overall distribution trends.	
4.10	**Analytics and radioactive components**	Unchanged dinotefuran in urine accounted for 92.5 - 97.2% of total urine radioactivity. A group of urinary metabolites, PHP and its isomers, 446-DO and its isomers, 446-CO, 446-DO-Ac, 446-OH-Ac and 446-OH+COOH, represented 2.08 - 5.95% administered radioactivity. Other minor urine metabolites, UF, FNG and DN, each accounted for no more than 0.53% administered radioactivity. Trace amounts (< 0.1% administered radioactivity) of 9 other metabolites were also detected in urine. Unchanged dinotefuran for 0.29 to 3.57 % of total administrated radiolabel was the major component of faecal radioactivity, but numerous minor metabolites were identified, and 0.01 - 1.75% administered radioactivity represented unidentified polar metabolites. Unchanged dinotefuran for 0.52 to 0.77 % of total administrated radiolabel was the major component of bile radioactivity, and minor metabolites were similar to those found in urine and faeces. Unchanged dinotefuran was the major component of plasma radioactivity (> 80%) with possible metabolites of MNG, 446-DO-Ac and PHPs. Overall, more than 90% of the radioactivity derived from [^{14}C]- dinotefuran is excreted as unchanged parent compound following oral or intravenous administration. Dinotefuran is the major radioactive component in most tissues examined, and less than 10% of dinotefuran is metabolised. There were no apparent differences related to treatment regimen or sex in the metabolic handling of dinotefuran. Initially, enzymatic hydroxylation on the tetrahydrofuran ring occurs to form PHP isomers, followed by further oxidation, reduction and acetylation of PHP to produce possible isomers of 446-CO, 446-DO, PHP-Ac and 446-OH+COOH. Other routes of metabolism involve desmethylation to FNG, nitro-reduction to 446-NH_2 and further hydrolysis to DN and UF. The combination of these reactions at certain stages produced numerous metabolites such as UF-DM, FNG-DN, BCDN, DN-OH and isomers and DN-CO. Trace amounts of MNG, MG and MG-Ac indicate a small degree of cleavage at the C-N bond to yield the furan and guanidine moieties.	X
		Figure 1 shows the proposed metabolic pathway for [^{14}C] dinotefuran in the rat.	X
		See Appendix 05_Chemical Structures for a list of names and structures of metabolites and parent compound.	X

Section A6.2-1 **Metabolism studies in mammals**

Annex Point IIA6.2 **Rat**

Oral

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

EPA-FIFRA, Subdivision F, § 85-1 and JMAFF 59 NohSan no. 4200

No relevant deviations from test guidelines.

Method:

The absorption, distribution, metabolism and elimination of radiolabelled dinotefuran (1:1 mixture of two radiolabel positions) were determined in rats after single intravenous or oral dose, or after multiple daily oral doses for 7 or 15 days. The absorption and excretion, pharmacokinetic profile, biliary elimination, whole-body autoradiography, tissue distribution and metabolite identification investigations were performed at dose levels of 50 and 1000 mg/kg. Other investigations, including milk transfer and placental transfer, were performed at 50 mg/kg only.

5.2 Results and discussion

Radioactivity derived from orally administered [^{14}C]-dinotefuran is rapidly and almost completely absorbed from the GI tract into the general circulation, and is widely distributed throughout the tissues and fluids of the body. Elimination is rapid, predominantly by urinary excretion and almost complete within 7 days after administration. [^{14}C]-dinotefuran is rapidly transferred to the foetus *in utero* and to maternal milk *post partum*, but is rapidly eliminated from them. More than 90% of orally and intravenously administered dinotefuran is eliminated as unchanged parent molecule, which is also the major radioactive component in plasma, milk, bile and most tissues. Unchanged dinotefuran accounted for 92.5 – 97.2% of total urine radioactivity.

The major route of metabolism appeared to be via initial hydroxylation of the furan ring to form isomers of PHP. Further oxidation, reduction, and acetylation of PHP produced additional metabolites. Other routes of metabolism involved desmethylation, nitro reduction, and deamination at various stages, producing numerous additional metabolites. A small degree of cleavage at the C-N bond also appeared to occur.

5.3 Conclusion

It is concluded that the absorption, distribution, metabolism and elimination of [^{14}C]-dinotefuran are unaffected by sex within dosing groups and also no apparent differences among the examined dosing regimens.

5.3.1 Reliability 1

5.3.2 Deficiencies No

Table A6.2.1-1 Treatment schedule and sampling regime

Group	Study element	Dose (mg/kg)	Number doses (1 dose/day)	Route	Position of radiolabel	No. animals (M + F)	Sampling regime
P-1	PRE	50	1[a]	oral	[F-^{14}C] dinotefuran	3 + 3	Expired air: 24-hour intervals for 7 days Urine / faeces: 0 - 6, 6 - 12, 12 - 24, 24 - 48, 48 - 72, 72 - 96, 96 - 120, 120 - 144 and 144 - 168hrs Whole blood / plasma: at termination Cage rinse: 0 - 24hrs Cage wash / wipe: after final excreta collection Termination: after final excreta collection, carcasses retained
P-2		50	1[a]	oral	[G-^{14}C] dinotefuran	3 + 3	
A	AE	50	1	intravenous	1:1 mixture	5 + 5	Urine / faeces: 0 - 6, 6 - 12, 12 - 24, 24 - 48, 48 - 72, 72 - 96, 96 - 120 and 120 - 144, 144 - 168hrs Whole blood / plasma: at termination Cage rinse: 0 - 24hrs Cage wash / wipe: after final excreta collection Termination: after final excreta collection, major organs / tissues + carcasses collected
B		50	1	oral	1:1 mixture	5 + 5	
C		50	14[b]+1	oral	1:1 mixture	5 + 5	
D		1000	1	oral	1:1 mixture	5 + 5	
C-1		50	7	oral	1:1 mixture	5 + 5	Urine / faeces: 0 -24, 24 - 48, 48 - 72, 72 - 96, 96 - 120, 120 - 144 and 144 - 168hrs + 0 - 6, 6 - 12, 12 - 24, 24 - 48, 48 - 72, 72 - 96, 96 - 120, 120 - 144 and 144 - 168hrs Whole blood / plasma: at termination Cage rinse: 24 hr intervals Cage wash / wipe: after final excreta collection Termination: after final excreta collection, major organs / tissues + carcasses collected
E	PK	50	1	oral	1:1 mixture	5 + 5	Blood plasma: pre-dose, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 4, 8, 12, 24, 48 and 72hrs Termination: after final collection, carcasses discarded
F		50	14[b]+1	oral	1:1 mixture	5 + 5	
F-1		50	7	oral	1:1 mixture	5 + 5	
G		1000	1	oral	1:1 mixture	5 + 5	
H	TD	50	1	oral	1:1 mixture	9 + 9	Whole blood and plasma: at termination Termination: 3 animals sacrificed at 0.5, 1.5 and 4hrs after final dose, 30 major organs / tissues + carcasses collected
I		50	14[b]+1	oral	1:1 mixture	9 + 9	
I-1		50	7	oral	1:1 mixture	9 + 9	
J		1000	1	oral	1:1 mixture	9 + 9	Whole blood and plasma: at termination Termination: 3 animals sacrificed at 1.5, 4 and 8hrs after dose, 30 major organs / tissues + carcasses collected

Group	Study element	Dose (mg/kg)	Number doses (1 dose/day)	Route	Position of radiolabel	No. animals (M + F)	Sampling regime
K	BE	50	1	bile duct	1:1 mixture	4 + 4	Urine / faeces: 0 - 6, 6 - 12, 12 - 24, 24 - 48hrs Whole blood / plasma: at termination Bile: 24hrs pre-dose, 0 -6, 6 - 12, 12 - 24 and 24 - 48hrs Cage rinse: 0 - 24hrs Cage wash / wipe: after final excreta collection Termination: after final excreta collection, major organs / tissues + carcasses collected
L		1000	1	bile duct	1:1 mixture	4 + 4	
M	PT	50	1	oral	1:1 mixture	0 + 9c	Whole blood and plasma: at termination Termination: 3 animals/time point sacrificed at 0.5, 1.5 and 4hrs after dose, 2 whole foetuses/animal, selected tissues from 2 further foetuses/animal (placenta, amniotic fluid, blood, brain, heart, liver, lungs, kidneys, carcass), maternal tissues collected
N	MT	50	1	oral	1:1 mixture	0 + 9d	Milk: 0.5, 1.5 and 4hrs Whole blood and plasma: at termination Termination: 3 animals/time point sacrificed at 0.5, 1.5 and 4hrs after milk collection, carcasses discarded
O	WBA	50	1	oral	1:1 mixture	4 + 4	Termination: one animal/sex/time point sacrificed at 0.5, 1.5, 4 and 24hrs post-dose and subjected to qualitative WBA
Q		1000	1	oral	1:1 mixture	4 + 4	Termination: one animal/sex/time point sacrificed at 1.5, 4, 8 and 72hrs post-dose and subjected to qualitative WBA
R	Vehicle control (0.5% CMC)	0	1	oral		2 + 2	Urine / faeces: 0 - 6, 6 - 12, 12 - 24, 24 - 48, 48 - 72, 72 - 96, 96 - 120, 120 - 144 and 144 - 168hrs Whole blood / plasma: at termination Termination: after final excreta collection, carcasses retained

[a] one group treated with [F-^{14}C]-dinotefuran, one group treated with [G-^{14}C]-dinotefuran; [b] 14 days non-radiolabelled treatment followed by one radiolabelled dose; pregnant females on day 10 of gestation at first dose; [c] pregnant females at approximately day 18 of gestation; [d] lactating females ca. 12 days *post partum*; PRE preliminary study; AE absorption and excretion; PK pharmacokinetic; TD tissue distribution; BE biliary excretion; PT placental transfer; MT milk transfer; WBA whole-body autoradiography (qualitative)

Table A6.2.1-2. Total recovery of radioactivity at 168 hours (preliminary study)

Sex	Test substance	% administered dose in:					
		Urine	Faeces	Cage	Expired air	Carcass	Total
Male	[F-^{14}C]-dinotefuran	90.3	1.39	5.73	0.01	0.09	97.5
	[G-^{14}C]-dinotefuran	90.6	4.02	2.97	0.05	0.10	97.8
Female	[F-^{14}C]-dinotefuran	82.0	7.32	8.23	0.02	0.44	98.0
	[G-^{14}C]-dinotefuran	93.5	1.22	2.51	0.05	0.08	97.3

Table A6.2.1-3. Total recovery of radioactivity at 168 hours (main study)

Sex	Dose / frequency / route (mg/kg x no. doses)	% administered dose in:				
		Urine	Feces	Cage	Tissues & carcass	Total
Male	50 x 1 i.v.	96.7	1.06	1.90	0.09	99.7
	50 x 1 oral	98.9	1.66	1.33	0.06	102
	50 x 15 oral	96.8	1.54	2.83	0.06	101
	50 x 7 oral	98.3	1.85	2.42	0.10	103
	1000 x 1 oral	90.1	2.15	2.52	0.10	94.7
Female	50 x 1 i.v.	96.6	1.26	1.42	0.05	99.2
	50 x 1 oral	99.8	1.19	0.62	0.08	102
	50 x 15 oral	89.7	3.16	6.42	0.21	99.3
	50 x 7 oral	95.8	1.53	4.88	0.10	102
	1000 x 1 oral	87.7	2.39	2.67	0.06	92.7

Table A6.2.1-4. Pharmacokinetic parameters calculated for [^{14}C]-dinotefuran

Sex	Dose/ frequency/ route (mg/kg x no. doses)	Mean values for:				
		C_{max} (ppm)	T_{max} (hrs)	$T_{1/2}$ (hrs)	AUC_{0-T} (ppm.hour)	$AUC0_{-\infty}$ (ppm.hour)
Male	50 x 1/ oral	40.8	0.50	3.64	83.3	83.3
	50 x 15/ oral	47.4	0.45	5.65	92.1	92.1
	50 x 7 oral	41.5	0.63	6.28	91.2	91.2
	1000 x 1 oral	566	2.10	13.8	2660	2660
Female	50 x 1 oral	45.6	0.25	7.86	110	110
	50 x 15 oral	42.2	0.38	6.89	76.0	76.0
	50 x 7 oral	43.8	0.31	16.1	69.0	69.2
	1000 x 1 oral	471	2.00	15.2	2360	2370

Table A6.2.1-5: Tissue concentration of [^{14}C]-dinotefuran after 1 oral dose and 7 single daily doses of 50 mg/kg

Sample	Concentration [^{14}C]- dinotefuran (ppm)									
	Single dosing								7-day dosing	
	Males at time (hours)[a]:				Females at time (hours) [a]:				Males at time (hours)[b]:	Females at time (hours) [b]:
	0.5	1.5	4	168	0.5	1.5	4	168	168	168
Adrenals	30.9	12.7	2.04	ND	29.9	12.9	1.81	ND	0.02	0.046
Blood	34.8	13.5	1.92	ND	35	12.5	1.4	ND	0.036	0.038
Bone (femur)	16.2	6.19	1.51	ND	11.9	4.98	1.06	ND	0.031	0.03
Bone marrow	29.9	10.3	1.82	ND	28.6	9.96	1.81	ND	0.023	ND
Eyes	14.8	8.88	2.16	ND	13	8.75	1.49	ND	0.021	0.016
Fat (reproductive)	7.88	2.36	0.44	ND	5.31	1.97	0.3	ND	0.075	0.045
Kidneys	79.4	33.5	3.98	0.01	72.4	28.9	3.9	ND	0.032	0.039
Liver	36.3	13.9	2.11	ND	37.6	12	1.54	ND	0.035	0.035
Lungs	32.9	12.4	1.99	ND	34.5	12.3	1.4	ND	0.024	0.04
Lymph node	29.2	10.4	1.73	ND	28.1	9.61	1.35	ND	0.025	0.024
Muscle (thigh)	31.4	12.4	2.13	ND	29.5	13	1.61	ND	0.006	0.02
Ovaries	N/A	N/A	N/A	N/A	28	10.6	1.16	ND	N/A	0.025
Pancreas	28.1	10.7	1.83	ND	29	10.1	1.5	ND	0.02	0.008
Pituitary gland	31.3	12	1.83	ND	32.6	11.7	1.19	ND	ND	ND
Skin	33.9	15.1	2.27	0.05	29.8	11.6	1.5	ND	0.193	0.054
Spleen	28.1	10.4	1.3	ND	28.1	9.83	1.07	ND	ND	ND
Stomach	67.3	27.5	2.39	ND	171	15.5	5.2	ND	0.022	0.007
Stomach contents	14.9	5	0.08	ND	28.9	1.46	0.67	ND	ND	ND
Testes	18.5	15.8	3.03	ND	N/A	N/A	N/A	N/A	ND	N/A
Thymus	32.6	12.3	1.95	ND	32.6	12	1.37	ND	ND	ND
Thyroid/parathy	24.5	9.99	1.56	ND	27.7	10.7	1.24	ND	ND	0.039
Urinary bladder	45.8	95.3	5.9	ND	32.4	19	6.31	ND	0.049	0.045
Uterus	N/A	N/A	N/A	N/A	33.5	13.1	1.18	ND	N/A	ND
Brain	2.92	2.06	0.32	ND	2.24	1.9	0.41	ND	ND	ND
Carcass	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.342	0.418
Heart	29.6	11.4	1.71	ND	26	11	1.24	ND	ND	ND
Intestinal tract	34.3	24.5	4.1	ND	47.5	18.2	4.15	ND	ND	ND
IT contents	8.28	7.26	4.12	ND	9.78	5.48	4	ND	ND	ND
Mammary gland	N/A	N/A	N/A	N/A	26.7	10.9	1.23	0.02	N/A	0.324
Plasma	40.6	15.9	2.24	ND	41.4	14.6	1.64	ND	0.002	0.003
Prostate	32.3	21.8	4.41	ND	N/A	N/A	N/A	N/A	ND	N/A

[a] group H: 0.5, 1.5 and 4 hours time points, group B: 168 hours time point. [b] group C-1.
N/A: not applicable. ND: not detected.

Table A6.2.1-6. Elimination of [^{14}C]-dinotefuran in bile duct-cannulated rats after one oral dose of 50 or 1000 mg/kg

Sex	Dose (mg/kg)	% administered dose in:					
		Urine	Feces	Bile	Cage	Carcass	Total
Male	50	94.7	1.08	0.62	2.70	0.39	99.5
	1000	85.2	1.33	0.78	7.04	0.38	94.7
Female	50	90.9	1.21	0.58	5.95	0.51	99.2
	1000	90.3	1.34	0.88	3.83	2.43	98.8

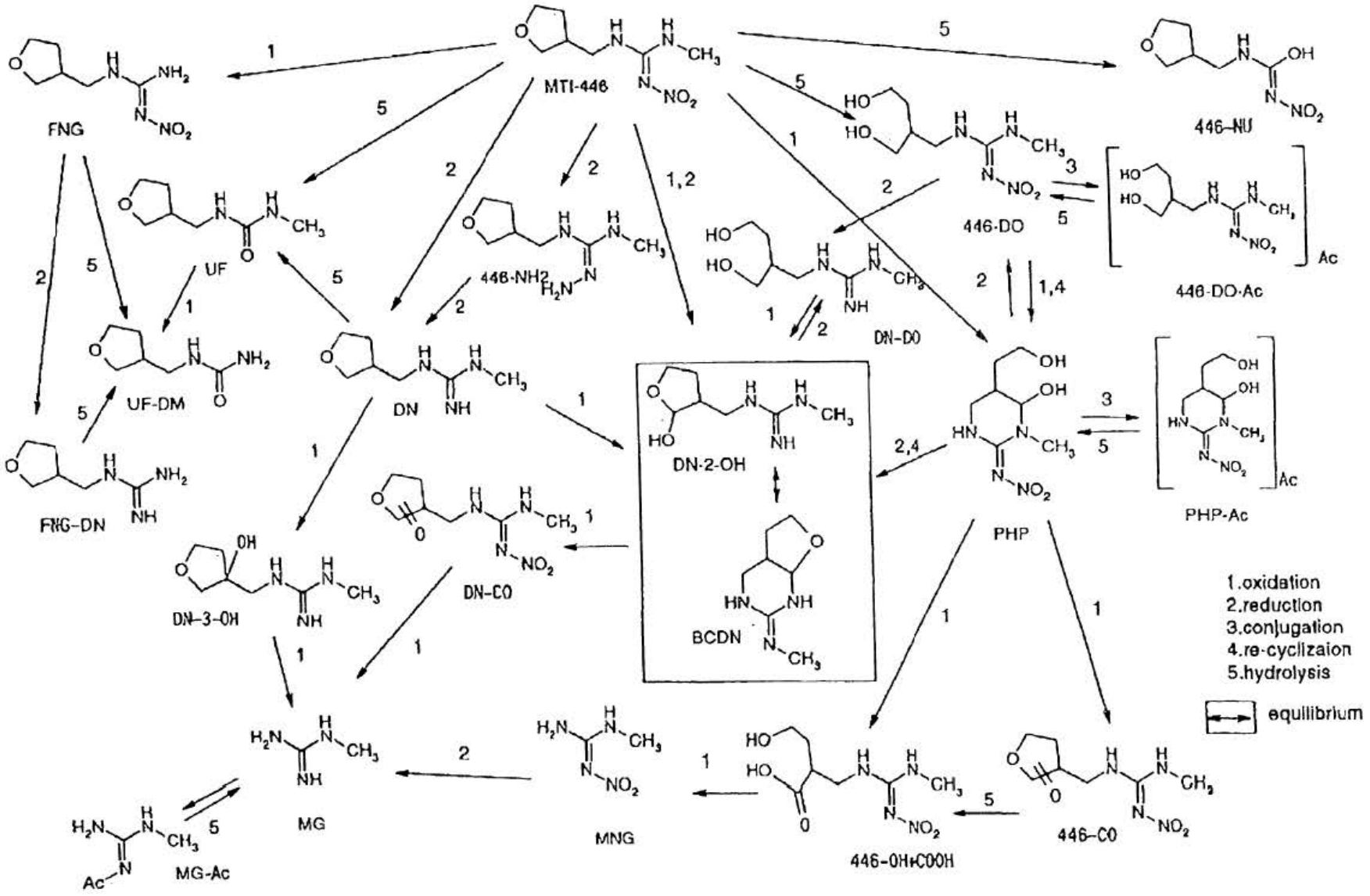
Table A6.2.1-7. Summary of results in pregnant (day 18 pc) females after one oral dose of 50mg/kg [^{14}C]-dinotefuran

Tissue	Concentration of [^{14}C]- dinotefuran (ppm) at (time post-dose):		
	0.5 hours	1.5 hours	4 hours
Foetal blood	23.9	17.3	3.63
Foetal brain	16.6	17.8	4.21
Foetal heart	27.1	18.2	3.74
Foetal kidneys	25.8	15.0	3.90
Foetal liver	18.9	11.8	2.62
Foetal lungs	23.1	16.7	3.41
Foetus	23.5	17.2	3.90
Maternal blood	38.1	18.5	3.99
Maternal plasma	44.3	21.3	4.65

Table A6.2.1-8. Summary of results in lactating (day 12 pp) females after one oral dose of 50 mg/kg [^{14}C]-dinotefuran

Tissue	Concentration of [^{14}C]- dinotefuran (ppm) at (time post-dose):		
	0.5 hours	1.5 hours	4 hours
Blood	24.8	14.3	2.87
Plasma	29.3	17.2	3.48
Milk	34.8	28.1	6.51

Figure 1: Proposed metabolic pathway for [^{14}C] dinotefuran in the rat



Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*26/09/12*
Materials and Methods	*As described by Applicant.*
Results and discussion	*As described by Applicant except,* *Section 4.10 – 'Unchanged dinotefuran for 0.29 to 3.57 % of total administrated radiolabel was the major component of faecal radioactivity' – the value of 3.57% is incorrect and should be 2.69% according to the test report amendment.* *Section 4.10 – The structure of DN-CO in the metabolic pathway figure is incorrect according to the test report amendment. The correct structure is shown in Doc IIA.* *Additional results are reported in Doc IIA.*
Conclusion	*Overall an oral absorption value of 100% will be used in the risk assessment of dinotefuran in the representative product (New Gok 1/Roachdown Gel).*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.2-2 Metabolism studies in mammals
Annex Point IIA6.2 Rat (neonatal rats)
Oral

Official use only

1 REFERENCE

1.1 Reference

[redacted] 2000c, Absorption, distribution, metabolism and excretion of [G-^{14}C]-MTI-446 following administration of a single oral dose to neonatal rats, [redacted], unpublished report no. [redacted] 6648-141, January 28, 2000.

[redacted] 2000d, First amendment to report - Absorption, distribution, metabolism and excretion of [G-^{14}C]-MTI-446 following administration of a single oral dose to neonatal rats, [redacted], unpublished report no. [redacted] 6648-141, March 28, 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

EPA-FIFRA, Subdivision F, § 85-1

JMAFF 59 NohSan no. 4200

2.2 GLP Yes

2.3 Deviations No

3 MATERIALS AND METHODS

3.1 Test material

1. Unlabelled dinotefuran
2. [^{14}C-guanidine] dinotefuran ([G-^{14}C])

3.1.1 Lot/Batch number

1. OFU-1265
2. VB9304

3.1.2 Specification

3.1.2.1 Description

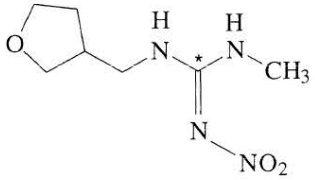
1. White solid

3.1.2.2 Purity

1. 99.47%
2. Radiochemical purity after repurification; 99.6 %, specific activity 27 mCi/mmol

3.1.2.3 Stability: Radiolabelled dinotefuran was purified prior to preparation, Non-radiolabel: expiration date; December 2002

3.1.2.4 Radiolabelling: G-^{14}C



* indicates position of radiolabel

Section A6.2-2 **Metabolism studies in mammals**

Annex Point IIA6.2 **Rat (neonatal rats)**

Oral

3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Crl:CD(SD) BR
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	12 days old pups, weighing 26.5 – 33.0g (time-mated female rats) at dosing
3.2.6	Number of animals per group	25 pups/sex/group and 3 pups/sex/group for whole-body autoradiographic analysis (WBA).
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	Single oral dose
3.3.2	Frequency of exposure	Once
3.3.3	Postexposure period	Up to 4 hours
3.3.4	Sampling time	0.5, 1.5 and 4.0 hours
3.3.5	Oral	
3.3.5.1	Type	Administered orally by gavage
3.3.5.2	Concentration	50 mg/kg
3.3.5.3	Vehicle	0.5% carboxymethyl cellulose (0.5% CMC)
3.3.5.4	Concentration in vehicle	Not applicable
3.3.5.5	Total volume applied	10mL/kg
3.4	**Samples**	Absorption, distribution, metabolism and excretion (group 1): Urine and faeces; -collected together as excreta from shoebox cages. Pup wipe and cage wipe; -following the excreta collection, the pups and the cages were wiped with gauze pads and added to the excreta. Terminal sacrifice and collection; - 5 pups/sex at 0.5 and 1.5 hours and 15 pups/sex at 4 hours were killed by cardiac puncture under halothane anesthesia. - blood samples were collected and transferred into heparinized tubes on ice. An aliquot of each blood sample was retained for radioanalysis. Plasma samples were taken from the residual blood sample by centrifugation. The cellular component was discarded. - liver, kidneys, intestinal tract and contents, stomach, stomach

Section A6.2-2 **Metabolism studies in mammals**
Annex Point IIA6.2 **Rat (neonatal rats)**
Oral

contents and residual carcass were excised, rinsed, blotted dry, weighed and placed on ice.

Excreta samples, blood/plasma samples, tissue samples were pooled to form a single sample for each sex and sampling interval. The weight of each pooled sample was recorded, and stored at -20°C except blood samples which were stored at 5 °C.

Qualitative whole body autoradiography (WBA) (group 2):

-one pup/sex from group 2 was killed at 0.5, 1.5 and 4 hours post-dose by halothane anesthesia and the carcasses prepared for whole body autoradiography.

3.5 Observation

Mortality/ moribundity: twice daily.

Clinical signs: daily.

Body weights: on the day of treatment.

3.6 Analytics

Radioanalysis procedures were validated in triplicate on fortified control samples of representative tissues and fluids. All sample combustion was performed using Packard model 306 or 307 sample oxidisers and analysed using Packard model 1900TR or 2300TR liquid scintillation counters for at least 5 minutes or 10^5 accumulated counts. All tissues and fluids collected were weighed, and homogenised unless the entire sample was used, sampled, and then analysed, in duplicate if sample size permitted. Scintillation counts were automatically corrected for counting efficiency.

Pooled samples of liver, kidneys, stomach, plasma, excreta, and intestinal tract with contents collected 4 hours post-dose from group 1 pups were extracted with methanol:water (8:2). The extracts were analysed by LSC to determine radioactivity recovery and by comparative HPLC to determine metabolite profiles. ^{14}C-UF and ^{14}C-DN were used for cochromatography with test sample extracts.

WBA: frozen carcasses were embedded in chilled carboxymethylcellulose, frozen at -20°C and sectioned at approximately 40µm. Sections from appropriate levels in the sagittal plane, including all major organs, tissues and fluids, were collected. Single section sets from each animal were exposed to Molecular Dynamics PhosphorImaging screens and scanned using a Molecular Dynamics 445 SI PhosphorImager. The autoradiographic standard image data were sampled using AIS software (Imaging Research Inc.), to create a calibrated standard curve. Tissue concentrations in specified tissues, organs and fluids were interpolated from each standard curve as nanoCuries/g and converted to µg equivalents/g based on specific activity.

4 RESULTS AND DISCUSSION

4.1 Toxic effects, clinical signs

There were no treatment-related clinical signs of toxicity in any of the study animals.

Section A6.2-2		**Metabolism studies in mammals**
Annex Point IIA6.2		**Rat (neonatal rats)**
		Oral
4.2	**Recovery of labelled compound**	See Table A6.2.2-1. Radioactivity was rapidly and extensively absorbed, widely distributed and rapidly eliminated from all tissues sampled. The mean total recovery of radioactivity from both sexes of pups was within the range 87.6 - 98.2% for all sampling intervals. The recovery of radioactivity at 4 hours post-dose amounted to 36.3 and 31.8% in excreta, 22.2 and 20.7% in stomach, and 30.6 and 31.8% in the residual carcass, in males and females, respectively. Absorption from the gastrointestinal tract amounted to at least 75% within 4 hours in both sexes. <u>Comparison of neonatal and young adult rats (see Section A6.2-1):</u> See Table A6.2.2-5. Plasma radioactivity in neonatal rats is approximately 52% that of the adult rat at 0.5 hours post-dose, whereas at 4 hours the neonatal plasma concentration is more than 4 times the adult concentration. A similar pattern is evident in liver. The concentrations in the kidneys and stomach contents of neonatal pups are markedly higher than in adults at 4 hours post-dose. These data indicate oral absorption and urinary elimination in neonates are slower than in the adult animal. Possible reasons for rate differences include incomplete development of the gastrointestinal tract and kidneys in the neonate, the absence of the maternal stimulus for neonatal micturition and the presence of food in the gut.
4.3	**Tissue distribution (TD)**	See Table A6_2_02-2. The maximum plasma concentration of radioactivity in both sexes was approximately 21ppm and occurred at 0.5 hours post-dose. Thereafter it declined to approximately 9 ppm at 4 hours post-dose. Concentrations in the stomach and kidneys were higher than in plasma at all sampling intervals, but were comparable in liver and plasma. Concentrations in blood and plasma were also comparable, indicating that radioactivity was not associated with the cellular elements of blood. There were no apparent sex differences in the absorption and elimination of radioactivity. <u>Comparison of neonatal and young adult rats (see section A6.2-1):</u> See Table A6.2.2-6. The gastrointestinal tract and contents of neonatal pups contained between 55 and 60% of administered radioactivity 0.5 hours post-dose, in contrast to 10 to 15% in adults. Concentrations had declined by 4 hours post-dose to 22 - 25% administered dose in pups and to less than 3% in adults. Thus, at 0.5 hours, oral absorption in pups amounted to approximately 50% compared to approximately 90% in adults. The kidneys of pups contained less radioactivity than adult kidneys at 0.5 hours and more after 4 hours. These data also support the contention that oral absorption and urinary elimination in neonatal pups are slower than in the adult animal.

Section A6.2-2		**Metabolism studies in mammals**
Annex Point IIA6.2		**Rat (neonatal rats)**
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4.4	**Analytics and radioactive components**	See Table A6.2.2-3. The recovery of radioactivity from all matrices for metabolite profiling ranged from 96.6 - 100%. In all matrices, [G-^{14}C]-dinotefuran was the major component and accounted for 97.0 - 100% of radioactivity in plasma, kidneys, stomach and excreta. In the liver, [G-^{14}C]-dinotefuran accounted for 61.1% (males) and 66.5% (females) of radioactivity, and in the intestinal tract with contents accounted for 83.3% (males) and 76.3% (females). Metabolism was limited Comparison of neonatal and young adult rats (see section A6.2-1): See Table A6.2.2-7. Metabolite patterns were similar in pups and adult when the metabolite profiles were comparable to those of the young adult, but fewer metabolites were detected in neonates. These data suggest slower metabolism of [G-^{14}C]-dinotefuran in pups, possibly due to incomplete development of neonatal liver function.
4.5	**Qualitative whole-body autoradiography (WBA)**	See Table A6.2.2-4. Autoradiographic data indicated that [G-^{14}C]-dinotefuran-derived radioactivity was widely distributed throughout the organs and tissues in male and female pups. Most of the radioactivity was contained in the stomach and contents, kidneys, urinary bladder and urine. Maximum tissue concentrations occurred either 0.5 or 1.5 hours post-dose. Overall, the concentration of radioactivity in most tissues declined during the 4 hours post-dose. In contrast, the concentrations in the renal medulla and cortex, the urinary bladder and urine increased from 0.5 to 4 hours post-dose indicating predominantly urinary elimination. Elimination from most tissues was incomplete at 4 hours post-dose. Low levels of radioactivity occurred in the brain. There were no apparent sex differences in the tissue distribution of radioactivity.

Section A6.2-2 **Metabolism studies in mammals**

Annex Point IIA6.2 **Rat (neonatal rats)**

Oral

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

EPA-FIFRA, Subdivision F, § 85-1 and JMAFF 59 NohSan no. 4200. No applicable EU guideline.

No relevant deviations from test guidelines.

Method:

The purpose of the study was to determine the absorption, distribution, excretion, and metabolism of radioactivity in 12-day-old rats following a single oral administration of [G-^{14}C]-dinotefuran. These data were compared to corresponding samples from young adult rats (unpublished report no. 6648-136, section A6.2_01).

Excreta, blood and selected tissues were collected from 5 pups/sex at 0.5 or 1.5 hours postdose and from 15 pups/sex at 4 hours postdose. Pooled samples of liver, kidneys, plasma, excreta, and gastrointestinal tract with contents collected 4 hours post-dose were extracted, and then analysed by LSC to determine radioactivity recovery and by comparative HPLC to determine metabolite profiles. One pup/sex/time point was sacrificed for WBA.

Section A6.2-2	**Metabolism studies in mammals**
Annex Point IIA6.2	**Rat (neonatal rats)**
	Oral

5.2 Results and discussion

The mean total recovery of radioactivity, at all time points, was 95.9% for males and 91.9% for females. At 4 hours postdose, 31.8% to 36.3% of the administered radioactivity was detected in excreta, 30.6% to 31.8% was found in residual carcass, and 20.7% to 22.2% was found in stomach contents.

Oral absorption of administered radioactivity was relatively fast; approximately half of the administered radioactivity was absorbed by 0.5 hours postdose and 80% was absorbed by 4 hours postdose. Concentrations of radioactivity in plasma were approximately 21 µg equivalents [G-^{14}C]-dinotefuran/g (ppm) at 0.5 hours postdose, and had declined to approximately 9 ppm by 4 hours postdose. Radioactivity was not distributed in the cellular fraction of the blood. Distribution of radioactivity throughout the body was confirmed by both tissue excision and WBA. High amounts of radioactivity in the bladder indicate that urine was the primary route of elimination of radioactivity. High amounts of radioactivity were also found in the stomach contents and kidneys.

Metabolism of [G-^{14}C]-dinotefuran was minimal, unchanged [G-^{14}C]-dinotefuran accounting for over 97% of the total radioactivity in samples of plasma, kidneys, stomach, and excreta. [G-^{14}C]-dinotefuran represented 61.6% to 66.5% of the radioactivity detected in liver and 76.3% to 83.3% of the administered radioactivity in intestinal tract with contents. These data were compared to corresponding samples for adult rats (unpublished report no. 6648-136, section A6.2_01) and the metabolite profiles were found to be similar.

There were no apparent sex differences in the absorption, distribution, metabolism, and elimination of radioactivity in neonatal rats after a single oral administration of [G-^{14}C]-dinotefuran at 50 mg/kg. Results were similar for neonatal and adult rats. However, absorption, elimination, and metabolism were slower in neonatal than in adults. This may be due to the incomplete development of the gastrointestinal system and kidneys in neonatal rats. Also the testing conditions were different. The neonatal rats were not fasted prior to dosing and had no maternal stimulation to encourage micturition, and thus elimination, while on test.

5.3 Conclusion

Absorption of [G-^{14}C]-dinotefuran from the neonatal gastrointestinal tract is rapid and extensive. It undergoes a wide distribution within the body tissues and is eliminated predominantly in the urine. [G-^{14}C]-dinotefuran undergoes minimal metabolism in the neonatal rat. The absorption, distribution, metabolism and elimination is not affected by the sex of the neonate.

The absorption, distribution, metabolism and elimination of [G-^{14}C]-dinotefuran are similar in neonatal and young adult rats. However, absorption and elimination in the neonate proceed at a slower rate than in young adults, and although metabolite profiles are similar, fewer metabolites are formed in the neonate.

5.3.1 Reliability: 1

5.3.2 Deficiencies: No

Table A6.2.2-1. Total recovery of radioactivity after a single oral dose of 50 mg/kg [G-^{14}C]-dinotefuran

Matrix	Pup sex	Mean % radioactive dose administered recovered at:		
		0.5hr	1.5hr	4hr
Blood	Male	1.84	1.38	0.69
Excreta		3.23	22.1	36.3
Intestinal tract + contents		2.91	2.28	1.80
Kidneys		0.95	0.28	1.15
Liver		1.47	1.05	0.64
Residual carcass		31.5	34.3	30.6
Stomach		1.14	0.45	0.88
Stomach contents		52.2	36.4	22.2
Total		95.3	98.2	94.2
Blood	Female	1.42	1.26	0.53
Excreta		5.75	26.3	31.8
Intestinal tract + contents		3.37	2.25	1.42
Kidneys		0.84	0.89	0.59
Liver		1.43	0.97	0.52
Residual carcass		25.1	26.1	31.8
Stomach		1.93	0.31	0.18
Stomach contents		54.1	36.0	20.7
Total		94.0	94.1	87.6

Table A6.2.2-2. Concentration of radioactivity in tissues after a single oral dose of 50 mg/kg [G-^{14}C]-dinotefuran

Matrix	Pup sex	µg equivalents [G-^{14}C]-dinotefuran /g (ppm) at:		
		0.5hr	1.5hr	4hr
Blood	Male	20.3	18.2	9.06
Intestinal tract + contents		28.8	21.8	14.2
Kidneys		36.1	10.2	45.8
Liver		23.7	15.1	10.2
Plasma		21.3	19.1	9.42
Residual carcass		18.7	20.5	17.9
Stomach		76.1	33.7	59.9
Stomach contents		70.8	48.3	33.4
Blood	Female	17.0	15.4	6.97
Intestinal tract + contents		28.3	20.2	12.3
Kidneys		30.9	34.3	22.6
Liver		21.3	15.1	7.66
Plasma		21.2	18.9	8.71
Residual carcass		15.3	16.1	19.5
Stomach		110	23.2	13.7
Stomach contents		78.1	38.4	32.7

Table A6.2.2-3. Metabolites detected in neonatal rats after a single oral dose of 50 mg/kg [G-^{14}C]-dinotefuran

Matrix	Sex	% radioactivity recovered in fraction:					
		dinotefuran	1	2	3	4	1+2+3+4
Liver	Male	61.1	6.69	4.89	19.5	7.82	38.9
Kidneys		97.1	1.62	0.73	0.53	0.00	2.88
Stomach		99.0	0.21	0.78	0.00	0.00	0.99
ITC[a]		83.3	0.00	15.4	1.40	0.00	16.8
Excreta		98.5	1.49	0.00	0.00	0.00	1.49
Plasma		100	0.00	0.00	0.00	0.00	0.00
Liver	Female	66.5	5.17	3.76	23.0	1.62	33.6
Kidneys		97.0	2.28	0.68	0.00	0.00	2.96
Stomach		100	0.00	0.00	0.00	0.00	0.00
ITC[a]		76.3	21.6	0.00	2.16	0.00	23.8
Excreta		100	0.00	0.00	0.00	0.00	0.00
Plasma		100	0.00	0.00	0.00	0.00	0.00

Fraction 1 (RT: 4.5 - 9.4) - Possible mixture of MNG, 446-DO-Ac and others

Fraction 2 (RT: 12.1 - 19.8) - Possible mixture of PHPs, 446-DO, 446-CO, 446-OH-COOH, UF-DM, PHP-Ac, UF, FNG

Fraction 3 (RT: 38.1 - 44.0) - Possible mixture of MG, MG-Ac, DN-DO, DN-Ohs, DN-CO, BCDN, DN

Fraction 4 (RT: 46.8 - 47.0) - unknown

[a] **intestinal tract with contents**

Table A6.2.2-4. Concentration of radioactivity in tissues after a single oral dose of 50 mg/kg [G-^{14}C]-dinotefuran determined by quantitative whole body autoradiography

Tissue	µg equivalents [G-^{14}C]-dinotefuran in:					
	Males at:			Females at:		
	0.5hr	1.5hr	4hr	0.5hr	1.5hr	4hr
Adrenal gland	33.6	38.1	18.3	30.1	29.1	19.3
Aorta	27.9	23.2	7.86	25.5	16.8	11.9
Axillary lymph node	17.8	18.4	6.45	16.9	NR	8.75
Blood	15.9	17.2	5.70	19.5	13.5	8.10
Bone	12.6	11.8	4.77	14.2	10.4	7.05
Bone marrow	15.0	15.3	5.48	17.9	12.3	7.44
Cerebellum	9.00	15.3	5.66	10.5	12.6	8.53
Cerebrum	7.32	13.9	6.04	8.62	12.0	8.40
Cervical lymph node	22.7	17.9	7.66	16.5	12.6	9.67
Diaphragm	13.7	27.3	9.88	30.7	18.8	13.1
Epididymis	25.6	14.9	6.47	-	-	-
Esophageal contents	58.4	19.2	NR	15.2	29.2	NR
Esophagus	86.7	27.6	NR	29.4	14.4	10.5
Exorbital lacrimal gland	21.5	24.1	9.69	21.8	14.3	12.6
Eye	10.7	14.2	7.14	13.0	11.8	10.7
Fat (abdominal)	5.74	8.58	5.66	7.83	2.82	2.48
Fat (brown)	13.0	13.2	5.59	17.8	11.1	7.82
GI tract	36.8	21.7	12.1	36.3	21.1	23.1
GIT contents	22.7	25.0	16.7	29.7	18.6	28.6
Harderian gland	17.7	21.4	8.71	20.8	16.5	10.7
Inguinal lymph node	11.8	14.2	5.49	13.6	6.63	7.48
Intraorbital lacrimal gland	NR	22.3	8.07	22.6	17.2	7.51
Kidney	71.1	389	172	84.4	61.2	96.9
Liver	31.8	24.7	12.1	33.0	21.5	14.0
Lung	20.7	17.4	7.04	21.7	14.3	9.03
Medulla	6.26	13.2	5.63	7.22	11.4	8.14
Mesenteric lymph node	32.6	23.6	7.13	30.1	19.9	10.3
Muscle	18.7	19.1	7.27	19.5	14.3	9.05
Myocardium	23.3	21.4	7.83	26.5	17.1	10.4
Nasal turbinates	11.6	17.3	6.20	16.9	15.2	8.71
Olfactory lobe	9.01	14.0	5.43	11.8	12.5	8.82
Ovary	-	-	-	28.8	16.3	14.2
Pancreas	28.8	26.2	19.6	34.2	24.7	21.4
Pineal gland	NR	20.6	NR	NR	NR	9.10
Pituitary gland	13.1	14.9	6.75	15.5	12.3	9.96
Popliteal lymph node	18.1	10.8	NR	15.3	10.0	5.33

Tissue	µg equivalents [G-^{14}C]-dinotefuran in:					
	Males at:			Females at:		
	0.5hr	1.5hr	4hr	0.5hr	1.5hr	4hr
Preputial gland	NR	NR	21.5	47.5	NR	NR
Renal cortex	62.5	138	137	78.7	52.3	76.5
Renal medulla	83.5	554	231	94.2	74.6	153
Salivary gland	18.9	21.7	7.82	23.5	16.3	10.2
Seminal vesicle	16.2	24.9	15.7	-	-	-
Skin	18.4	23.0	9.03	18.9	17.4	13.0
Spinal cord	8.32	12.3	4.56	9.86	10.2	6.21
Spleen	26.0	26.1	11.4	29.4	19.4	14.6
Stomach	52.0	40.7	35.1	74.9	48.0	35.5
Stomach contents	222	238	261	517	328	296
Testes	22.6	24.7	19.3	-	-	-
Thymus	18.3	18.0	7.39	19.9	14.3	9.00
Thyroid gland	NR	NR	8.12	21.5	15.9	10.2
Trachea	NR	16.1	NR	21.7	38.4	NR
Urinary bladder	64.9	111	1420	222	653	1240
Urine	45.0	164	291	35.6	166	338
Uterus	-	-	-	19.1	11.0	18.6

NR not represented (tissue not present in section)

Table A6.2.2-5. Comparison of tissue radioactivity in neonatal and adult rats after a single oral dose of 50 mg/kg [^{14}C]-dinotefuran

Tissue	µg equivalents [G-^{14}C]-dinotefuran at:					
	0.5hr		1.5hr		4hr	
	Neonate	Adult[a]	Neonate	Adult[a]	Neonate	Adult[a]
Males						
Blood	20.3	34.8	18.2	13.5	9.06	1.92
Plasma	21.3	40.6	19.1	15.9	9.42	2.24
Kidneys	36.1	79.4	10.2	33.5	45.8	3.98
Liver	23.7	36.3	15.1	13.9	10.2	2.11
Stomach	76.1	67.3	33.7	27.5	59.9	2.39
Stomach contents	70.8	14.9	48.3	5.00	33.4	0.083
Females						
Blood	17.0	35.0	15.4	12.5	6.97	1.40
Plasma	21.2	41.4	18.9	14.6	8.71	1.64
Kidneys	30.9	72.4	34.3	28.9	22.6	3.90
Liver	21.3	37.6	15.1	12.0	7.66	1.54
Stomach	110	171	23.2	15.5	13.7	5.20
Stomach contents	78.1	28.9	38.4	1.46	32.7	0.667

[a] adult data derived from [redacted], 2000a (unpublished report no. [redacted] 6648-136, section A6.2_01)

Table A6.2.2-6. Comparison of tissue radioactivity in neonatal and adult rats after a single oral dose of 50 mg/kg [^{14}C]-dinotefuran

Tissue	% administered dose of radioactivity at:					
	0.5hr		1.5hr		4hr	
	Neonate	Adult[a]	Neonate	Adult[a]	Neonate	Adult[a]
Males						
Blood	1.84	2.70	1.38	1.07	0.69	0.13
Kidneys	0.95	1.55	0.28	0.65	1.15	0.08
Liver	1.47	2.98	1.05	1.10	0.64	0.17
Stomach contents	52.2	2.41	36.4	0.64	22.2	0.02
Stomach	1.14	0.88	0.45	0.34	0.88	0.03
Intestine with contents	2.91	7.62	2.28	4.48	1.80	2.75
Females						
Blood	1.42	2.69	1.26	0.88	0.53	0.09
Kidneys	0.84	1.21	0.89	0.46	0.59	0.06
Liver	1.43	2.41	0.97	0.79	0.52	0.10
Stomach contents	54.1	4.24	36.0	0.19	20.7	0.14
Stomach	1.93	1.88	0.31	0.16	0.18	0.05
Intestine with contents	3.37	8.24	2.25	3.86	1.42	2.17

[a] adult data derived from [redacted], 2000a (unpublished report no. [redacted] 6648-136, section A6.2_01)

Table A6.2.2-7. Comparison of extent of metabolism in neonatal and adult rats after a single oral dose of 50 mg/kg [^{14}C]-dinotefuran

Matrix	Sex	% radioactivity recovered from matrix in:			
		Young adult[b]		Neonate	
		dinotefuran	Total metabolites	dinotefuran	Total metabolites
Plasma	Male	87.1	6.80	100	0.00
Liver		4.66	87.0	61.1	38.9
Kidneys		62.6	21.1	97.1	2.88
Stomach		56.7	43.3	99.0	0.99
Excreta		88.2	8.09	98.5	1.49
ITC[a]		NS	NS	83.3	16.8
Plasma	Female	NA	NA	100	0.00
Liver		0.00	92.9	66.5	33.5
Kidneys		72.2	16.7	97.0	2.96
Stomach		NA	NA	100	0.00
Excreta		93.1	5.23	100	0.00
ITC[a]		NS	NS	76.3	23.7

[a] intestinal tract with contents; NS no sample with contents; NA data not available (low radioactivity in sample).

[b] adult data derived from [redacted], 2000a (unpublished report no. [redacted] 6648-136, section A6.2_01)

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*28/09/12*
Materials and Methods	*As described by the Applicant.*
Results and discussion	*As described by the Applicant.*
Conclusion	*As described by the Applicant.*
Reliability	*As described by the Applicant.*
Acceptability	*Acceptable.*
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.2-3 Percutaneous absorption (*in vivo* test)

Annex Point IIA6.2 **Rat**

Official use only

1 REFERENCE

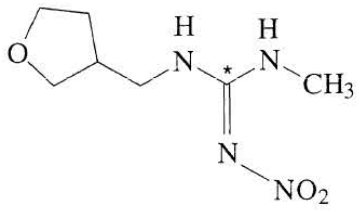
1.1	**Reference**	[redacted] 2006b, Dermal absorption of [^{14}C]MTI-446 formulated as aqueous solution in the rat (*in vivo*), [redacted], unpublished report no. A25975, April 5, 2006.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes EPA OPPTS 870.7600 (1998)
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	1. Unlabelled dinotefuran 2. [^{14}C- guanidine]dinotefuran ([G-^{14}C])
3.1.1	Lot/Batch number	1. 5500310 2. CP-2499
3.1.2	Specification	
3.1.2.1	Description	1. Solid
3.1.2.2	Purity	1. 97.26% 2. Radiochemical purity after purification 98.5% by HPLC.
3.1.2.3	Stability	1. Expiry date: December 31, 2005 2. The radiochemical purity was checked at the time of administration
3.1.2.4	Radiolabelling	G-^{14}C



* indicates position of radiolabel

3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague Dawley (outbred)
3.2.3	Source	[redacted]
3.2.4	Sex	Male

Section A6.2-3 Percutaneous absorption (*in vivo* test)

Annex Point IIA6.2 **Rat**

3.2.5	Age/weight at study initiation	Age: 8 – 9weeks at acclimatization Body weight: approximately 260 g
3.2.6	Number of animals per group	24 males/6 subgroups/group
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	
3.3.1	Administration route	Dermal, non-occlusive
3.3.2	Preparation of test site	Clipped and wiped with acetone under anaesthesia with isofluorane. O-ring (inside area of approximately 25 - 35 cm^2) was used.
3.3.3	Concentration of test substance	3.2, 30 and 302 $\mu g/cm^2$
3.3.4	Specific activity of test substance	156 µCi/mg (5760 kBq/mg)
3.3.5	Volume applied	100µL of application solution was applied to the skin inside the O-ring using a syringe and spread evenly
3.3.6	Size of test site	10 cm^2
3.3.7	Exposure period	24 hours, with interim kills of 4 rats per group after 0.5, 1, 2, 4 and 10 hours exposure.
3.3.8	Sampling time	0.5, 1, 2, 4, 10 and 24 hours after initiation of skin contact
3.3.9	Samples	<u>Urine and faeces:</u> -urine was collected into dry-ice-cooled vessels and volume was measured. -faeces were collected at ambient temperature and weights were measured. <u>Blood, untreated skin, the gastrointestinal tract (GI) and residual carcass:</u> -each sample was removed at necropsy and weighed. <u>Treated skin:</u> - after sacrifice, the application site was washed 4 times with 10g/L of a mild soap solution and once with deionized water using soft cotton swabs. -before and after skin wash, soft cotton swabs were used for removing the remaining test substance and drying the moist skin area. All cotton swabs were extracted with 50 mL methanol for analysis -the treated skin after washing was repeatedly tape-stripped until the stratum corneum was completely removed, and the tapes were retained for analysis. -subsequently, treated skin was carefully removed from animals. <u>Other non-biological samples:</u> - O-ring and cover tape was removed and extracted with 50 mL methnol. - the cages were washed with water/ethanol (1:1 v/v) and the wash retained for analysis.

Section A6.2-3 **Percutaneous absorption (*in vivo* test)**

Annex Point IIA6.2 **Rat**

3.3.10	Observation	Appearance/behavior: on arrival and at each sampling time point. Treated skin sites: examined for signs of irritation prior to the dermal application, at the end of exposure and at sacrifice. Body weights: measured at the start of exposure and at necropsy.
3.3.11	Necropsy	At necropsy, the animals were killed by CO_2 inhalation and exsanguination into heparinized tubes.
3.3.12	Analytics	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), urine (1 mL), cage wash (1 mL), skin wash (0.2-0.5 mL) and O-ring and cover extract (1 mL) were added directly to the scintillant for the measurement of radioactivity. Aliquots of blood (about 0.1 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 scintillant was added prior to LSC. Faeces samples were homogenised with 3 parts of water, and carcass and GI tract with contents were separately homogenized. Aliquots of homogenized samples (0.1-0.2g each) were dissolved in tissue solubilizer and then mixed with scintillant prior to LSC. The skin particles attached to the adhesive tapes were dissolved by 20 mL in tissue solubilizer. Aliquots of 0.5 mL each were mixed with scintillant prior to LSC. The RA in the treated and non-treated skin was determined after digestion with tissue solubilizer. Aliquots of the digested specimens (1 mL) were mixed with scintillant prior to LSC. Aliquots of each skin wash (2 mL for group1 and 0.5 mL for group 2 and 3) were pooled according to dose level and exposure time. The total volume of each skin wash pool was reduced to dryness by rotary evaporation and dissolved in 1 mL water (MilliQ) for measurements of radioactivity. An aliquot of each pool was analyzed by HPLC to check the radiochemical purity of the test substance after exposure. All measurements were performed in triplicate (faeces, GI and carcass) or duplicate (all other samples) for the calculations of extent of systemic absorption, excretion, total recovery, dislodged dose, and tissue residues. All radioactivity counting of specimens were corrected for background by subtraction to give net "dpm" per specimen.

4 RESULTS AND DISCUSSION

4.1	**Toxic effects, clinical signs**	There were no clinical signs of toxicity or abnormal reactions to treatment. The slight weight loss of animals was attributed to the stress and discomfort during the experiment. At dissection urinary calculi were found in the urinary bladder with cloudy urine of one animal of group 3.
4.2	**Dermal irritation**	There was no evidence of skin irritation.
4.3	**Recovery of labelled compound**	See Table A6.2.6-1 Mean total recoveries of radioactivity were high (95.4-99.1%) in all dose groups at all time points and the systemically absorbed dose was rapidly excreted, predominantly in urine.

Section A6.2-3 Percutaneous absorption (*in vivo* test)

Annex Point IIA6.2 **Rat**

4.4 Percutaneous absorption

Low dose group (see Table A6.2.6-2):

After dermal application, systemic absorption was increased with the exposure time from 0.5 hours to 10 hours, but thereafter it remained almost constant. Therefore systemic absorption during a 24-hour exposure was calculated to be 0.81% of the applied dose. The concentration of radioactivity (RA) in blood was below the limit of quantification (LOQ) at all sampling time points. A major part of the applied RA was found in the skin wash which decreased with exposure time from 85% of dose after 0.5 hours to 60% of dose after 10 hours but no further change was observed between 10 hours and 24 hours. Correspondingly, the RA in the stratum corneum (tape strips) increased from 7.4% after 0.5 hours to 35% of the applied dose after 10 hours of exposure. The lower skin layers, corium and subcutis, showed very low amounts of RA (less than 1%) and 0.3-4.8% of the dose was recovered in the cover and O-ring. The systemically absorbed test item was rapidly excreted with the urine and only 0.11 % of the dose remained in the animals 24 hours after the start of exposure. The mean penetration rate for the 24-hour exposure time was calculated to be 0.0011 µg/cm^2/hour.

Middle dose group (See Table A6.2.6-3):

After dermal application, absorption was only 0.48% during an exposure of 24 hours. The highest values were found 1 and 4 hours after start of exposure accounting for 1.43% and 1.15% of the applied dose, respectively, but a high inter-individual variation was observed. The concentrations of RA in blood were below the limit of quantification (LOQ) at most of the sampling time points, and 0.0033 and 0.0134 ppm dinotefuran equivalents were determined after 0.5 and 1 hour, respectively. A major part of the applied RA was recovered in the skin wash which was decreased with exposure time from 90% of dose after 0.5 hours to 69% of dose after 10 hours but no further change was observed between 10 hours and 24 hours. Correspondingly, the RA in the stratum corneum increased with exposure time and reached 26% of the applied dose after an exposure period of 24 hours. The lower skin layers, corium and subcutis, showed very low amounts of RA (less than 0.5%) and in the range 0.05-3.2% of the dose were recovered in the cover and O-ring. The systemically absorbed test item was rapidly excreted with the urine and only 0.09 % of the dose remained in the animals 24 hours after the start of exposure. The mean penetration rate for the 24 hours exposure time was calculated to be 0.0060 µg/cm^2/hour.

Section A6.2-3 **Percutaneous absorption (*in vivo* test)**

Annex Point IIA6.2 **Rat**

		High dose group (see Table A6.2.6-4): After dermal application, absorption was 1.04% during an exposure of 24 hours. Higher values of dermal absorption were found at 1, 4 and 10 hours after start of exposure accounting for 1.41%, 2.10% and 1.44% of the applied dose, respectively, but a high inter-individual variation was observed. The highest concentration in blood was found 1 hour after the start of exposure accounting for 0.1773 ppm dinotefuran equivalents. Thereafter the concentrations decreased rapidly to values close to the limit of quantification. A large amount of RA was found in the skin wash. It decreased only slightly with ongoing exposure time, i.e. 93% of the dose after 0.5 hours to 86% of the dose after 10 hours. After 1 hour of exposure 8% of the applied dose had penetrated into the stratum corneum. This level was almost constant until 24 hours. The lower skin layers, corium and subcutis, showed very low amounts of RA. The systemically absorbed test item was rapidly excreted with the urine and only 0.09 % of the dose was still remaining in the animals 24 hours after start of exposure. The mean penetration rate for the 24 hours exposure time was calculated to be 0.1309 µg/cm^2/hour.
4.5	**Analysis of skin wash**	See Table A6.2.6-5 The skin wash samples of the individual animals were pooled for every sampling time point and dose level, and analyzed by HPLC. In all analyses more than 96% of the RA was found as unchanged dinotefuran. Therefore it was concluded that the applied test substance remained stable at the application site over the whole exposure period.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Guidelines: EPA OPPTS 870.7600 (1998) No relevant deviations from test guidelines. Method: Dermal, non-occlusive exposure with radiolabelled test substance, 3 dose groups of 24 male SD rats, each comprising 6 subgroups of 4 animals, 24 hours exposure, with interim kills of 4 rats per group after 0.5, 1, 2, 4 and 10 hours exposure, mass balance to determine direct and indirect absorption.

Section A6.2-3 Annex Point IIA6.2	Percutaneous absorption (*in vivo* test) Rat	
5.2 Results and discussion	During 24 hours of dermal exposure to [^{14}C]-dinotefuran, 0.81% of the low dose, 0.48% of the middle dose and 1.04% of the high dose was systemically absorbed. The penetration rates were calculated to be 0.0011, 0.0060 and 0.1309 µg/cm^2/hour for the low, middle and high dose level, respectively. Mean total recoveries of radioactivity were high (95.4-99.1%) in all dose groups at all time points and the systemically absorbed dose was rapidly excreted, predominantly in urine. Measured concentrations of [^{14}C]-dinotefuran in blood were below or very close to the limit of quantification for the low and middle dose levels, confirming the low extent of dermal absorption. At all dose levels a major portion of the applied test item could be washed from the application site after the exposure period. In total 61 - 89% of the low dose, 71 - 91% of the middle dose and 86 - 93% of the high dose was washed from the application site at the end of exposure. The applied dinotefuran remained unchanged at the application site during the application period. The dermally applied dinotefuran penetrated rapidly into the stratum corneum, up to 35% for the low dose, 26% for the middle dose and 9% for the high dose, within 24 hours. At all sampling times very low amounts were found in the deeper skin layers (corium and subcutis), not exceeding 0.8, 0.5 and 0.2% of the low, middle and high dose, respectively, indicating the major part of RA in the stratum corneum is not available for systemic absorption during the first 24 hours after the start of exposure.	X
5.3 Conclusion	A large proportion of the dermally applied dinotefuran (60.7 – 93.3%) remained on the surface of the skin and could be dislodged by washing, and was not available for absorption into the skin or systemic circulation. Therefore, systemic absorption of dinotefuran was very low at all time points for all dose levels.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Table A6.2.6-1: Summary of mean mass balance and systemic absorption

Dose level ($\mu g/cm^2$)	Matrix	Radioactivity in % of applied dose:					
		0-0.5 h	0-1 h	0-2 h	0-4 h	0-10 h	0-24 h
3.2	Systemic absorption	0.2	0.2	0.4	0.7	1.2	0.8
	Dislodged dose	89.5	78.7	67.4	68.9	61.5	60.7
	Stratum corneum (outer skin)	7.4	18.1	31.0	29.2	34.8	34.6
	Corium+subcutis (inner skin)	0.1	0.2	0.3	0.5	0.6	0.8
	Total	97.2	97.2	99.1	99.1	97.9	96.9
30	Systemic absorption	0.3	1.4	0.3	1.2	0.8	0.5
	Dislodged dose	90.5	83.8	74.5	77.6	71.9	71.2
	Stratum corneum (outer skin)	5.6	12.5	21.9	18.8	24.7	25.6
	Corium+subcutis (inner skin)	0.2	0.5	0.2	0.1	0.2	0.4
	Total	96.6	98.3	96.9	97.7	97.6	97.7
302	Systemic absorption	0.2	1.4	0.9	2.1	1.4	1.0
	Dislodged dose	93.3	85.8	88.9	86.7	86.1	86.1
	Stratum corneum (outer skin)	3.4	8.0	7.8	6.4	7.8	9.0
	Corium+subcutis (inner skin)	0.1	0.5	0.1	0.2	0.1	0.2
	Total	97.0	95.7	97.7	95.4	95.4	96.3

Table A6.2.6-2: Balance of radioactivity and excretion pattern – low dose group (3.2 µg/cm^2)

Matrix	Radioactivity in % of applied dose:											
	0-0.5 hours		0-1 hours		0-2 hours		0-4 hours		0-10 hours		0-24 hours	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	<0.01	<0.01	0.02	0.01	0.14	0.16	0.45	0.49	0.97	0.50	0.62	0.48
Faeces	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01
Cage wash	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.03	0.01	0.02	0.01	0.05	0.07
TOTAL EXCRETION	0.01	<0.01	0.03	0.02	0.16	0.18	0.48	0.49	1.00	0.51	0.69	0.53
Blood	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Untreated skin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
GI tract + contents	0.02	<0.01	0.02	<0.01	0.03	<0.01	0.03	0.01	0.03	<0.01	0.02	<0.01
Carcass	0.13	0.01	0.15	0.02	0.16	0.03	0.14	0.05	0.12	0.02	0.09	0.02
Sub-total	0.16	0.02	0.18	0.03	0.19	0.04	0.18	0.06	0.15	0.02	0.11	0.02
SYSTEMIC ABSORPTION	0.17	0.02	0.20	0.03	0.35	0.21	0.65	0.55	1.15	0.52	0.81	0.52
Tape Strips (stratum corneum)	7.42	0.31	18.06	6.04	30.97	4.40	29.15	2.88	34.77	5.40	34.63	1.08
Remaining treated skin	0.09	0.03	0.17	0.03	0.31	0.10	0.45	0.37	0.56	0.25	0.77	0.47
APPRICATION SITE	7.51	0.33	18.24	6.07	31.27	4.37	29.60	3.20	35.33	5.28	35.40	1.55
Skin wash	84.70	6.55	78.04	3.04	66.83	3.72	68.60	4.11	59.78	6.09	59.10	5.46
O-ring/covers	4.79	6.08	0.67	1.07	0.60	0.76	0.30	0.30	1.68	3.04	1.62	2.70
DISLODGED DOSE	89.49	0.68	78.71	2.70	67.43	3.40	68.90	3.98	61.46	5.43	60.72	2.99
TOTAL RECOVERY	97.18	0.56	97.15	3.38	99.05	0.98	99.14	0.80	97.93	0.58	96.93	1.63

Table A6.2.6-3: Balance of radioactivity and excretion pattern – middle dose group (30 µg/cm^2)

Matrix	Radioactivity in % of applied dose:											
	0-0.5 hours		0-1 hours		0-2 hours		0-4 hours		0-10 hours		0-24 hours	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	0.04	0.04	0.40	0.42	0.12	0.02	0.88	0.88	0.65	0.66	0.36	0.26
Faeces	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
Cage wash	<0.01	<0.01	0.03	0.05	<0.01	<0.01	0.05	0.05	<0.01	<0.01	<0.01	<0.01
TOTAL EXCRETION	0.04	0.04	0.43	0.46	0.13	0.02	0.93	0.93	0.67	0.67	0.38	0.27
Whole blood	<0.01	<0.01	0.03	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Untreated skin	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
GI tract + contents	0.02	0.01	0.09	0.09	0.02	<0.01	0.03	0.02	0.02	<0.01	0.01	<0.01
Carcass	0.20	0.10	0.87	0.57	0.14	0.01	0.17	0.04	0.10	<0.01	0.08	<0.01
Sub-total	0.24	0.12	1.00	0.67	0.17	0.02	0.21	0.06	0.12	0.01	0.09	<0.01
SYSTEMIC ABSORPTION	0.28	0.16	1.43	1.13	0.30	0.03	1.15	0.98	0.78	0.68	0.48	0.27
Tape Strips (stratum corneum)	5.59	1.62	12.51	4.33	21.87	1.35	18.75	3.43	24.73	5.40	25.62	3.34
Remaining treated skin	0.15	0.12	0.49	0.44	0.20	0.07	0.14	0.03	0.16	0.05	0.40	0.22
APPRICATION SITE	5.74	1.70	13.00	4.63	22.06	1.39	18.89	3.43	24.89	5.35	26.02	3.31
Skin wash	90.49	3.23	83.62	5.58	71.30	1.75	74.44	4.48	69.12	3.75	70.07	4.67
O-ring/covers	0.05	0.02	0.22	0.27	3.22	2.70	3.17	6.11	2.82	2.81	1.12	1.95
DISLODGED DOSE	90.54	3.23	83.84	5.69	74.51	1.02	77.61	2.90	71.94	5.58	71.19	3.72
TOTAL RECOVERY	96.55	3.37	98.27	0.96	96.88	0.83	97.65	1.34	97.62	1.22	97.69	1.04

Table A6.2.6-4 Balance of radioactivity and excretion pattern – high dose group (302 µg/cm²)

Matrix	Radioactivity in % of applied dose:											
	0-0.5 hours		0-1 hours		0-2 hours		0-4 hours		0-10 hours		0-24 hours	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	0.02	0.03	0.43	0.25	0.54	0.58	1.76	1.49	1.31	1.01	0.89	0.55
Faeces	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.02	<0.01
Cage wash	<0.01	<0.01	0.01	<0.01	0.02	0.02	0.11	0.08	0.01	<0.01	0.03	0.01
TOTAL EXCRETION	0.02	0.03	0.45	0.25	0.57	0.60	1.87	1.57	1.33	1.02	0.94	0.56
Blood	<0.01	<0.01	0.03	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Untreated skin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
GI tract + contents	0.02	0.01	0.10	0.06	0.05	0.04	0.05	0.03	0.02	<0.01	0.01	<0.01
Carcass	0.15	0.07	0.83	0.35	0.26	0.13	0.18	0.05	0.08	0.01	0.07	<0.01
Sub-total	0.17	0.09	0.97	0.42	0.31	0.17	0.24	0.08	0.11	0.02	0.09	<0.01
SYSTEMIC ABSORPTION	0.19	0.12	1.41	0.67	0.88	0.77	2.10	1.64	1.44	1.04	1.04	0.56
Tape Strips (stratum corneum)	3.37	0.54	8.01	1.91	7.82	2.09	6.43	1.17	7.82	0.77	9.00	0.95
Remaining treated skin	0.10	0.07	0.50	0.24	0.09	0.05	0.20	0.06	0.10	0.06	0.24	0.13
APPRICATION SITE	3.47	0.58	8.51	1.69	7.92	2.14	6.63	1.20	7.92	0.82	9.24	0.89
Skin wash	93.30	0.98	85.75	1.55	88.86	2.65	86.63	2.28	85.93	1.08	85.93	0.63
O-ring/covers	0.03	<0.01	0.05	0.04	0.06	0.04	0.04	<0.01	0.12	0.15	0.12	0.06
DISLODGED DOSE	93.32	0.99	85.80	1.53	88.92	2.64	86.68	2.28	86.05	1.08	86.06	0.66
TOTAL RECOVERY	96.98	1.29	95.72	1.09	97.72	1.25	95.41	0.27	95.42	0.50	96.33	0.76

Table A6.2.6-5 Analysis of skin wash

Dose level (µg/cm²)	Unchanged dinotefuran (%) during exposure time (hours)					
	0.5	1	2	4	10	24
3.2	97.4	96.5	96.9	97.3	97.2	97.3
30	97.0	97.5	96.9	96.4	96.9	97.3
302	97.0	96.8	97.4	97.1	97.8	96.1

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*14.08.12*
Materials and Methods	*As described by the Applicant. In addition, the stratum corneum was removed by gluing an adhesive surgical tape to the application site (Section 3.3.9). This process was repeated 1 or 2 times.*
Results and discussion	*As described by the Applicant with the following exception:* *5.2 The amount of radiolabel in the deeper skin layers of the high dose group did not exceed 0.25%.*
Conclusion	*UK CA considers the overall dermal absorption values for each dose level at 24h to be as follows:* *3.2 µg/cm^2 (equivalent to 0.03% a.s.) – 36%* *((Excreta + cage wash + carcass - 0.81%) + (corium + subcutis – 0.77%) + (stratum corneum – 34.63%))* *30 µg/cm^2 (equivalent to 0.3% a.s.) – 27%* *((Excreta + cage wash + carcass - 0.48%) + (corium + subcutis – 0.4%) + (stratum corneum – 25.62%)))* *302 µg/cm^2 (equivalent to 3% a.s.) – 10%* *((Excreta + cage wash + carcass – 1.04%) + (corium + subcutis – 0.24%) + stratum corneum – 9.0%))* *N.B. These values are applicable to aqueous solutions of 0.03, 0.3 and 3% dinotefuran.* *Although the representative product contains 2% dinotefuran which is within the range of concentrations tested, as an emulsion-type formulation, it also contains co-formulants that may enhance absorption. Therefore for the risk assessment of* ***the representative product a default value of 75%*** *will be used.*
Reliability	*2*
Acceptability	*Acceptable study but not applicable to the representaive product.*
Remarks	*The study did not include a post-exposure sampling period.*
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.3.1-1 **Repeated dose toxicity**

Annex Point IIA6.3 **Oral**

Rat, 28-day

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1997a, 4-week dietary toxicity study with MTI-446 in rats, [redacted], unpublished report no. [redacted] 6648-125, December 15, 1997.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD guideline no. 407 (1995) which is equivalent to 92/69/EEC (method B.7)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Deviations from 92/69/EEC - none; special functional observations and motor activity assessment required by OECD 407 not performed.
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	22-00110
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	96.5% + 2.0% water, purity of dried material 99.1%
3.1.2.3	Stability	Expiration date: May 14, 2001
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Crl:CD® [SD]BR VAF/Plus ®
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	About 7 weeks old, weighing 241-287 g for males and 137-176 g for females
3.2.6	Number of animals per group	5 male and 5 female rats per group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral

Section A6.3.1-1		**Repeated dose toxicity**
Annex Point IIA6.3		**Oral**
		Rat, 28-day
3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	7 days per week
3.3.3	Postexposure period	None
3.3.4	Oral	
3.3.4.1	Type	Administered in the diet by direct admixture.
3.3.4.2	Concentration	Nominal in diet: 0, 5000, 25000 and 50000 ppm in the diet Mean achieved dose levels: 0, 390, 1814 and 3720 mg/kg bw/day in males 0, 450, 2183 and 4222 mg/kg bw/day in females
3.3.4.3	Vehicle	No vehicle, added to basal diet
3.3.4.4	Concentration in vehicle	Not applicable
3.3.4.5	Total volume applied	Not applicable
3.3.4.6	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, weekly
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, weekly
3.4.3	Food consumption	Yes, weekly
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	Yes: - Number of animals: all animals - Time points: end of study - Parameters: Red blood cell count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, prothrombin time, activated partial thromboplastin time, white blood cell count, blood cell morphology, differential blood cell count.
3.4.7	Clinical Chemistry	Yes: - Number of animals: all animals - Time points: end of study - Parameters: Glucose, urea nitrogen, creatinine, total protein, albumin, globulin (calculated), albumin/globulin ratio, total bilirubinm cholesterol, aspartate aminotransferase, alanine aminotransferase,

Section A6.3.1-1 **Repeated dose toxicity**

Annex Point IIA6.3 **Oral**

Rat, 28-day

		alkaline phosphatase, gamma-glutamyl transferase, calcium, inorganic phosphorus, sodium, potassium, chloride.	
3.4.8	Urinalysis	Yes: - Number of animals: all animals - Time points: end of study - Parameters: Volume, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, specific gravity, appearance, microscopic examiniation of urine sediment.	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes: The following organs were weighed; paired organs were weighed separately: Adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus, thyroids with parathyroid.	
3.5.2	Gross and histopathology	Yes: - All preserved tissues from animals treated at 0 or 50000ppm were examined by light microscopy. Gross lesions were also examined from animals in the groups treated at 5000 or 25000ppm.	
3.5.3	Statistics	Where appropriate, data were analysed statistically at the 5% level by one-way ANOVA on homogeneous or transformed data followed by Dunnett's multiple comparison t-test where ANOVA proved significant. Levene's test was used to evaluate the homogeneity of variance.	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	No effects	
4.1.2	Mortality	No mortalities at any dose level	
4.2	**Body weight gain**	- Females treated at 50000ppm lost weight during the first week of treatment, but subsequently gained weight. - Males and females treated at 50000ppm and males at 25000ppm showed significant ($p < 0.05$) reductions in overall weight gain of 46.9, 21.5 and 49.3%, respectively. - Although the female group treated at 25000ppm showed a 25.4% reduction in overall weight gain, the difference from the controls was not statistically significant. - Reduced weight gain in both sexes at 50000ppm and males at 25000ppm was associated with reduced overall food consumption of 10.4 to 17.6%. See Table A6.3.1.1-1	X1
4.3	**Food consumption and compound intake**	- The food consumption of these groups was significantly lower than the controls during the first week of treatment and also during the second week in males at 50000ppm. - Therefore, reduced weight gain is considered to be due to reduced diet palatability and not to be an adverse effect of dinotefuran. - There were no treatment-related effects on weight gain and food	X2

Section A6.3.1-1 — **Repeated dose toxicity**
Annex Point IIA6.3 — **Oral**
Rat, 28-day

		consumption at 5000ppm. See Table A6.3.1.1-1	
4.4	**Ophtalmoscopic examination**	Not applicable	
4.5	**Blood analysis**		
4.5.1	Haematology	No effects on any parameter at any dose level.	
4.5.2	Clinical chemistry	Treatment-related effects on serum clinical chemistry were confined to the male groups treated at 25000 and 50000ppm: - The group mean glucose concentration at 50000ppm was slightly, but significantly ($p < 0.05$) reduced, and the group mean cholesterol concentrations at 25000ppm and 50000ppm were significantly ($p < 0.05$) increased relative to the controls. - However, these minor differences from the controls were not apparent in the females at any dose level and were not associated with overt histopathological alterations at 50000ppm. - Therefore, they are considered not to be adverse effects but a manifestation of minor alterations in carbohydrate and lipid metabolism associated with reduced body weight gain. - There were no other treatment-related effects on serum clinical chemistry parameters at any dose level.	X3
4.5.3	Urinalysis	No effects on any parameter at any dose level.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	- There were no effects on organ weights and ratios considered to be directly related to treatment with dinotefuran. - A number of absolute organ weights were slightly, but significantly ($p < 0.05$), lower in males at 50000ppm (spleen, heart, kidneys, liver) and females at 50000ppm (heart and ovaries). - However, these differences were not apparent in the corresponding body weight ratios. - Therefore, the differences in absolute organ weights are considered to be a consequence of the lower terminal body weights	
4.6.2	Gross and histopathology	- There were no treatment-related macroscopic lesions at any dose level and no treatment-related microscopic findings at 50000ppm. - The nature and incidences of all histopathological alterations were comparable in the control and treated groups.	
4.7	**Other**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Guidelines: OECD guideline no. 407 (1995), which is equivalent to 92/69/EEC (method B.7) Deviations from 92/69/EEC - none; special functional observations	

Section A6.3.1-1	Repeated dose toxicity		
Annex Point IIA6.3	Oral		
	Rat, 28-day		
		and motor activity assessment required by OECD 407 not performed. Method: Four groups of 5 male and 5 female rats were treated orally for at least 4 weeks by admixture in the diet at constant nominal concentrations of 0, 5000, 25000 and 50000ppm. Mean achieved dose levels were 0, 390, 1814 and 3720mg/kg bw/day (males) and 0, 450, 2183 and 4222mg/kg bw/day (females). Morbidity/mortality checks were performed twice daily and a detailed clinical examination was performed weekly. Body weights and food consumption were recorded weekly. Hematology, serum clinical chemistry and urine analyses were performed in week 5. All animals were subjected to necropsy and *post mortem* examination of major organs and tissues. Selected organs were weighed and all major organs and tissues from animals treated at 0 or 50000ppm were examined by light microscopy. Gross lesions were also examined from animals in the groups treated at 5000 or 25000ppm.	
5.2	**Results and discussion**	No target organs were identified in either sex at the highest dose level employed. The no-observed-effect-level (NOEL) for all effects was established as 5000ppm diet in males and 25000ppm in females, equivalent to dose levels of 390mg/kg bw/day (males) and 2183mg/kg bw/day (females), based on the occurrence of reduced weight gain and increased serum cholesterol in males at 25000ppm, reduced weight gain in females at 50000ppm and reduced serum glucose concentration in males at 50000ppm.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	Not determined	
5.3.2	NO(A)EL	A no-observed-adverse-effect-level (NOAEL) was established as 50000ppm, equivalent to a dose level of 3720mg/kg bw/day (males) and 4222mg/kg bw/day (females), based on the absence of toxicologically significant effects at 50000ppm.	X4
5.3.3	Reliability	1	
5.3.4	Deficiencies	Yes, special functional observations and motor activity assessment required by OECD 407 not performed	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*10/10/12*
Materials and Methods	*As described by Applicant.*
Results and discussion	*X1 Section 4.2 The statistically significant reductions in overall body weight gain were 21.5% in males exposed to 25000 ppm and 46.9% and 49.3% in males and females exposed to 50000 ppm dinotefuran respectively compared to controls.* *X2 Section 4.3 Reduced palatability of the test diet may have contributed to the reduced bodyweight gain, but in the opinion of the RMS this should conservatively be regarded as a treatment-related adverse effect..* *X3 Section 4.5.2 The male group mean glucose concentration at 50000 ppm was 14.4% lower than controls. The male group mean cholesterol concentrations at 25000 and 50000 ppm were 25 and 42.9% greater than controls respectively.*
Conclusion	*X4 Section 5.3.2 As noted above the RMS considers the reduced bodyweight gain and food consumption at to be adverse, and therefore a LOAEL of 25000 ppm and a NOAEL of 5000 ppm are established in this study.*
Reliability	*As described by Applicant.*
Acceptability	*Acceptable.*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.3.1.1-1 Summary of body weight gain and food consumption

Sex	Dose level (ppm)	Overall group mean weight gain (g)	Group mean terminal body weight (g)	Overall mean food consumption (g/wk)a
Male	0	177	404.0	193
	5000	173	403.1	193
	25000	139*	378.6	173
	50000	94*	335.5*	159
Female	0	67	199.2	119
	5000	53	197.6	120
	25000	50	188.2	111
	50000	34*	172.6	99

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

Annex Point IIA6.3 **Mouse**

Oral, 28-day

Official use only

	1 REFERENCE	
1.1	**Reference**	[redacted], 1997b, 4-week dietary toxicity study with MTI-446 in mice, [redacted], unpublished report no. [redacted] 6648-124, December 15, 1997.
1.2	**Data protection**	Yes
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD guideline no. 407 (1995) which is equivalent to 92/69/EEC (method B.7)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Deviations from 92/69/EEC - none; special functional observations and motor activity assessment required by OECD 407 not performed.
	3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	22-00110
3.1.2	Specification	
3.1.2.1	Description	White powder
3.1.2.2	Purity	96.5% + 2.0% water, purity of dried material 99.1%
3.1.2.3	Stability	Expiration date: May 14, 2001
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Mouse
3.2.2	Strain	Crl:CD1®[ICR]BR VAF/Plus®
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	About 7 weeks old, weighing 18.5 – 36.2 g for males and 22.4 – 28.9 g for females
3.2.6	Number of animals per group	10 male and 10 female mice per group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral

Section A6.3.1-2		**Repeated dose toxicity**
Annex Point IIA6.3		**Mouse**
		Oral, 28-day
3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	7 days per week
3.3.3	Post exposure period	None
3.3.4	Oral	
3.3.4.1	Type	Administered in the diet by direct admixture.
3.3.4.2	Concentration	Nominal in food: 0, 5000, 25000 and 50000 ppm in the diet Mean achieved dose levels: 0, 901, 4612 and 10303 mg/kg bw/day in males 0, 1043, 5359 and 12289 mg/kg bw/day in females
3.3.4.3	Vehicle	No vehicle, added to basal diet
3.3.4.4	Concentration in vehicle	Not applicable
3.3.4.5	Total volume applied	Not applicable
3.3.4.6	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, weekly
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, weekly
3.4.3	Food consumption	Yes, weekly
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	Yes: - Number of animals: all animals - Time points: end of study - Parameters: Red blood cell count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, white blood cell count, blood cell morphology, differential blood cell count.
3.4.7	Clinical Chemistry	Yes: - Number of animals: all animals - Time points: end of study - Parameters: Glucose, urea nitrogen, creatinine, total protein, albumin, globulin (calculated), albumin/globulin ratio, cholesterol, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase.

Section A6.3.1-2 — **Repeated dose toxicity**
Annex Point IIA6.3 — **Mouse**
Oral, 28-day

3.4.8	Urinalysis	No	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes: The following organs were weighed, paired organs were weighed separately: Adrenals, brain, epididymides, heart, kidneys, liver with gallbladder, ovaries, pituitary, spleen, testes, thymus, thyroids with parathyroid.	
3.5.2	Gross and histopathology	Yes: - All preserved tissues from animals treated at 0 or 50000ppm were examined by light microscopy. Gross lesions were also examined from animals in the groups treated at 5000 or 25000ppm.	
3.5.3	Statistics	Where appropriate, data were analysed statistically at the 5% level by one-way ANOVA on homogeneous or transformed data followed by Dunnett's multiple comparison t-test where ANOVA proved significant. Levene's test was used to evaluate the homogeneity of variance.	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	No effects	
4.1.2	Mortality	No mortality at any dose level	
4.2	**Body weight gain**	Both sexes treated at 25000 or 50000ppm showed a dose-related depression of overall body weight gain of between 46.2 and 85.5%. Males and females treated at 50000ppm lost weight during the first week of treatment but subsequently gained weight at a comparable rate to the controls. See Table A6.3.1.2-1	
4.3	**Food consumption and compound intake**	Marked food spillage by the groups treated at 25000 or 50000ppm suggested these test diets were less palatable than untreated diet, and precluded a valid assessment of food consumption for most time points. Therefore, initial weight loss and/or overall depressed weight gain at 25000 and 50000ppm is considered to be a reflection of reduced palatability of the diets. At 5000ppm, there was no evidence of an effect on food consumption.	X1
4.4	**Ophthalmoscopic examination**	Not applicable	
4.5	**Blood analysis**		
4.5.1	Haematology	There were no treatment-related effects on the hematological profile at any dose level.	
4.5.2	Clinical chemistry	Treatment-related effects on serum chemistry were confined to the male group treated at 50000ppm and comprised slightly, but significantly ($p < 0.05$), higher total serum protein and albumin concentrations. These minor differences from the controls were not associated with overt histopathological changes and are considered not to be adverse effects. All other serum chemistry values were	