DINOTEFURAN

First draft prepared by
Rudolf Pfei1[1] and Maria Tasheva[2]

[1] Toxicology of Pesticides and Biocides, Federal Institute for Risk Assessment, Berlin, Germany
[2] Associate Professor Toxicologist, Sofia, Bulgaria

Explanation....167
Evaluation for acceptable daily intake....168
1. Biochemical aspects....168
1.1 Absorption, distribution and excretion....168
1.2 Biotransformation....177
2. Toxicological studies....180
2.1 Acute toxicity....180
(a) Lethal doses....180
(b) Dermal and ocular irritation and dermal sensitization....185
2.2 Short-term studies of toxicity....188
(a) Oral administration....188
(b) Dermal application....200
(c) Exposure by inhalation....201
2.3 Long-term studies of toxicity and carcinogenicity....203
2.4 Genotoxicity....207
2.5 Reproductive and developmental toxicity....210
(a) Multigeneration studies....210
(b) Developmental toxicity....219
2.6 Special studies....225
(a) Neurotoxicity....225
(b) Developmental neurotoxicity and immunotoxicity....229
(c) Immunotoxicity....236
(d) Transfer into milk of lactating rats....240
3. Observations in humans....241
Comments....241
Toxicological evaluation....243
References....246
Appendix 1: Dinotefuran and metabolite key....249

Explanation

Dinotefuran is the International Organization for Standardization (ISO)–approved common name for (*EZ*)-(*RS*)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service No. 165252-70-0), a novel neonicotinic insecticide used in various crops. Dinotefuran acts as an agonist at the insect nicotinic acetylcholine receptor and exhibits broad insecticidal activity via ingestion and contact.

Dinotefuran has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues.

All critical studies were certified as complying with good laboratory practice (GLP).

Evaluation for acceptable daily intake

Unless otherwise stated, the studies evaluated in this monograph were performed by laboratories that were certified for GLP and complied with the relevant Organisation for Economic Co-operation and Development (OECD) test guideline(s) or similar guidelines of the European Union (EU) or United States Environmental Protection Agency (USEPA). As these guidelines specify the clinical pathology tests normally performed and the tissues normally examined, only significant exceptions to these guidelines are reported here, to avoid repetitive listing of study parameters.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption, distribution, metabolism and elimination (ADME) of ^{14}C-labelled dinotefuran (purity of non-radiolabelled test material: 98.92% and 99.47%; radiochemical purity: tetrahydrofuran-labelled > 98%, guanidine-labelled > 99%) were investigated in male and female Crl:CD(SD) BR rats and Hla(SD)CVF rats (bile duct–cannulated animals) (Cheng, 2000a,b, 2001). The study was conducted according to Japanese Ministry of Agriculture, Forestry, and Fisheries (JMAFF) test guideline 59 NohSan No. 4200. The test substances were suspended in 0.5% carboxymethylcellulose for oral administration or in 0.9% saline for intravenous administration. The animals were treated at a constant dose volume of 10 ml/kg body weight (bw), according to the schedule in Table 1.

In two preliminary groups treated with (^{14}C-tetrahydrofuran)-labelled (group P-1) or (^{14}C-guanidine)-labelled (group P-2) dinotefuran, the distribution of radioactivity between urine, faeces and carcass was similar, and radioactivity in expired air amounted to only 0.05% of the administered dose. Therefore, the definitive studies were performed with an approximate 1:1 ratio of both radiolabelled forms. Expired air was not collected in subsequent experiments. The schedule for sample collection and the terminal procedures are shown in Table 2.

There were no treatment-related clinical signs of toxicity, although one animal in group F died shortly after a difficult 4-hour blood sample.

Treatment of the two preliminary groups with tetrahydrofuran-labelled and guanidine-labelled dinotefuran demonstrated that the distribution of radioactivity between urine, faeces and carcass was similar and that radioactivity in expired air amounted to only 0.01–0.05% of the administered dose (Table 3).

In the main study, the total mean recovery of radioactivity ranged from 92.7% to 103% of the administered dose, with 87.7–99.8% recovered in urine, 1.06–3.16% recovered in faeces and 0.62–6.42% recovered from the cages. The similar, and extensive, recoveries in urine following oral and intravenous administration indicate almost complete absorption of ^{14}C-labelled dinotefuran from the gastrointestinal tract. Radioactivity was eliminated rapidly, with 84.3–98.9% 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 (Table 4).

In bile duct–cannulated rats (groups K and L), a small amount of radioactivity (0.58–0.88% of the administered dose) was detected in bile samples after single doses of either 50 or 1000 mg/kg bw, indicating very limited enterohepatic recirculation of radioactivity (Table 5).

The mean maximum plasma concentration (C_{max}) of [^{14}C]dinotefuran ranged from 40.8 to 47.4 parts per million (ppm) at 0.25–0.625 hour after administration of single or repeated low oral doses (Table 6). A single high oral dose produced C_{max} values of 566 and 471 ppm 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, respectively. A single high oral dose produced half-life values of 13.8 and 15.2 hours in males and females, respectively. Area under the plasma concentration–time curve (AUC) values following single and repeated low oral doses were in the range 69.0–110 ppm·h, compared with values of 2660 and 2360 ppm·h in males and females, respectively, after a single high oral dose. As the ratios of dose to AUC were comparable for the low

and high dose levels, absorption and pharmacokinetic characteristics of [^{14}C]dinotefuran were considered to be linear within the dose range 50–1000 mg/kg bw.

Table 1. Treatment schedule in the ADME study in rats

Group	Dose (mg/kg bw)	No. of doses	No. of males/females	Study element
P-1	50	1	3/3	Oral low dose—[F-^{14}C]dinotefuran[a]
P-2	50	1	3/3	Oral low dose—[G-^{14}C]dinotefuran[a]
A	50	1	5/5	Intravenous low dose
B	50	1	5/5	Oral low dose
C	50	14 + 1	5/5	Oral repeated low dose[b]
C-1	50	7	5/5	Oral repeated low dose[c]
D	1000	1	5/5	Oral high dose
E	50	1	5/5	PK—oral low dose
F	50	14 + 1	5/5	PK—oral repeated low dose[b]
F-1	50	7	5/5	PK—oral repeated low dose[c]
G	1000	1	5/5	PK—oral high dose
H	50	1	9/9	TD—oral low dose
I	50	14 + 1	9/9	TD—oral repeated low dose[b]
I-1	50	7	9/9	TD—oral repeated low dose[c]
J	1000	1	9/9	TD—oral high dose
K	50	1	4/4	BE—oral low dose
L	1000	1	4/4	BE—oral high dose
M	50	1	0/9	PT—oral low dose[d]
N	50	1	0/9	MT—oral low dose[e]
O	50	1	4/4	WBA—oral low dose
Q	1000	1	4/4	WBA—oral high dose
R	0	1	2/2	Vehicle control, oral

From Cheng (2000a)

BE, biliary excretion; [F-^{14}C]dinotefuran, [tetrahydrofuran-^{14}C]dinotefuran; [G-^{14}C]dinotefuran, [guanidine-^{14}C]dinotefuran; MT, milk transfer; PK, pharmacokinetic; PT, placental transfer; TD, tissue distribution; WBA, whole-body autoradiography (qualitative)

[a] Expired air collected to determine the radiolabelled material.

[b] Fourteen days of non-radiolabelled treatment followed by one radiolabelled dose.

[c] Seven days of radiolabelled treatment.

[d] Pregnant females at approximately day 18 of gestation.

[e] Lactating females approximately 12 days postpartum.

Table 2. Sample collection schedule and terminal procedures in the ADME study in rats

Group identity	Samples collected, collection schedule and terminal procedures
P-1, P-2	Expired air: 24 h intervals for 7 days
(oral low dose)	Urine/faeces: 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168 h
	Whole blood/plasma: at termination
	Cage rinse: 0–24 h
	Cage wash/wipe: after final excreta collection
	Termination: after final excreta collection, carcasses retained

Table 2 (continued)

Group identity	Samples collected, collection schedule and terminal procedures
A, B, C, D	Urine/faeces: 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168 h
(iv low dose)	Whole blood/plasma: at termination
(oral low dose)	Cage rinse: 0–24 h
(oral high dose)	Cage wash/wipe: after final excreta collection
(repeated low dose)	Termination: after final excreta collection, major organs/tissues + carcasses collected
C-1	Urine/faeces: 0–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168 h until day 7; then 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168 h
(repeated low dose—7 radioactive doses)	Whole blood/plasma: at termination
	Cage rinse: 24 h intervals
	Cage wash/wipe: after final excreta collection
	Termination: after final excreta collection, major organs/tissues + carcasses collected
E, F, F-1, G	Blood plasma: predose, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 4, 8, 12, 24, 48 and 72 h
(PK)	Termination: after final collection, carcasses discarded
H, I, I-1	Whole blood and plasma: at termination
(TD—low dose + repeat)	Termination: 3 animals sacrificed at 0.5, 1.5 and 4 h after final dose, 30 major organs/tissues + carcasses collected
J	Whole blood/plasma: at termination
(TD—high dose)	Termination: 3 animals sacrificed at 1.5, 4 and 8 h after dose, 30 major organs/tissues + carcasses collected
K, L	Urine/faeces: 0–6, 6–12, 12–24, 24–48 h
(BE)	Whole blood/plasma: at termination
	Bile: 24 h predose, 0–6, 6–12, 12–24, 24–48 h
	Cage rinse: 0–24 h
	Cage wash/wipe: after final excreta collection
	Termination: after final excreta collection, major organs/tissues + carcasses collected
M	Whole blood/plasma: at termination
(PT)	Termination: 3 animals per time point sacrificed at 0.5, 1.5 and 4 h after dose, 2 whole fetuses per animal, selected tissues from 2 further fetuses per animal (placenta, amniotic fluid, blood, brain, heart, liver, lungs, kidneys, carcass), maternal tissues collected
N	Milk: 0.5, 1.5 and 4 h
(MT)	Whole blood/plasma: at termination
	Termination: 3 animals per time point sacrificed at 0.5, 1.5 and 4 h after milk collection, carcasses discarded
O	Termination: 1 animal of each sex per time point sacrificed at 0.5, 1.5, 4 and 24 h post-dose and subjected to qualitative WBA
(WBA—low dose)	
Q	Termination: 1 animal of each sex per time point sacrificed at 1.5, 4, 8 and 72 h post-dose and subjected to qualitative WBA
(WBA—high dose)	
R	Urine/faeces: 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168 h
(vehicle control)	Whole blood/plasma: at termination
	Termination: after final excreta collection, carcasses retained

From Cheng (2000a)
BE, biliary excretion; iv, intravenous; MT, milk transfer; PK, pharmacokinetic; PT, placental transfer; TD, tissue distribution; WBA, whole-body autoradiography (qualitative)

Table 3. Recovery of radioactivity at 168 hours in the preliminary ADME study in rats

Sex	Dose (mg/kg bw); label	Recovery of radioactivity (% of administered dose)					
		Urine	Faeces	Cage	Expired air	Tissues and carcass	Total
Males	1 × 50; [F-^{14}C]	90.3	1.39	5.73	0.01	0.09	97.5
	1 × 50; [G-^{14}C]	90.6	4.02	2.97	0.05	0.10	97.8
Females	1 × 50; [F-^{14}C]	82.0	7.32	8.23	0.02	0.44	98.0
	1 × 50; [G-^{14}C]	93.5	1.22	2.51	0.05	0.08	97.3

From Cheng (2000a)
[F-^{14}C]dinotefuran, [tetrahydrofuran-^{14}C]dinotefuran; [G-^{14}C]dinotefuran, [guanidine-^{14}C]dinotefuran

Table 4. Recovery of radioactivity at 168 hours in the ADME study in rats

Sex	Dose (mg/kg bw); route	Recovery of radioactivity (% of administered dose)				
		Urine	Faeces	Cage	Tissues and carcass	Total
Males	1 × 50; intravenous	96.7	1.06	1.90	0.09	99.7
	1 × 50; oral	98.9	1.66	1.33	0.06	102
	15 × 50; oral	96.8	1.54	2.83	0.06	101
	7 × 50; oral	98.3	1.85	2.42	0.10	103
	1 × 1000; oral	90.1	2.15	2.52	0.10	94.7
Females	1 × 50; intravenous	96.6	1.26	1.42	0.05	99.2
	1 × 50; oral	99.8	1.19	0.62	0.08	102
	15 × 50; oral	89.7	3.16	6.42	0.21	99.3
	7 × 50; oral	95.8	1.53	4.88	0.10	102
	1 × 1000; oral	87.7	2.39	2.67	0.06	92.7

From Cheng (2000a)

Table 5. Recovery of radioactivity at 48 hours in bile duct–cannulated rats

Sex	Dose (mg/kg bw); route	Recovery of radioactivity (% of administered dose)					
		Urine	Faeces	Bile	Cage	Tissues and carcass	Total
Males	1 × 50; oral	94.7	1.08	0.62	2.70	0.39	99.5
	1 × 1000; oral	85.2	1.33	0.78	7.04	0.38	94.7
Females	1 × 50; oral	90.9	1.21	0.58	5.95	0.51	99.2
	1 × 1000; oral	90.3	1.34	0.88	3.83	2.43	98.8

From Cheng (2000a)

Radioactivity was widely distributed in all tissues examined 0.5 hour after a single oral dose of 50 mg/kg bw (Table 7). At this time, only the concentrations in the kidneys (79.4 ppm), stomach (67.3 ppm) and urinary bladder (45.8 ppm) were higher than those in plasma (40.6 ppm). Tissue concentrations declined quickly, and at 168 hours after dosing, concentrations in all tissues, with the exception of male skin (0.05 ppm), kidneys (0.01 ppm) and mammary gland (0.02 ppm), were below the limit of detection (0.001 ppm). All tissues were below the limit of detection 168 hours after a single intravenous dose. With the exceptions of male and female skin (0.007 and 0.014 ppm, respectively), female bone (0.004 ppm), female intestinal tract (0.003 ppm) and female mammary gland (0.018 ppm), all tissues were below the limit of detection 168 hours after 15 oral doses of 50 mg/kg bw. Radioactivity was widely distributed in most tissues examined following 7 daily oral doses of 50 mg/kg bw and after a single oral dose of 1000 mg/kg bw. In these groups, low concentrations

occurred in plasma (0.002–0.028 ppm), and the highest concentrations occurred in female mammary gland (0.324–0.703 ppm) and in the skin (0.193–0.692 ppm). The results indicate that the disposition of radioactivity is similar following single or multiple dosing regimens and after low or high doses.

Table 6. Toxicokinetic parameters calculated for [^{14}C]dinotefuran in rats

Sex	Dose (mg/kg bw); route	C_{max} (ppm)	T_{max} (h)	$t_{½}$ (h)	AUC_{0-t} (ppm·h)	$AUC_{0-\infty}$ (ppm·h)
Males	1 × 50; oral	40.8	0.50	3.64	83.3	83.3
	15 × 50; oral	47.4	0.45	5.65	92.1	92.1
	7 × 50; oral	41.5	0.63	6.28	91.2	91.2
	1 × 1000; oral	566	2.10	13.8	2660	2660
Females	1 × 50; oral	45.6	0.25	7.86	110	110
	15 × 50; oral	42.2	0.38	6.89	76.0	76.0
	7 × 50; oral	43.8	0.31	16.1	69.0	69.2
	1 × 1000; oral	471	2.00	15.2	2360	2370

From Cheng (2000a)
AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; $t_{½}$, half-life; T_{max}, time to reach C_{max}

In pregnant rats (group M), radioactivity was rapidly transferred to fetuses and rapidly distributed to the fetal tissues. Maximum fetal concentrations occurred in all tissues examined (except fetal brain) within 0.5 hour of maternal treatment (Table 8). Subsequently, radioactivity declined rapidly to low levels within 4 hours. Similar concentrations occurred in maternal and fetal blood, suggesting a rapid equilibration and similar tissue distribution in maternal and fetal tissues.

In lactating rats (group N), radioactivity was rapidly transferred from maternal blood to the milk of lactating animals at day 12 postpartum. C_{max} values for maternal plasma and milk were 29.3 and 34.8 ppm, respectively, 0.5 hour post-dosing (Table 9). Concentrations in milk declined rapidly to 6.51 ppm after 4 hours. Calculation of pharmacokinetic parameters gave an elimination half-life 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 (0.002 ppm).

Whole-body autoradiography after single oral doses of 50 or 1000 mg/kg bw (groups O and Q) 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 hour and 1.5 hours, for 50 and 1000 mg/kg bw, respectively. Thereafter, levels of radioactivity were declining 1.5 hours after administration of 50 mg/kg bw and 8 hours after administration of 1000 mg/kg bw. Elimination was almost complete after 24 hours (50 mg/kg bw) and 72 hours (1000 mg/kg bw); no detectable radioactivity was apparent in either sex. The highest levels of radioactivity were generally found in the urine, followed by the gastrointestinal contents and renal medulla. Low levels of radioactivity were detected in the brain and gonads of both sexes after 50 or 1000 mg/kg bw. There were no apparent sex-related or dose-related differences in the overall distribution trends (Cheng, 2000a).

Table 7. Concentrations of radioactivity in organs/tissues of rats after a single oral dose of 50 mg/kg bw

Tissue	Concentration of radioactivity (ppm)							
	Males[a]				Females[a]			
	0.5 h	1.5 h	4 h	168 h	0.5 h	1.5 h	4 h	168 h
Blood	34.8	13.5	1.92	ND	35.0	12.5	1.40	ND
Plasma	40.6	15.9	2.24	ND	41.4	14.6	1.64	ND
Eyes	14.8	8.88	2.16	ND	13.0	8.75	1.49	ND
Skin	33.9	15.1	2.27	0.05	29.8	11.6	1.50	ND
Mammary gland	NA	NA	NA	NA	26.7	10.9	1.23	0.02
Urinary bladder	45.8	95.3	5.9	ND	32.4	19.0	6.31	ND
Fat (reproductive)	7.88	2.36	0.44	ND	5.31	1.97	0.30	ND
Prostate	32.3	21.8	4.41	ND	NA	NA	NA	NA
Testes	18.5	15.8	3.03	ND	NA	NA	NA	NA
Ovaries	NA	NA	NA	NA	28.0	10.6	1.16	ND
Uterus	NA	NA	NA	NA	33.5	13.1	1.18	ND
Spleen	28.1	10.4	1.3	ND	28.1	9.83	1.07	ND
Adrenals	30.9	12.7	2.04	ND	29.9	12.9	1.81	ND
Kidneys	79.4	33.5	3.98	0.01	72.4	28.9	3.90	ND
Liver	36.3	13.9	2.11	ND	37.6	12.0	1.54	ND
Thymus	32.6	12.3	1.95	ND	32.6	12.0	1.37	ND
Heart	29.6	11.4	1.71	ND	26.0	11.0	1.24	ND
Lungs	32.9	12.4	1.99	ND	34.5	12.3	1.40	ND
Thyroid/parathyroid	24.5	9.99	1.56	ND	27.7	10.7	1.24	ND
Brain	2.92	2.06	0.32	ND	2.24	1.90	0.41	ND
Pituitary gland	31.3	12.0	1.83	ND	32.6	11.7	1.19	ND
Muscle (thigh)	31.4	12.4	2.13	ND	29.5	13.0	1.61	ND
Bone marrow	29.9	10.3	1.82	ND	28.6	9.96	1.81	ND
Bone (femur)	16.2	6.19	1.51	ND	11.9	4.98	1.06	ND
Pancreas	28.1	10.7	1.83	ND	29.0	10.1	1.50	ND
Lymph node	29.2	10.4	1.73	ND	28.1	9.61	1.35	ND
Stomach contents[b]	14.9	5.00	0.08	ND	28.9	1.46	0.67	ND
Intestinal tract contents[b]	8.28	7.26	4.12	ND	9.78	5.48	4.00	ND
Stomach	67.3	27.5	2.39	ND	171	15.5	5.20	ND
Intestinal tract	34.3	24.5	4.1	ND	47.5	18.2	4.15	ND

From Cheng (2000a)
NA, not applicable; ND, not detected
[a] Group H: 0.5-hour, 1.5-hour and 4-hour time points; Group B: 168-hour time point.
[b] Includes wash.

Table 8. Concentrations of radioactivity in pregnant rats (day 18 post-coitum) after a single oral dose of 50 mg/kg bw

Tissue	Concentration of radioactivity (ppm)		
	0.5 h	1.5 h	4 h
Fetal blood	23.9	17.3	3.63
Fetal brain	16.6	17.8	4.21
Fetal heart	27.1	18.2	3.74
Fetal kidneys	25.8	15.0	3.90
Fetal liver	18.9	11.8	2.62
Fetal lungs	23.1	16.7	3.41
Fetus	23.5	17.2	3.90
Maternal blood	38.1	18.5	3.99
Maternal plasma	44.3	21.3	4.65

From Cheng (2000a)

Table 9. Concentrations of radioactivity in lactating rats (day 12 postpartum) after a single oral dose of 50 mg/kg bw

Tissue	Concentration of radioactivity (ppm)		
	0.5 h	1.5 h	4 h
Blood	24.8	14.3	2.87
Plasma	29.3	17.2	3.48
Milk	34.8	28.1	6.51

From Cheng (2000a)

In a special study in neonatal (i.e. 12-day-old) rats, the ADME of (^{14}C-guanidine)-labelled dinotefuran (purity 99.47%; radiochemical purity 99.6%) were investigated in 28 male and 28 female Crl:CD(SD) BR rats (Cheng, 2000c,d). The study was conducted according to JMAFF test guideline 59 NohSan No. 4200. The test substance was administered as a suspension in 0.5% carboxymethylcellulose by oral gavage at a single dose of 50 mg/kg bw using a dose volume of 10 ml/kg bw. For analysis of excreta, blood and selected tissues, 25 pups of each sex were used (group 1), whereas 3 pups of each sex (group 2) were used for whole-body autoradiographic analysis. Morbidity and mortality checks were performed twice daily, clinical signs were recorded daily and body weights were recorded on the day of treatment. Urine and faeces were collected together as excreta from group 1 animals. After each sampling interval, the pups and cages were wiped with gauze pads and the pads added to the excreta. Excreta samples were pooled to form a single sample for each sex and sampling interval. Five pups of each sex from group 1 were killed at 0.5 or 1.5 hours post-dosing, and 15 pups of each sex at 4 hours post-dosing. Blood samples were collected and pooled to form a single sample for each sex and sampling interval. An aliquot of each blood sample was retained for radioanalysis, and the residue was centrifuged to separate plasma and cellular components. The cellular component was discarded. Liver, kidneys, intestinal tract and contents, stomach, stomach contents and residual carcass were excised, rinsed, blotted dry and weighed, and each tissue was pooled to form a single sample for each sex and sampling interval. The weight of each pooled sample was recorded. One pup of each sex from group 2 was killed at 0.5, 1.5 and 4 hours post-dosing, and the carcasses were prepared for whole-body autoradiography.

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 (Table 10). The recovery of radioactivity at 4 hours post-dosing amounted to 36.3% and 31.8% in excreta, 22.2% and 20.7% in stomach contents 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.

Table 10. Recovery of radioactivity in neonatal rats after a single oral dose of 50 mg/kg bw

Tissue	Recovery of radioactivity (% of administered dose)					
	Males			Females		
	0.5 h	1.5 h	4 h	0.5 h	1.5 h	4 h
Blood	1.84	1.38	0.69	1.42	1.26	0.53
Excreta	3.23	22.1	36.3	5.75	26.3	31.8
Intestinal tract + contents	2.91	2.28	1.80	3.37	2.25	1.42
Kidneys	0.95	0.28	1.15	0.84	0.89	0.59
Liver	1.47	1.05	0.64	1.43	0.97	0.52
Residual carcass	31.5	34.3	30.6	25.1	26.1	31.8
Stomach	1.14	0.45	0.88	1.93	0.31	0.18
Stomach contents	52.2	36.4	22.2	54.1	36.0	20.7
Total	95.3	98.2	94.2	94.0	94.1	87.6

From Cheng (2000c)

The maximum plasma concentration of radioactivity in both sexes was approximately 21 ppm and occurred at 0.5 hour post-dosing (Table 11). Thereafter, it declined to approximately 9 ppm at 4 hours post-dosing. Concentrations in the stomach and kidneys were higher than those in plasma at all sampling intervals, but were comparable to those 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-related differences in the absorption or elimination of radioactivity.

Table 11. Concentrations of radioactivity in neonatal rats after a single oral dose of 50 mg/kg bw

Tissue	Concentration of radioactivity (ppm)					
	Males			Females		
	0.5 h	1.5 h	4 h	0.5 h	1.5 h	4 h
Blood	20.3	18.2	9.06	17.0	15.4	6.97
Intestinal tract + contents	28.8	21.8	14.2	28.3	20.2	12.3
Kidneys	36.1	10.2	45.8	30.9	34.3	22.6
Liver	23.7	15.1	10.2	21.3	15.1	7.66
Plasma	21.3	19.1	9.42	21.2	18.9	8.71
Residual carcass	18.7	20.5	17.9	15.3	16.1	19.5
Stomach	76.1	33.7	59.9	110	23.2	13.7
Stomach contents	70.8	48.3	33.4	78.1	38.4	32.7

From Cheng (2000c)

Autoradiographic data indicated that radioactivity derived from (^{14}C-guanidine)-labelled dinotefuran was widely distributed throughout the organs and tissues in male and female pups (Table 12). 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-dosing. Overall, the concentration of radioactivity in most tissues declined during the 4 hours post-dosing. In contrast, the

concentrations in the renal medulla and cortex, the urinary bladder and urine increased from 0.5 to 4 hours post-dosing, indicating predominantly urinary elimination. Elimination from most tissues was incomplete at 4 hours post-dosing. Low levels of radioactivity occurred in the brain. There were no apparent sex differences in the tissue distribution of radioactivity.

Table 12. Concentrations of radioactivity in neonatal rats after a single oral dose of 50 mg/kg bw determined by quantitative whole-body autoradiography

Tissue	Concentration of radioactivity (ppm)					
	Males			Females		
	0.5 h	1.5 h	4 h	0.5 h	1.5 h	4 h
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	—	—	—
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
Gastrointestinal tract	36.8	21.7	12.1	36.3	21.1	23.1
Gastrointestinal tract 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
Oesophageal contents	58.4	19.2	NR	15.2	29.2	NR
Oesophagus	86.7	27.6	NR	29.4	14.4	10.5
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

Tissue	Concentration of radioactivity (ppm)					
	Males			Females		
	0.5 h	1.5 h	4 h	0.5 h	1.5 h	4 h
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
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

From Cheng (2000c)
NR, not represented (tissue not present in section)

The gastrointestinal tract and contents of neonatal pups contained 56–60% of the administered radioactivity 0.5 hour post-dosing, in contrast to 10–15% in adults (Table 13). Concentrations had declined by 4 hours post-dosing to 22–25% of the administered dose in pups and to less than 3% in adults. Thus, at 0.5 hour, oral absorption in pups amounted to approximately 50%, compared with approximately 90% in adults. The kidneys of pups contained less radioactivity than adult kidneys at 0.5 hour and more after 4 hours. These data also support the contention that oral absorption and urinary elimination are slower in neonatal pups than in the adult animal.

The concentration of plasma radioactivity in neonatal rats was approximately 52% of that of the adult rat 0.5 hour post-dosing, whereas at 4 hours, the neonatal plasma concentration was more than 4 times the adult concentration (Table 14). A similar pattern was evident in liver. The concentrations in the kidneys and stomach contents of neonatal pups were markedly higher than in adults at 4 hours post-dosing. These data indicate that 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 feed in the gut.

1.2 Biotransformation

In the ADME study of ^{14}C-labelled dinotefuran described above (Cheng, 2000a,b, 2001), pooled tissue, fluid and excreta samples for metabolite identification were analysed for radioactivity either by direct liquid scintillation counting (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). The total radioactive content of each fraction was determined by LSC. Samples with sufficient radioactivity for further

analysis were subjected to high-performance liquid chromatography (HPLC) to determine metabolite profiles by comparison with reference standards. Urine was also analysed by two-dimensional thin-layer chromatography. Urine and faecal samples were used for isolation/fractionation of radioactive metabolites by sequential SPE. Selected column eluates were subjected to analysis by liquid chromatography with mass spectrometry or tandem mass spectrometry to obtain spectral data from the isolated metabolites.

Table 13. Recovery of radioactivity in neonatal and adult rats after a single oral dose of 50 mg/kg bw

Sex	Tissue	Recovery of radioactivity (% of administered dose)					
		0.5 h		1.5 h		4 h	
		Neonate	Adult	Neonate	Adult	Neonate	Adult
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 + 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 + contents	3.37	8.24	2.25	3.86	1.42	2.17

From Cheng (2000a,c)

Table 14. Comparison of radioactivity concentrations in neonatal and adult rats after a single oral dose of 50 mg/kg bw

Sex	Tissue	Concentration of radioactivity (ppm)					
		0.5 h		1.5 h		4 h	
		Neonate	Adult	Neonate	Adult	Neonate	Adult
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

From Cheng (2000a,c)

Unchanged dinotefuran in urine accounted for 92.5–97.2% of total urine radioactivity (Table 15). A group of urinary metabolites, PHP and isomers, 446-DO and isomers, 446-CO, 446-DO-Ac, 446-OH-Ac and 446-OH+COOH, represented 2.08–5.95% of the administered radioactivity (see Appendix 1). Other minor urine metabolites, UF, FNG and DN, each accounted for no more than 0.53% of the administered radioactivity. Trace amounts (< 0.1% of the administered radioactivity) of nine other metabolites were also detected in urine.

Table 15. Detected metabolites of dinotefuran in urine in rats

Dose (mg/kg bw)	% of radioactivity in sample												
	Parent	F1[a]	F2[b]	F3[c]	UF	FNG	F4[d]	F5[e]	DN-2-OH	DN-3-OH[f]	BCDN	DN	446-NH_2
Males													
1 × 50, iv	94.4	0.03	2.18	2.31	0.32	0.48	0.05	0.10	0.02	0.00	0.02	0.04	0.00
1 × 50, oral	92.5	0.16	2.80	3.47	0.15	0.56	0.00	0.16	0.08	0.00	0.05	0.03	0.03
15 × 50, oral	94.0	0.14	2.48	2.53	0.21	0.37	0.07	0.00	0.00	0.09	0.00	0.13	0.00
7 × 50, oral	95.0	0.10	0.90	2.38	0.18	0.27	0.00	0.00	0.00	0.06	0.00	0.02	0.02
1 × 1000, oral	92.8	0.17	2.48	3.34	0.29	0.49	0.10	0.14	0.05	0.04	0.00	0.04	0.03
Females													
1 × 50, iv	97.2	0.10	1.07	1.33	0.13	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1 × 50, oral	95.3	0.07	1.71	2.19	0.17	0.30	0.00	0.09	0.03	0.00	0.00	0.09	0.03
15 × 50, oral	95.3	0.18	1.71	2.07	0.16	0.39	0.00	0.07	0.00	0.07	0.00	0.08	0.00
7 × 50, oral	95.8	0.19	1.63	1.65	0.12	0.32	0.00	0.11	0.00	0.00	0.00	0.13	0.00
1 × 1000, oral	95.8	0.12	1.36	1.90	0.21	0.27	0.17	0.09	0.00	0.08	0.01	0.03	0.00

From Cheng (2000a)
iv, intravenous; RT, retention time
[a] Fraction 1 (RT: 8.2–9.7 min): mixture of MNG, 446-DO-Ac.
[b] Fraction 2 (RT: 12.0–14.0 min): mixture of PHPs, UF-DM, 446-OH+COOH.
[c] Fraction 3 (RT: 14.7–16.8 min): mixture of 446-CO, 446-DO, PHP-Ac.
[d] Fraction 4 (RT: 21.1–22.4 min).
[e] Fraction 5 (RT: 26.7–33.9 min): mixture of MG, MG-Ac.
[f] FNG-DN with an RT between that of DN-3-OH and BCDN also detected in some samples.

Unchanged dinotefuran was the major component of faecal radioactivity, but numerous minor metabolites were identified, and 0.01–1.75% of the administered radioactivity represented unidentified polar metabolites. Unchanged dinotefuran was also 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.

In conclusion, dinotefuran was the major radioactive component in most tissues examined, and less than 10% of dinotefuran was metabolized. 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. A proposed metabolic pathway and the structures of the putative metabolites are shown in Figure 1.

In the special ADME study in neonatal (i.e. 12-day-old) rats described above (Cheng, 2000c,d), pooled samples of liver, kidneys, stomach, plasma, excreta and intestinal tract with contents collected 4 hours post-dosing from group 1 pups were extracted. 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 co-chromatography with test sample extracts.

The recovery of radioactivity from all matrices for metabolite profiling ranged from 96.6% to 100%. In all matrices, [^{14}C-guanidine]dinotefuran was the major component and accounted for 97.0–100% of the radioactivity in plasma, kidneys, stomach and excreta (Table 16). In the liver, [^{14}C-guanidine]dinotefuran accounted for 61.1% (males) and 66.5% (females) of radioactivity, and in the intestinal tract with contents, it accounted for 83.3% (males) and 76.3% (females). Putative metabolites in the neonate are shown in the legend to Table 16.

Metabolism in the neonatal rat was limited, and the metabolite profiles were comparable to those of the young adult rat (Cheng, 2000a), but fewer metabolites were detected in neonates (Table 17). These data suggest slower metabolism of [^{14}C-guanidine]dinotefuran, possibly due to incomplete development of neonatal liver function.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of acute toxicity studies with dinotefuran are summarized in Table 18.

In an acute oral toxicity study conducted according to OECD Test Guideline No. 401, male and female Crl:CD[SD]BR (SPF) rats received dinotefuran (purity 96.5%) in 0.5% aqueous methylcellulose. Initially, a dose range–finding study was performed comprising four groups of one animal of each sex treated with a dose level of 500, 1000, 3000 or 5000 mg/kg bw at a volume of 20 ml/kg bw. In phase I of the main study, five animals of each sex were treated at a volume of 10 ml/kg bw with 5000 mg/kg bw, and two groups of females only were treated at 3000 or 1000 mg/kg bw. In phase II of the main study, groups of five male animals were treated with a dose level of 1000, 2000, 3000 or 5000 mg/kg bw, and groups of five females were treated with a dose level of 1000, 2000, 3000 or 4000 mg/kg bw, each at a volume of 20 ml/kg bw. The main study animals were checked twice daily for morbidity or mortality. Clinical signs were recorded frequently on the day of treatment (main study animals) and then daily for 14 days. Body weights were recorded predosing and on days 7 and 14 or on the day of death. Decedents and survivors from the dose range–finding study were discarded without necropsy. Animals dying during the observation period and all survivors from the main study were submitted for necropsy and abbreviated postmortem examination.

In the range-finding study, the females treated at 3000 or 5000 mg/kg bw died on day 1; all other animals survived the observation period (Table 19). In phase I of the main study, there were no deaths at any dose level administered at a treatment volume of 10 ml/kg bw. The median lethal dose (LD_{50}) for dinotefuran administered at 10 ml/kg bw was greater than 5000 mg/kg bw. In phase II of the main study, deaths occurred in females treated at and above 2000 mg/kg bw and in males treated at and above 3000 mg/kg bw. All deaths in phase II occurred on the day of dosing or on the day following dosing.

Figure 1. Proposed metabolic scheme for dinotefuran (MTI-446) in rats

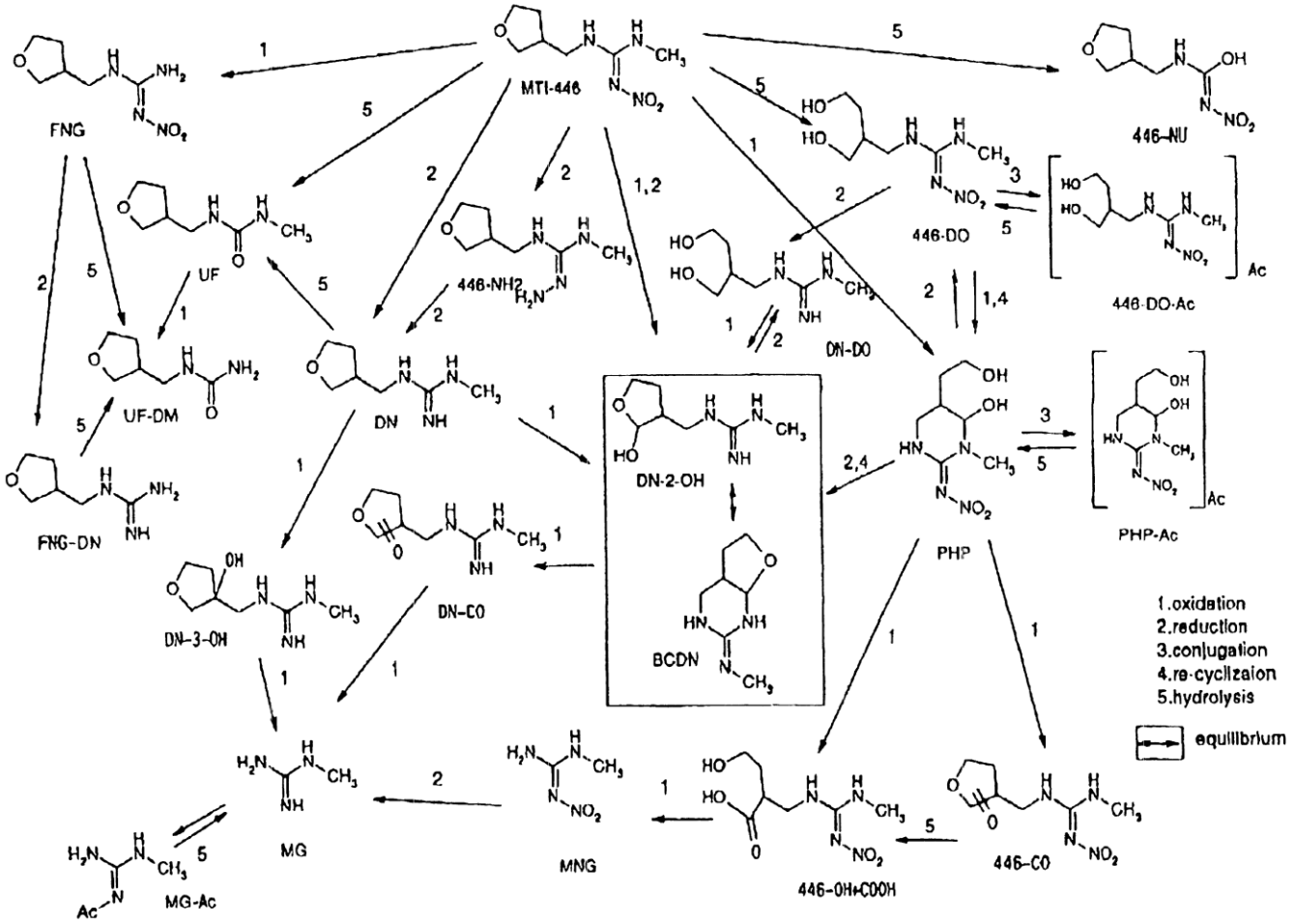


Table 16. Detected metabolites of dinotefuran in neonatal rats after a single oral dose of 50 mg/kg bw

Tissue	% of radioactivity in sample / ppm					
	Dinotefuran	Fraction 1[a]	Fraction 2[b]	Fraction 3[c]	Fraction 4[d]	1+2+3+4
Males						
Liver	61.1/6.23	6.69/0.69	4.89/0.50	19.5/1.99	7.82/0.80	38.9/3.98
Kidneys	97.1/44.5	1.62/0.74	0.73/0.34	0.53/0.24	0.00/0.00	2.88/1.32
Stomach	99.0/59.3	0.21/0.13	0.78/0.47	0.00/0.00	0.00/0.00	0.99/0.60
ITC	83.3/11.8	0.00/0.00	15.4/2.19	1.40/0.20	0.00/0.00	16.8/2.39
Excreta	98.5/NA	1.49/NA	0.00/NA	0.00/NA	0.00/NA	1.49/NA
Plasma	100/9.42	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00
Females						
Liver	66.5/5.09	5.17/0.39	3.76/0.29	23.0/1.76	1.62/0.12	33.6/2.56
Kidneys	97.0/21.9	2.28/0.52	0.68/0.15	0.00/0.00	0.00/0.00	2.96/0.67
Stomach	100/13.7	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00
ITC	76.3/9.38	21.6/2.65	0.00/0.00	2.16/0.26	0.00/0.00	23.8/2.91
Excreta	100/NA	0.00/NA	0.00/NA	0.00/NA	0.00/NA	0.00/NA
Plasma	100/8.71	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00

From Cheng (2000c)

ITC, intestinal tract with contents; NA, not calculated, because the net sample weight without cage wash was not known; RT, retention time

[a] Fraction 1 (RT: 4.5–9.4 min): Possible mixture of MNG, 446-DO-Ac and others.

[b] Fraction 2 (RT: 12.1–19.8 min): Possible mixture of PHPs, 446-DO, 446-CO, 446-OH+COOH, UF-DM, PHP-Ac, UF, FNG.

[c] Fraction 3 (RT: 38.1–44.0 min): Possible mixture of MG, MG-Ac, DN-DO, DN-OHs, DN-CO, BCDN, DN.

[d] Fraction 4 (RT: 46.8–47.0 min): Unknown.

Table 17. Comparison of extent of metabolism of dinotefuran in neonatal and adult rats

Matrix	% of radioactivity in sample							
	Males				Females			
	Neonate		Adult		Neonate		Adult	
	Parent	Metabolites	Parent	Metabolites	Parent	Metabolites	Parent	Metabolites
Plasma	100	0.00	87.1	6.80	100	0.00	NA	NA
Liver	61.1	38.9	4.66	87.0	66.5	33.5	0.00	92.9
Kidneys	97.1	2.88	62.6	21.1	97.0	2.96	72.2	16.7
Stomach	99.0	0.99	56.7	43.3	100	0.00	NA	NA
Excreta	98.5	1.49	88.2[a]	8.09[a]	100	0.00	93.1[a]	5.23[a]
ITC[a]	83.3	16.8	NS	NS	76.3	23.7	NS	NS

From Cheng (2000a) for neonatal rats, Cheng (2000c) for adult rats

ITC, intestinal tract with contents; NA, data not available (low radioactivity in sample); NS, no sample with contents

[a] Calculated by combining data from urine and faeces.

Table 18. Summary of acute toxicity studies with dinotefuran

Species	Strain	Sex	Route	Purity (%)	LD_{50} / LC_{50}	Reference
Rat	Crl:CD[SD]BR	M+F	Oral	96.5	2450 mg/kg bw	Glaza (1997a)
Mouse	Crl:CD-1[ICR]BR	M+F	Oral	96.5	2371 mg/kg bw	Glaza (1997b)
Rat	Crl:CD[SD]BR	M+F	Dermal	96.5	> 2000 mg/kg bw	Glaza (1997c)
Rat	Crl:WI[Glx/BRL/Han]BR	M+F	Inhalation	93.0	> 4.09 mg/l	Shepherd (1999)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

Table 19. Results of the acute oral toxicity study in rats

Dose (mg/kg bw)	No. of animals dying/no. tested					
	Range-finding study (dose volume 20 ml/kg bw)		Main study – phase I (dose volume 10 ml/kg bw)		Main study – phase II (dose volume 20 ml/kg bw)	
	Males	Females	Males	Females	Males	Females
500	0/1	0/1	—	—	—	—
1000	0/1	0/1	—	0/5	0/5	0/5
2000	—	—	—	—	0/5	3/5
3000	0/1	1/1	—	0/5	3/5	4/5
4000	—	—	—	—	—	5/5
5000	0/1	1/1	0/5	0/5	—	—

From Glaza (1997a)

In phase I of the main study, two females at 5000 mg/kg bw showed transient staggering gait on the day of treatment only and red staining of the face persisting for up to 3 days. One female treated at 3000 mg/kg bw also showed transient staggering gait on the day of treatment. A male at 5000 mg/kg bw showed transient excessive salivation, and a female at 1000 mg/kg bw showed red staining of the face. All other animals were of normal appearance and behaviour. In phase II of the main study, at 1000 mg/kg bw, three females exhibited red-stained face on the day of treatment, and one male exhibited a scab on its face on days 2 through 14. The onset of clinical signs at dose levels of 2000 mg/kg bw and higher was dose related; clinical signs included hypoactivity, staggering gait, hunched posture, prostration, red-stained face, miosis, lacrimation, salivation, tachypnoea, dyspnoea, soft faeces, yellow staining of the urogenital area, tonic or clonic convulsions and tremors. The surviving animals at 2000 and 3000 mg/kg bw returned to a normal appearance by day 3 after treatment.

The acute oral LD_{50} (and 95% confidence limits) were calculated to be 2804 mg/kg bw (1947–4037 mg/kg bw) for males, 2000 mg/kg bw (1354–2954 mg/kg bw) for females and 2450 mg/kg bw (1942–3090 mg/kg bw) for both sexes combined (Glaza, 1997a).

In an acute oral toxicity study conducted according to OECD Test Guideline No. 401, male and female Crl:CD-1[ICR]BR (SPF) mice received dinotefuran (purity 96.5%) in 0.5% aqueous methylcellulose. Initially, a dose range–finding study was performed comprising four groups of one animal of each sex treated at 20 ml/kg bw with a dose level of 500, 1000, 3000 or 5000 mg/kg bw. In the main study, three groups of five animals of each sex were treated at 20 ml/kg bw with 1000, 2000 or 3000 mg/kg bw. The animals were deprived of feed for approximately 5 hours before treatment. Range-finding study animals were checked for mortality once daily for 14 days, and necropsy was not performed. In the main study, animals were checked twice daily for morbidity or mortality. Clinical signs were recorded frequently on the day of treatment and then daily for 14 days. Body weights were recorded predosing and on days 7 and 14 or on the day of death. The animals dying during the

observation period and all survivors were submitted for necropsy and abbreviated postmortem examination.

In the range-finding study, both animals treated at 5000 mg/kg bw and the male treated at 3000 mg/kg bw died on the day of treatment; all other animals survived the observation period (Table 20). In the main study, deaths occurred at dose levels of 2000 mg/kg bw and above, but not at 1000 mg/kg bw. All deaths in the main study occurred on the day of treatment. Transient clinical signs of toxicity, on the day of treatment only, were apparent at dose levels of 2000 mg/kg bw and higher and included hypoactivity, staggering gait, dyspnoea, tonic convulsions and tremors. Survivors treated at 2000 or 3000 mg/kg bw gained weight throughout the observation period. Necropsy and postmortem examination revealed no gross lesions in either decedents or survivors killed at the end of the observation period.

Table 20. Results of the acute oral toxicity study in mice

Dose (mg/kg bw)	No. of animals dying/no. tested			
	Range-finding study		Main study	
	Males	Females	Males	Females
500	0/1	0/1	—	—
1000	0/1	0/1	0/5	0/5
2000	—	—	1/5	2/5
3000	1/1	0/1	4/5	4/5
5000	1/1	1/1	—	—

From Glaza (1997b)

The acute oral LD_{50} (and 95% confidence limits) were calculated to be 2450 mg/kg bw (1801–3331 mg/kg bw) for males, 2275 mg/kg bw (1537–3369 mg/kg bw) for females and 2371 mg/kg bw (1884–2983 mg/kg bw) for both sexes combined (Glaza, 1997b).

In an acute dermal toxicity study conducted according to OECD Test Guideline No. 402, a group of five male and five female Crl:CD[SD]BR (SPF) rats was exposed to dinotefuran (purity 96.5%) at a single dose of 2000 mg/kg bw, formulated as a paste in aqueous carboxymethylcellulose solution, by occluded dermal application for 24 hours to an area of 16 cm^2 of intact clipped dorsal skin. The animals were observed for 14 days and then subjected to necropsy and postmortem examination. All animals survived to the end of the study, and there were no treatment-related clinical signs of toxicity. All male animals gained weight throughout the study, but four females during the 1st week and two females during the 2nd week showed minor weight losses of up to 9 g. Transient slight to moderate erythema, associated with slight oedema in one animal, occurred in 8 of the 10 animals on the day of patch removal. Slight erythema persisted in two animals until day 7, but thereafter no dermal reactions were evident. Necropsy and postmortem examination did not reveal any gross lesions. Based on these results, the acute dermal LD_{50} was estimated to be greater than 2000 mg/kg bw in both sexes (Glaza, 1997c).

In an acute inhalation toxicity study conducted according to OECD Test Guideline No. 403, groups of five male and five female Crl:WI[Glx/BRL/Han]BR (SPF) rats were exposed (nose-only) to a dust aerosol of dinotefuran (purity 93.0%) at a concentration of 0 or 4.09 mg/l for 4 hours. The animals were checked twice daily for morbidity and mortality. The animals were observed for clinical signs hourly during and following exposure and daily thereafter for 14 days. Body weights were recorded before and after exposure on day 1 and on days 2, 8 and 15 of the test. After a 14-day observation period, all animals were submitted for necropsy and a full internal and external

postmortem examination. The nasal cavity and respiratory tract were assessed for evidence of irritation, and the weight of the lungs with trachea was recorded. All gross lesions were preserved in fixative, but not examined histologically. The mean achieved concentration in the exposure chamber of the treated group was 4.09 mg/l (range 2.0–5.0 mg/l), which is considered to be the maximum technically possible; the mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) was 4.74 ± 2.79 µm.

No deaths occurred during the exposure or observation periods, and no treatment-related clinical signs of an adverse reaction to treatment were apparent. Body weight gains were not affected by exposure to dinotefuran. Necropsy and postmortem examination did not reveal any treatment-related lesions in either sex. The group mean absolute and relative lung weights were considered to be unaffected by treatment.

Under the conditions of the study, the acute median lethal concentration (LC_{50}) for male and female rats was estimated to be greater than 4.09 mg/l air (Shepherd, 1999, 2000a,b).

(b) Dermal and ocular irritation and dermal sensitization

The results of the studies on acute dermal and eye irritation and skin sensitization are summarized in Table 21.

Table 21. Summary of acute dermal and eye irritation and skin sensitization studies with dinotefuran

Species	Strain	Sex	Endpoint	Purity (%)	Result	Reference
Rabbit	New Zealand White	M+F	Skin irritation	96.5	Not irritating	Glaza (1998a)
Rabbit	New Zealand White	M+F	Eye irritation	96.5	Slightly irritating	Glaza (1998b)
Rabbit	New Zealand White	M+F	Eye irritation	98.9	Minimally irritating	Kuhn (2004)
Guinea-pig	Crl:[HA]BR	M	Skin sensitization (M&K test)	96.5	Not sensitizing	Glaza (1997d)

F, female; M, male; M&K, Magnusson and Kligman

In an acute dermal irritation study conducted according to OECD Test Guideline No. 404, 0.5 g of dinotefuran (purity 96.5%) moistened with distilled water was applied once for 4 hours, under semi-occlusive dressing, to the shaved intact dorsal and/or flank skin (2.5 × 2.5 cm) of six (five male, one female) New Zealand White (Hra:(NZW)SPF) rabbits. After 4 hours, the occlusive dressings were removed and the test sites washed with water to remove all traces of test article. Skin reactions were evaluated 30 minutes after patch removal and subsequently at 24, 48 and 72 hours. The untreated skin of each animal served as the reference site for grading. Dermal erythema, eschar formation and oedema were graded according to the Draize scoring method, and the scores were calculated for each animal.

Three of the six test animals showed very slight (grade 1) erythema reactions 30 minutes after patch removal. Very slight erythema persisted in one of these animals until 24 hours, but was not apparent in the other two animals at this time. The remaining three animals showed no evidence of dermal irritation at any of the observation intervals. Oedema and other manifestations of dermal irritation did not occur in any animal at any observation interval. According to the EU and Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification system, five of the six animals had index scores of 0.0, and the remaining animal had an index score of 0.33. Consequently, dinotefuran is not classified as irritating to skin according to EU and GHS criteria. According to the USEPA classification system, the primary dermal irritation index for dinotefuran was calculated as 0.2, a score defined as indicating slight irritation (category IV) (Glaza, 1998a).

In an acute eye irritation study conducted according to OECD Test Guideline No. 405, 0.1 g of dinotefuran powder (purity 96.5%) was introduced into the right conjunctival sac of nine (six male and three female) New Zealand White (Hra:(NZW)SPF) rabbits. The left eye remained untreated as a reference control. Both eyes of three male animals were flushed with water for 1 minute starting 30 seconds after instillation of the test article. The eyes of the other six animals remained unwashed. The treated eyes of all animals were examined for ocular irritation reactions 1, 24, 48, 72 and 96 hours and 7 and 14 days after instillation. Irritation reactions were graded and scored according to the Draize technique. Sodium fluorescein examinations were performed to assist the visualization of possible corneal lesions at 24, 48, 72 and 96 hours or until a negative response was evident.

The unwashed eyes of four of six animals showed slight (grade 1) corneal opacity with epithelial peeling and iridial irritation in two of six animals within 24 hours of instillation of dinotefuran. The reaction was accompanied by slight to moderate (grade 1 or 2) erythema, chemosis and clear or purulent discharge in all six animals. The eyes of four of the animals had returned to normal in appearance within 96 hours of instillation, and the remaining two animals had returned to normal 14 days after instillation (Tables 22 and 23).

Table 22. Summary of positive ocular reactions[a] in the acute eye irritation study in rabbits

Observation	1 h	24 h	48 h	72 h	96 h	7 days	14 days
Unwashed group							
Corneal opacity	1/6	4/6	1/6	1/6	1/6	1/6	0/6
Iris lesion	1/6	2/6	1/6	0/6	0/6	0/6	0/6
Conjunctival redness	6/6	6/6	2/6	1/6	0/6	1/6	0/6
Chemosis	6/6	2/6	1/6	0/6	0/6	0/6	0/6
Washed group							
Corneal opacity	0/3	3/3	1/3	0/3	0/3	0/3	0/3
Iris lesion	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Conjunctival redness	3/3	3/3	2/3	1/3	1/3	0/3	0/3
Chemosis	3/3	2/3	1/3	1/3	1/3	0/3	0/3

From Glaza (1998b)

[a] A positive reaction for each parameter is defined as any corneal opacity or iris lesion score of 1 or greater or any conjunctival redness or chemosis score of 2 or greater.

Table 23. Mean scores (according to EU criteria) in the acute eye irritation study in rabbits

Observation	Unwashed group					Washed group				
	1 h	24 h	48 h	72 h	96 h	1 h	24 h	48 h	72 h	96 h
Corneal opacity	0.2	0.7	0.2	0.2	0.2	0.0	1.0	0.0	0.0	0.0
Iris lesion	0.2	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erythema	2.0	2.0	1.3	0.8	0.3	2.0	2.0	1.7	1.3	1.0
Oedema	2.0	1.5	1.2	0.3	0.2	2.3	1.7	1.3	0.7	0.7

From Glaza (1998b)

A positive reaction occurred in three of three animals in the washed group and was characterized by slight corneal opacity and slight to moderate erythema, chemosis and discharge. With the exception of one of three animals that showed slight erythema and chemosis on day 7, the washed eyes of all animals returned to normal in appearance by 96 hours. The appearance of the eyes of the third animal had returned to normal by day 14 (Tables 22 and 23).

Based on these results, dinotefuran is slightly irritating to the eyes of the rabbit but does not require classification according to EU and GHS criteria (Glaza, 1998b).

In an acute eye irritation study conducted according to USEPA guideline OPPTS 870.2400, 0.1 ml (37.8 mg) of dinotefuran powder (purity 98.9%) was introduced into the right conjunctival sac of three (one male and two female) New Zealand White rabbits. The left eye remained untreated to serve as a reference control. All treated eyes were washed with deionized water at room temperature for 1 minute immediately after recording the 24-hour observation. The treated eyes of all animals were examined for ocular irritation reactions 1, 24, 48 and 96 hours after instillation according to the Draize technique. Fluorescein dye retention was assessed for the corneas at 24 hours. One hour after test substance instillation, two animals exhibited minimal corneal effects (slight dulling of normal luster), all three animals had slight conjunctival discharge (grade 1) and one animal exhibited slight conjunctival redness (grade 1). Based on the irritation scoring method of Kay & Calandra (1962), a maximum average irritation score of 2.7 was obtained at 1 hour after treatment. All animals were free of eye irritation at 24 hours after instillation. Fluorescein staining did not occur in any of the eyes. Based on these results, dinotefuran is considered to be minimally irritating to the eyes of the rabbit but does not require classification according to EU and GHS criteria (Kuhn, 2004).

The skin sensitization potential of dinotefuran (purity 96.5%) was investigated in male albino (Crl:[HA]BR) guinea-pigs using a Magnusson and Kligman maximization test according to OECD Test Guideline No. 406. The concentrations of dinotefuran used in the main study were determined in a preliminary irritation study in which two groups of four guinea-pigs were exposed to dinotefuran by occluded topical application for 24 hours at concentrations of 5%, 10%, 15% and 25% weight per weight (w/w) in petrolatum (with each animal receiving two different concentrations) or intradermally at concentrations of 1%, 5%, 10% and 15% weight per volume (w/v) in 0.5% aqueous carboxymethylcellulose (with each animal receiving all four concentrations of the test material). Dermal reactions were evaluated 24 and 48 hours after treatment.

No dermal irritation occurred at any concentration of dinotefuran administered by topical application, up to 25% w/w in petrolatum. In the four animals treated by intradermal injection, dinotefuran produced mild erythema (grade 1) at 1% w/v, mild to moderate diffuse erythema (grades 1–2) reactions at 5% w/v and moderate to marked erythema (grades 2–3) at 10% and 15% w/v.

In the main study, a test group and a negative control group of 20 male guinea-pigs each were used. On day 1, the test group received three pairs of intradermal injections of 50% Freund's complete adjuvant in water, 5% w/v dinotefuran in aqueous carboxymethylcellulose and 10% w/v dinotefuran in aqueous carboxymethylcellulose diluted 1:1 with Freund's complete adjuvant. The control group received similar injections, but without dinotefuran. On day 7, the application sites of both groups of animals were treated topically with 10% sodium lauryl sulfate. On day 8, the animals were treated topically, over the injection sites, under occlusive dressing for 48 hours with 25% w/w dinotefuran in petrolatum (treated group) or petrolatum alone (irritation control group). Two weeks later, both groups were challenged topically, under occlusive dressing for 24 hours, with 25% w/w dinotefuran in petrolatum (right side) and petrolatum alone (left side). The challenge sites were scored for dermal reactions 24 and 48 hours after removal of the challenge dressings. Clinical observations were recorded daily, and individual body weights were recorded pretest and at termination.

There were no treatment-related clinical signs or adverse effects on body weight. None of the test or control group animals exhibited a dermal response to the challenge application of the test or control articles either 24 or 48 hours after patch removal. Therefore, all dermal reaction scores were zero.

It was concluded that dinotefuran does not have a sensitizing effect on the skin of the guinea-pig in the maximization test under the test conditions chosen (Glaza, 1997d).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a study of toxicity conducted according to OECD Test Guideline No. 407, groups of 10 male and 10 female Crl:CD-1®(ICR)BR VAF/Plus® mice were fed diets containing dinotefuran (purity 96.5%) at a concentration of 0, 5000, 25 000 or 50 000 ppm (equal to 0, 901, 4612 and 10 303 mg/kg bw per day for males and 0, 1043, 5359 and 12 289 mg/kg bw per day for females, respectively) for at least 4 weeks. Morbidity/mortality checks were performed twice daily, and a detailed clinical examination was performed weekly. Body weights and feed consumption were recorded weekly throughout the study. Haematology and serum clinical chemistry analyses were performed in week 5, each on five mice of each sex per group, which had feed withdrawn for at least 4 hours prior to blood sampling. Decedents and all surviving animals killed at the end of the study were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed, and all preserved tissues from animals treated at 0 or 50 000 ppm were examined by light microscopy. Gross lesions were also examined from all animals treated at 5000 and 25 000 ppm.

There were no treatment-related deaths or clinical signs at any dose level. Both sexes treated at 25 000 or 50 000 ppm showed a dose-related depression of overall body weight gain of between 46.2% and 85.5% (Table 24). Males and females treated at 50 000 ppm lost weight during the 1st week of treatment but subsequently gained weight at a comparable rate to the controls. Marked feed spillage by the groups treated at 25 000 or 50 000 ppm suggested that these test diets were less palatable than untreated diet and precluded a valid assessment of feed consumption for most time points. Therefore, initial weight loss and/or overall depressed weight gain at 25 000 and 50 000 ppm might be a reflection of reduced palatability of the diets. At 5000 ppm, there was no evidence of an effect on feed consumption.

Table 24. Summary of selected findings in the 4-week mouse study

	Males				Females			
	0 ppm	5000 ppm	25 000 ppm	50 000 ppm	0 ppm	5000 ppm	25 000 ppm	50 000 ppm
Body weight (g), week 1	30.7	32.1	32.6	32.8	25.5	26.1	26.5	26.0
Body weight (g), week 5	36.2	37.0	35.4	33.6	29.4	30.4	28.6	27.8
Body weight gain (g), weeks 1–5	5.5	4.9	2.8*	0.3*	3.9	4.3	2.0*	1.8*
Serum total protein (g/dl)	4.7	4.8	5.0	5.1*	4.8	5.0	5.1	4.9
Serum albumin (g/dl)	3.2	3.2	3.3	3.6*	3.5	3.4	3.7	3.5

From Weiler (1997b)
* $P < 0.05$

There were no treatment-related effects on the haematological profile at any dose level. Treatment-related effects on serum chemistry were confined to the male group treated at 50 000 ppm and comprised slightly higher serum total protein and albumin concentrations (Table 24). 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 unaffected by treatment at all dose levels.

There were no treatment-related effects on absolute or relative organ weights and no treatment-related macroscopic or microscopic histopathological alterations at any of the dose levels employed.

The no-observed-adverse-effect level (NOAEL) was 5000 ppm (equal to 901 mg/kg bw per day in males and 1043 mg/kg bw per day in females), based on reduced body weight gain in both sexes at 25 000 ppm and above (Weiler, 1997b).

In a study of toxicity conducted according to OECD Test Guideline No. 408, groups of 10 male and 10 female Crl:CD-1®(ICR)BR VAF/Plus® mice were fed diets containing dinotefuran (purity 96.5%) at concentrations of 0, 500, 5000, 25 000 or 50 000 ppm (equal to 0, 81, 844, 4442 and 10 635 mg/kg bw per day for males and 0, 102, 1064, 5414 and 11 560 mg/kg bw per day for females, respectively) for at least 13 weeks. Morbidity/mortality checks were performed twice daily, and a detailed clinical examination was performed weekly. Body weights and feed consumption were recorded weekly throughout the study. Ophthalmic examinations were conducted pretest and in week 14 on all animals. Haematology, serum clinical chemistry and urine analysis were performed in week 14 on five mice of each sex per group (haematology and clinical chemistry) and on all animals for urine analysis. Blood samples were withdrawn after a period of at least 4 hours of feed deprivation. All animals were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed, and all preserved tissues from animals treated at 0 or 50 000 ppm were examined by light microscopy. Gross lesions, kidneys, lungs and liver with gall bladder were also examined from all animals treated at 500, 5000 or 25 000 ppm.

There were no deaths and no treatment-related clinical signs at any dose level, but both sexes at 50 000 ppm lost weight during the 1st week of treatment and subsequently showed a treatment-related depression of body weight gain. The overall mean weight gains and body weights at termination of both sexes were reduced. The overall weight gains of males at 25 000 ppm and females at 500, 5000 and 25 000 ppm were 15.9–31.4% lower than, but not significantly different from, those of the controls (Table 25). Increased feed spillage occurred during the 1st week in the groups treated at 25 000 or 50 000 ppm and continued throughout the study at 50 000 ppm. Spillage at concentrations of 25 000 ppm and higher is considered to indicate reduced diet palatability, and any apparent increase in feed consumption at these concentrations is considered to represent spillage. There was no evidence of an effect on feed consumption or feed efficiency at dietary concentrations up to 5000 ppm.

There were no treatment-related ophthalmological or haematological effects at any dose level. Treatment-related effects on serum chemistry after 13 weeks of treatment were confined to males treated at 50 000 ppm, which showed slightly raised serum albumin concentration. Urinary pH was slightly lower in both sexes at 50 000 ppm. These minor differences from the controls were not associated with overt histopathological alterations and are considered not to be adverse effects.

There were no treatment-related effects on absolute or relative organ weights at any dose level, with the exception of lower absolute weights of the heart and liver in females and of the kidneys in both sexes at 50 000 ppm. The differences are considered to be a consequence of growth retardation, as the organ to body weight ratios were not affected. There were no treatment-related macroscopic findings at necropsy at any dose level. There were no treatment-related histopathological alterations, and the nature, severity and incidence of microscopic findings were similar in all treated and control groups.

The NOAEL was 25 000 ppm (equal to 4442 mg/kg bw per day for males and 5414 mg/kg bw per day for females), based on reduced body weight and body weight gain in both sexes at 50 000 ppm (equal to 10 635 mg/kg bw per day for males and 11 560 mg/kg bw per day for females) (Weiler, 1997d, 2000b).

Table 25. Summary of selected findings in the 13-week mouse study

	Males					Females				
	0 ppm	500 ppm	5000 ppm	25 000 ppm	50 000 ppm	0 ppm	500 ppm	5000 ppm	25 000 ppm	50 000 ppm
Body weight (g)										
- week 1	31.0	31.6	30.2	30.1	31.1	26.0	26.1	25.3	26.1	25.6
- week 2	32.7	33.2	32.0	31.2	30.2	27.3	27.2	26.5	26.8	24.0
- week 3	34.4	35.0	33.7	32.9	30.4*	28.9	28.5	27.6	28.0	24.7*
- week 4	34.6	34.9	34.2	32.8	30.9*	29.1	28.5	27.8	28.1	25.1*
- week 8	37.9	38.1	37.0	36.1	33.2*	31.5	30.7	30.0	30.2	26.6*
- week 14	41.7	41.9	40.3	39.1	35.3*	36.5	34.1	32.5*	33.3	28.5*
Body weight gain (g), weeks 1–14	10.7	10.3	10.1	9.0	4.2*	10.5	8.0	7.2	7.2	2.9*
Feed consumption (g)										
- weeks 1–4	45.1	43.6	43.6	43.7	49.6	41.4	43.2	43.6	44.5	46.1
- weeks 5–8	45.6	44.4	45.1	45.8	51.4	45.0	46.1	45.1	45.9	42.3
- weeks 9–13	40.5	40.4	40.2	41.7	45.3	42.6	42.1	42.6	43.7	41.9
Serum albumin (g/dl)	2.9	3.1	3.2	3.3	3.4*	3.4	3.4	3.4	3.5	3.6
Urinary pH	7.3	7.5	7.5	6.9	6.3	7.2	7.2	7.2	6.8	6.6*

From Weiler (1997d)
* $P < 0.05$

Rats

In a study of toxicity conducted according to OECD Test Guideline No. 407, groups of five male and five female Crl:CD®(SD)BR VAF/Plus® rats were fed diets containing dinotefuran (purity 96.5%) at a concentration of 0, 5000, 25 000 or 50 000 ppm (equal to 0, 390, 1814 and 3720 mg/kg bw per day for males and 0, 450, 2183 and 4222 mg/kg bw per day for females, respectively) for at least 4 weeks. Morbidity/mortality checks were performed twice daily, and a detailed clinical examination was performed weekly. Body weights and feed consumption were recorded weekly. Haematology, serum clinical chemistry and urine analyses were performed in week 5. All animals were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed, and all major organs and tissues from animals treated at 0 or 50 000 ppm were examined by light microscopy. Gross lesions were also examined from animals in the groups treated at 5000 or 25 000 ppm.

There were no deaths and no treatment-related clinical signs at any dose level. Overall reduced weight gain occurred at 25 000 and 50 000 ppm (Table 26). Feed consumption of both sexes at 50 000 ppm and of males at 25 000 ppm was significantly lower during the 1st week of the study, but the differences lessened by the 3rd and 4th weeks. The lower feed consumption data suggest that the diets at 25 000 and/or 50 000 ppm were less palatable, but were eventually accepted by the animals. There were no treatment-related effects on weight gain or feed consumption at 5000 ppm.

Table 26. Summary of selected findings in the 4-week rat study

	Males				Females			
	0 ppm	5000 ppm	25 000 ppm	50 000 ppm	0 ppm	5000 ppm	25 000 ppm	50 000 ppm
Body weight (g)								
- week 1	256	257	264	262	152	161	158	155
- week 5	433	430	403	356*	219	215	208	189
Body weight gain (g), weeks 1–5	177	173	139*	94*	67	53	50	34*
Feed consumption (g)								
- week 1	196	184	161*	115*	115	121	103	80*
- week 2	207	207	186	159*	116	118	113	105
- week 3	205	204	188	179	117	113	110	109
- week 4	164	176	157	184	129	127	117	102
Serum glucose (mg/dl)	118	119	111	101*	105	105	103	100
Serum cholesterol (mg/dl)	56	52	70*	80*	85	70	83	86

From Weiler (1997a)
* $P < 0.05$

There were no treatment-related effects on haematology or urine analysis parameters at any dose level. Treatment-related effects on serum clinical chemistry were confined to the male groups treated at 25 000 and 50 000 ppm: glucose concentration at 50 000 ppm was slightly reduced, and cholesterol concentrations at 25 000 and 50 000 ppm were increased relative to the controls. These differences from the controls were not apparent in the females at any dose level and were not associated with overt histopathological alterations at 50 000 ppm.

There were no effects on organ weights or weight 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 50 000 ppm (spleen, heart, kidneys, liver) and females at 50 000 ppm (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. There were no treatment-related macroscopic lesions at any dose level and no treatment-related microscopic findings at 50 000 ppm.

The NOAEL in males was 5000 ppm (equal to 390 mg/kg bw per day), based on reduced body weight gain and increased serum cholesterol at 25 000 ppm (equal to 1914 mg/kg bw per day) and above. The NOAEL in females was 25 000 ppm (equal to 2183 mg/kg bw per day), based on reduced body weight gain at 50 000 ppm (equal to 4222 mg/kg bw per day) (Weiler, 1997a).

In a study of toxicity conducted according to OECD Test Guideline No. 408, groups of 10 male and 10 female Crl:CD®(SD)BR VAF/Plus® rats were fed diets containing dinotefuran (purity 96.5%) at a concentration of 0, 500, 5000, 25 000 or 50 000 ppm (equal to 0, 34, 336, 1623 and 3156 mg/kg bw per day for males and 0, 38, 384, 1871 and 3616 mg/kg bw per day for females, respectively) for at least 13 weeks. Morbidity/mortality checks were performed twice daily, and a detailed clinical examination was performed weekly. Body weights and feed consumption were recorded weekly throughout the study. Ophthalmic examinations were conducted pretest and in week 14 on all animals. Haematology, serum clinical chemistry and urine analysis were performed on all animals pretest and in week 14, following overnight feed deprivation. All animals were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed, and samples of all major organs and tissues were preserved. All preserved tissues from animals treated at 0 or 50 000 ppm were examined by light microscopy. Gross lesions, adrenal glands,

kidneys, liver and lungs were also examined from all animals in the groups treated at 500, 5000 and 25 000 ppm.

There were no deaths and no treatment-related clinical signs during the study. Males and females treated at 50 000 ppm lost weight during week 1, but subsequently gained weight. The animals of both sexes treated at 25 000 or 50 000 ppm and females treated at 5000 ppm showed statistically significant and dose-related reductions in overall body weight gain (Table 27). At termination, the mean body weights of these groups were 7.2–24.1% lower than control values. The mean weekly feed consumption of both sexes at 25 000 or 50 000 ppm was significantly reduced for at least 11 weeks of the treatment period, and the overall mean feed consumption was 11.5–24.5% lower than control values.

There were no treatment-related ocular lesions at any dose level. Treatment-related effects on haematology and serum clinical chemistry after 13 weeks of treatment were confined to the group treated at 50 000 ppm. Activated partial thromboplastin times (APTT) were slightly shorter than control values and urea nitrogen concentrations were slightly elevated in both sexes, but were significantly different from the controls in males only. Males at 50 000 ppm also showed slightly, but significantly, lower blood glucose, total protein and globulin concentrations. These minor differences from the controls were not associated with overt histopathological alterations and are considered not to be of toxicological significance. All other haematological and serum clinical chemistry parameters were comparable to control group values. Urine analysis profiles were unaffected by treatment at all dose levels.

There were no treatment-related macroscopic findings at necropsy. There were no effects on absolute organ weights or ratios that are considered to be a direct effect of treatment at any dose level. However, absolute heart, kidney, liver and spleen weights were significantly ($P < 0.05$) lower than those of the controls and their ratios relative to body weight and/or brain weight were significantly different from the controls in the groups treated at 25 000 and 50 000 ppm. The absolute weights of the adrenals and pituitary were lower in females and the relative weights of brain and testes were higher in animals at 50 000 ppm. As the body weights of these groups were significantly reduced at termination and because there were no correlating histopathological changes in these organs, the differences from control values are considered to be incidental to treatment or secondary to substantially reduced weight gain.

Treatment-related histopathological alterations were confined to increased cytoplasmic vacuolation of the adrenal cortex in both sexes treated at 25 000 and 50 000 ppm and in males at 5000 ppm (Table 27). The vacuolation was apparent in both the zona glomerulosa and zona fasciculata of the males, but was confined to the zona glomerulosa in the females. The severity of the lesion was graded as minimal or slight in all instances except for one female at 50 000 ppm, which was graded moderate. The nature, incidence and severity of all other histopathological findings in animals treated at 0 or 50 000 ppm did not indicate an effect of treatment with dinotefuran.

The NOAEL was 500 ppm (equal to 38 mg/kg bw per day), based on reduced body weight and body weight gain in females at 5000 ppm (equal to 384 mg/kg bw per day) and above (Weiler, 1997c, 2000a).

Dogs

In a range-finding study of toxicity, not conducted in accordance with GLP, groups of one male and one female Beagle dog were fed diets containing dinotefuran (purity 92.9%) at a concentration of 0, 1250, 5000 or 20 000 ppm for 7 days. After 1 week of treatment, the intermediate- and high-dose groups were killed and subjected to necropsy, and the control and low-dose groups were continued on treatment for a further 7 days at increased dietary concentrations of 40 000 and 30 000 ppm, respectively. The dietary concentration of 40 000 ppm was calculated to provide approximate dose levels of 770 and 924 mg/kg bw per day in males and females, respectively, Morbidity/mortality checks were performed twice daily, and clinical signs of a reaction to treatment were recorded 1 hour before and after treatment. Body weights were recorded before treatment and

feeding on days 0, 2, 4 and 7 and additionally on days 10 and 14 for animals treated for 2 weeks. Feed consumption was recorded daily. Blood and urine were sampled predosing and after 7 and 14 days (40 000 and 30 000 ppm only) of treatment. Urine samples were collected from water-loaded (200 ml) animals and semiquantitatively analysed. Blood samples were withdrawn after overnight feed withdrawal and analysed for haematological and clinical chemistry parameters. All animals were killed after 7 days (1250 and 5000 ppm) or 14 days (0/40 000 and 1250/30 000 ppm) of treatment and subjected to necropsy and postmortem examination. Major organs were weighed, and a comprehensive range of tissues was processed for histological examination. No statistical analyses were performed.

Table 27. Summary of selected findings in the 13-week rat study

	Males					Females				
	0 ppm	500 ppm	5000 ppm	25 000 ppm	50 000 ppm	0 ppm	500 ppm	5000 ppm	25 000 ppm	50 000 ppm
Body weight (g)										
- week 1	257	252	257	258	256	203	195	201	195	195
- week 2	309	305	306	290*	252*	227	222	226	207*	190*
- week 14	572	568	552	515*	446*	345	326	320*	291*	262*
Body weight gain (g), weeks 1–14	315	316	295	257*	190*	141	131	118*	96*	67*
Feed consumption (g)										
- week 1	192	186	188	157*	100*	151	139	140	119*	89*
- week 2	200	193	194	178*	145*	153	147	149	135	117*
- week 4	219	214	205	191*	171*	170	156*	171	142*	147
- week 7	218	217	212	196*	173*	164	158	158	139*	124*
- week 13	197	194	197	171*	155*	152	135*	136*	123*	112*
Adrenal weight, left										
- absolute (mg)	29.4	32.1	27.8	28.8	25.4	40.5	36.2	35.3*	34.3	31.9*
- relative (% of body weight × 1000)	5.6	6.1	5.5	6.1	6.2	12.9	12.2	12.2	13.0	13.3
Adrenal weight, right										
- absolute (mg)	30.1	29.4	27.2	27.4	24.8	38.2	33.3	36.3	33.3	29.8*
- relative (% of body weight × 1000)	5.8	5.5	5.4	5.8	6.0	12.2	11.2	12.5	12.6	12.5
Adrenal cortex (n = 10)										
Zona glomerulosa, increased vacuolation										
- total	0	0	3	2	4	0	0	0	6	10
- minimal	0	0	3	1	3	0	0	0	6	2
- slight	0	0	0	1	1	0	0	0	0	7
- moderate	0	0	0	0	0	0	0	0	0	1
Zona fasciculata, increased vacuolation										
- total	1	0	2	3	5	0	0	0	0	0
- minimal	1	0	1	3	2	0	0	0	0	0
- slight	0	0	1	0	3	0	0	0	0	0

From Weiler (1997c)
* $P < 0.05$

There were no deaths or treatment-related clinical signs during the study. Males and females treated at 40 000 ppm and females treated at 30 000 or 20 000 ppm showed slight weight loss and reduced feed consumption during the week of exposure (Table 28). As reduced feed consumption in the female groups was concentration dependent, the effects might be attributable to reduced palatability of the diet.

Table 28. Summary of selected findings in the range-finding dog study (dietary administration)

	Males				Females			
	0/40 000 ppm	1250/30 000 ppm	5000 ppm	20 000 ppm	0/40 000 ppm	1250/30 000 ppm	5000 ppm	20 000 ppm
Body weight (kg)								
- day 0	9.2	9.0	9.6	9.4	8.8	8.4	9.2	8.6
- day 2	9.2	9.0	9.8	9.4	9.0	8.4	9.4	8.6
- day 4	9.4	9.0	10.0	9.8	9.2	8.4	9.4	8.8
- day 7	9.0	8.8	10.0	9.8	9.0	8.6	9.4	8.4
- day 10	9.4	9.0	—	—	9.0	8.6	—	—
- day 14	8.8	9.0	—	—	8.8	8.2	—	—
Feed consumption (g/day)								
- week 1	400	357	400	400	339	400	400	265
- week 2	181	257	—	—	208	231	—	—

From Teramoto (1998a)

There were no treatment-related effects at any dose level on urine analysis, haematological or clinical chemistry profiles and no treatment-related gross lesions at necropsy or effects on organ weights. There were no treatment-related histopathological alterations in any tissue at any dose level. The nature and incidence of microscopic changes indicated that they were spontaneous in origin and incidental to treatment with dinotefuran.

In conclusion, administration of dinotefuran by the diet resulted in reduced feed consumption at 30 000 ppm and above in males and at 20 000 ppm and above in females and in decreased body weight at 40 000 ppm in the male dog and at 20 000 ppm and above in female dogs (Teramoto, 1998a).

In a second range-finding study of toxicity, not conducted in accordance with GLP, groups of one male and one female Beagle dog were administered dinotefuran (purity 92.9%) by capsule at a dose level of 0, 30, 100 or 300 mg/kg bw per day for 7 days. Morbidity/mortality checks were performed twice daily, and clinical signs of a reaction to treatment were recorded 1 hour before and after treatment. Body weights were recorded before treatment and feeding on days 0, 2, 4 and 7. Feed consumption was recorded daily. Blood and urine were sampled prior to and after 7 days of treatment. Urine samples were collected from water-loaded (200 ml) animals and semiquantitatively analysed. Blood samples were withdrawn after overnight feed withdrawal and analysed for haematological and clinical chemistry parameters. All animals were killed at the end of the treatment period and subjected to necropsy and postmortem examination. Major organs were weighed, and a comprehensive range of tissues was processed for histological examination. No statistical analyses were performed.

There were no deaths during the study; treatment-related clinical signs were confined to diarrhoea and vomiting in both sexes treated at 300 mg/kg bw per day. Feed consumption and body weight gain were unaffected by treatment at all dose levels (Table 29). No biologically relevant changes in urine analysis, haematological and blood biochemistry profiles occurred at any dose level.

Table 29. Summary of selected findings in the range-finding dog study (capsule administration)

	Males				Females			
	0 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	0 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day
Body weight (kg)								
- day 0	9.8	10.0	10.0	10.0	8.2	7.6	9.4	8.4
- day 7	10.4	10.6	10.4	10.4	8.0	7.6	9.6	8.6
Feed consumption (g/day), days 1–6	400	400	400	400	270	256	400	400
Testes weight								
- absolute (g)	6.87	4.33	5.29	3.29	—	—	—	—
- relative (% of body weight × 100)	6.61	4.08	5.09	3.16	—	—	—	—
Prostate weight								
- absolute (g)	3.00	2.05	1.33	1.80	—	—	—	—
- relative (% of body weight × 100)	2.88	1.94	1.28	1.73	—	—	—	—
Uterus weight								
- absolute (g)	—	—	—	—	0.89	0.79	1.24	0.49
- relative (% of body weight × 100)	—	—	—	—	1.12	1.04	1.29	0.57

From Teramoto (1998b)

There were no treatment-related gross lesions or organ weight changes at necropsy in any of the dinotefuran-treated groups. The absolute and relative organ weights of dinotefuran-treated animals were variable, but there were no trends indicative of an effect of treatment. Although the absolute and relative weights of testis, prostate and uterus in dinotefuran-treated animals were generally lower than control values, the differences are considered to reflect differences in the stage of sexual maturity rather than an effect of treatment (Table 29).

There were no treatment-related histopathological alterations in any tissue at any dose level. The nature and incidence of microscopic changes indicated that they were spontaneous in origin and incidental to treatment with dinotefuran.

In conclusion, administration of dinotefuran by capsules resulted in higher incidences of vomiting and diarrhoea at 300 mg/kg bw per day (Teramoto, 1998b).

In a study of toxicity conducted according to OECD Test Guideline No. 409, groups of four male and four female Beagle dogs were fed diets containing dinotefuran (purity 93.0%) at a concentration of 0, 1600, 8000 or 40 000 ppm for 13 weeks. Because of a marked reduction in feed consumption, the highest dietary concentration was reduced to 30 000 ppm on day 5 and further to 24 000 ppm on day 12. Mean achieved dose levels were 0, 58, 307 and 862 mg/kg bw per day for males and 0, 58, 323 and 950 mg/kg bw per day for females, respectively. Morbidity and mortality were checked twice daily, and clinical signs were recorded daily. Feed consumption was measured daily for the first 2 weeks of treatment and weekly thereafter. Water consumption was recorded for 2 days/week. Body weights were recorded weekly and at necropsy. Ophthalmic examinations were conducted pretest and in week 14 on all animals. Haematology, serum clinical chemistry and urine analysis were performed on all animals, after overnight feed withdrawal, pretest and in weeks 5 and 14. All animals were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed, and samples of all preserved tissues from all animals were examined by light microscopy.

There were no deaths during the study. Treatment-related clinical signs were confined to animals treated at 40 000/30 000 ppm. Discoloured faeces, few or no faeces, thinness, slightly reduced activity and pale gums occurred in some animals. Two males and one female initially treated at 40 000 ppm showed black faeces for 1 or 2 days, but the occurrence may be related to stress resulting from offering unpalatable diet, rather than a primary effect of dinotefuran. One male treated at 40 000/30 000 ppm showing liquid/mucoid faeces was diagnosed with enteritis on day 8 and was taken off dose for 4 days. There were no other clinical signs after reduction of the dietary concentration to 24 000 ppm.

Body weights were significantly reduced in both sexes at 24 000 ppm from week 2, resulting in overall reductions in weight gain of 33.3% and 31.4% in males and females, respectively (Table 30). However, a large proportion of the reduced weight gain was associated with the administration of 40 000/30 000 ppm during the first 12 days of treatment, and subsequent treatment at 24 000 ppm resulted in only marginally lower weight gains. Females at 1600 and 8000 ppm also showed significantly lower body weights during the last 8 weeks of treatment and lower overall weight gains (34.3% and 31.4%, respectively).

Feed consumption was markedly and significantly reduced in both sexes treated at 40 000/30 000 ppm, indicating reduced diet palatability. Although feed consumption increased after the reduction of the dietary concentration to 24 000 ppm, reduced feed consumption persisted in both sexes throughout the study (Table 30).

There was a concomitant decrease in water consumption during the first 11 days of treatment at 40 000/30 000 ppm. Subsequently, the water consumption of the males remained depressed, but water consumption of the females was comparable to, or higher than, that of the control females from day 24. The feed consumption of females treated at 1600 and 8000 ppm was also low in comparison with the feed consumption of the female controls. However, the differences in feed consumption are considered not to be toxicologically relevant because they were not dose related and the predosing feed consumption at 1600 ppm was 16.4% lower than the control consumption. Water consumption at 1600 or 8000 ppm was not affected by treatment for both sexes.

There were no ocular lesions in any animal after 13 weeks of treatment, and there were no treatment-related haematological changes in either sex at any dose level.

Males and females at 24 000 ppm showed slightly but significantly reduced serum alanine aminotransferase (ALT) activity relative to both predosing values and the controls after 5 and 13 weeks of treatment. Urinary pH of males at 24 000 ppm was marginally lower than control values in weeks 5 and 14 (Table 30). There were no other treatment-related changes in the haematology, serum chemistry or urine analysis profiles at any dose level.

All organ weights and weight ratios were unaffected by treatment at all dose levels, and none at 24 000 ppm was significantly different from the control values. There were no treatment-related macroscopic or microscopic histopathological alterations at any of the dose levels employed. Although haemorrhage in the mesenteric and/or mandibular lymph nodes occurred in three of the four males treated at 24 000 ppm, its occurrence is considered incidental to treatment with dinotefuran, as haemorrhage was not evident in other organs, and none of the macroscopic or clinical pathology observations suggested a haemorrhagic condition in these animals. All other histopathological alterations occurred sporadically and without regard to sex or dose level.

The NOAEL in males was 8000 ppm (equal to 307 mg/kg bw per day), based on a reduction in feed and water consumption, body weight and body weight gain at 24 000–40 000 (equal to an average dose of 862 mg/kg bw per day). In females, a NOAEL could not be identified; the lowest-observed-adverse-effect level (LOAEL) was 1600 ppm (equal to 58 mg/kg bw per day), based on a reduction in feed consumption, body weight and body weight gain at all doses administered (Weiler, 1999a,b).

Table 30. Summary of selected findings in the 13-week dog study

	Males				Females			
	0 ppm	1600 ppm	8000 ppm	24 000 ppm	0 ppm	1600 ppm	8000 ppm	24 000 ppm
Body weight (kg)								
- week 1	8.1	8.0	8.2	8.3	7.1	7.0	7.2	7.0
- week 2	8.9	9.1	8.9	7.9*	7.9	7.7	7.8	6.8*
- week 4	9.9	9.9	9.6	9.1*	8.7	8.2*	8.4	7.8*
- week 8	10.8	11.0	10.6	10.0*	9.8	8.8*	9.0*	8.6*
- week 13	11.7	11.8	11.5	10.7*	10.6	9.3*	9.6*	9.4*
Body weight gain (kg), weeks 1–14	3.5	3.6	3.3	2.5*	3.5	2.3*	2.5	2.3*
Feed consumption (g/week)								
- week 1	2951	2913	2555	1394*	2712	2406	2588	869*
- week 2	2919	2671	2603	2381	2863	2311*	2557	2240*
- week 4	2875	2645	2686	2570	2773	2212*	2392*	2295*
- week 8	2958	2528	2708	2391*	2722	2203*	2350	2207*
- week 13	2990	2722	2896	2411*	2657	2102*	2631	2402
Water consumption (g/week)								
- day 2	915	780	743	138*	573	503	390	38*
- day 10	1455	958*	1260	798*	1030	903	818	748
- day 24	1423	1090	1295	1165	945	790	735	1035
- day 52	1550	980*	1270	963*	977	658	683	940
- day 86	1505	1095	1313	1000*	980	833	747	1103
Urinary pH, mean (range)								
- week −1	7.0 (7.0–7.0)	6.9 (6.5–7.5)	6.9 (6.5–7.0)	7.0 (6.5–7.5)	6.8 (6.5–7.5)	7.1 (6.0–7.5)	7.1 (7.0–7.5)	6.6 (6.5–7.0)
- week 5	8.1 (7.5–8.5)	7.5 (6.5–8.5)	6.8 (6.0–7.5)	6.5 (6.0–7.0)	6.9 (6.5–7.0)	6.8 (6.5–7.0)	6.5 (6.0–7.0)	6.5 (6.0–7.0)
- week 14	7.6 (7.0–8.5)	7.6 (7.5–8.0)	7.8 (7.0–8.5)	6.8 (6.0–7.5)	6.6 (6.5–7.0)	7.3 (6.5–8.0)	7.1 (7.0–7.5)	6.6 (6.0–7.0)
Lymph nodes, mesenteric; haemorrhage	0	0	0	3	1	0	1	2
Lymph nodes, mandibular; haemorrhage	0	0	0	1	0	1	0	0

From Weiler (1999a)
* $P < 0.05$

In a study of toxicity conducted according to OECD Test Guideline No. 452, groups of four male and four female Beagle dogs were fed diets containing dinotefuran (purity 93.0%) at a concentration of 0, 640, 3200 or 16 000 ppm (equal to 0, 20, 111 and 559 mg/kg bw per day for males and 0, 22, 108 and 512 mg/kg bw per day for females, respectively) for 52 weeks. The animals were observed twice daily for morbidity or mortality and daily for clinical signs of poor health or abnormal behaviour. Body weights and feed and water consumption were recorded weekly for 16 weeks and at 4-week intervals thereafter. Ophthalmoscopic examinations were conducted on all animals pretest and during week 52. Haematology, serum clinical chemistry and urine analysis were performed on all

animals pretest and in weeks 14, 27 and 53. Feed was withdrawn from the animals overnight prior to and during sampling. A sample of plasma was retained at each blood sampling interval from each animal and stored deep-frozen for possible future analysis. The animals were killed after at least 52 weeks of treatment and subjected to necropsy and postmortem examination. Feed was withdrawn from the animals overnight prior to necropsy. Organ weights were recorded, and samples of organs and tissues were preserved and prepared for histological evaluation and then examined microscopically from all animals.

All animals survived to the end of the treatment period, and no treatment-related clinical signs of toxicity or ocular defects occurred at any dose level. There was a treatment-related decrease in the overall body weight gains of both sexes at 16 000 ppm and of females at 3200 ppm. Body weight gains were 30–37% lower than the control values and statistically significant in the female groups. The body weight gains of males at 3200 ppm and both sexes at 640 ppm were unaffected by treatment (Table 31). Females treated at 3200 and 16 000 ppm showed 8% and 12% decreases, respectively, in feed consumption, but consumption was unaffected by treatment in the other groups. Water consumption was unaffected by treatment at all dose levels.

Table 31. Summary of selected findings in the 52-week dog study

	Males				Females			
	0 ppm	640 ppm	3200 ppm	16 000 ppm	0 ppm	640 ppm	3200 ppm	16 000 ppm
Body weight (kg)								
- week 1	8.7	8.7	8.9	8.5	8.0	7.9	7.9	7.9
- week 14	11.4	11.4	11.0	11.0	10.8	10.2	9.9*	9.7*
- week 28	11.6	11.7	11.5	10.7	11.3	10.6	10.3	10.2
- week 40	11.7	11.9	12.1	11.1	12.0	11.1	10.4*	10.2*
- week 52	11.7	11.6	11.9	10.6	11.5	11.4	10.1*	10.2*
Body weight gain (kg), weeks 1–52	3.0	2.9	3.0	2.1	3.5	3.6	2.3*	2.3*
Feed consumption (g/day), weeks 1–52	349	330	368	363	350	348	322	307
Thymus weight								
- absolute (g)	7.23	4.88	3.58	3.55	5.61	6.78	3.89	3.88
- relative (% of body weight × 100)	6.16	4.12	3.09	3.36	4.83	5.96	3.93	4.00
Thymus microscopic findings								
- cyst, ultimobranchial	0/4	0/3	3/4	2/4	1/4	1/4	1/4	1/4
- depletion, lymphocytic	0/4	1/3	2/4	0/4	1/4	1/4	1/4	0/4

From Weiler (1999c)
* $P < 0.05$

There were no treatment-related effects at any dose level on the haematological, serum clinical chemistry or urine analysis profiles. The only statistically significant differences between the 16 000 ppm group and the controls were higher urinary pH at week 27 and slightly higher serum albumin and potassium ion concentrations at week 53 in females. These differences are considered incidental to treatment with dinotefuran because they were not evident at other sampling intervals and were not associated with correlative clinical or histopathological alterations. There were no treatment-related effects at any dose level on the incidence of macroscopic findings at necropsy or on organ weights or ratios. An apparent treatment-related effect on the group mean thymus weight of all male treated groups was noted (Table 31). Statistically significant higher uterus to body weight ratios in

females treated at 3200 ppm and higher ovary to body weight ratios in females at 3200 and 16 000 ppm were attributed to lower body weights at termination. There were no treatment-related histopathological alterations at any dose level. All microscopic findings were typical of those occurring spontaneously in Beagle dogs.

A detailed examination of the individual thymus weights in male dogs showed that there was a single high value in one control animal (11.71 g), which represents the highest thymus weight recorded in 18-month-old control dogs at the study performing laboratory (Table 32). This value increases the contemporary control mean thymus weight to an unrepresentative value of 7.23 g (cf. historical control mean value of 5.94 g). Excluding this weight reduces the contemporary control mean to a value of 5.74 g, which is very close to the historical control mean value of 5.94 g. Furthermore, examination of the individual thymus weights showed that there are two dogs, one low-dose and one high-dose animal, with thymus weights (2.00 and 2.09 g, respectively) outside the historical control range (minimum value = 2.33 g). The low-dose male dog, with a thymus weight of 2.00 g, showed minimal lymphocytic depletion in the organ, an alteration that was not apparent in any of the high-dose males. This animal gained only 0.3 kg in weight during the entire 52-week treatment period, in contrast to all other treated males, which gained 1.4–4.4 kg, and to control males, which gained 2.3–3.9 kg. Although the animal gained 2.0 kg up to week 24, thereafter it progressively lost 1.7 kg in weight. As none of the other mid- or high-dose male animals showed a similar pattern of weight loss during the second half of the treatment period, the weight loss in this low-dose animal does not appear to be an effect of treatment, but the reason for it is unclear. Minimal kidney mineralization was the only other notable histomorphological change in this animal. Whatever the cause of weight loss during the latter part of the study was, this animal cannot be regarded as representative of the low-dose group. Therefore, excluding its unusually low thymus weight from the calculation of the group mean value, the low-dose group mean thymus weight is 5.83 g (cf. historical control mean value of 5.94 g and contemporary control mean [excluding aberrant value] of 5.74 g).

Table 32. Mean and individual thymus weights of males in the 52-week dog study[a]

	0 ppm	640 ppm	3200 ppm	16 000 ppm
Thymus weight (g), individual values	6.17	4.19	4.25	4.74
	11.71#	8.08	3.09	3.61
	5.67	5.23	4.37	3.74
	5.37	2.00#	2.60	2.09
Thymus weight (g), mean value	7.23	4.88	3.58	3.55
(mean, excluding one high/low value)	(5.74)	(5.83)		
Thymus weight (% of body weight × 100), individual values	5.5	3.4	3.4	4.6
	9.3#	6.6	2.6	3.3
	4.9	4.5	4.0	3.6
	4.9	2.0#	2.4	2.0
Thymus weight (g), mean value	6.2	4.1	3.1	3.4
(mean, excluding one high/low value)	(5.1)	(4.8)		

From Weiler (1999c); Gale (2005)

Value excluded for calculation of mean value

[a] Historical control data for thymus weights, mean [range]:

- Absolute weight (g): Males (n = 39): 5.94 [2.33–11.71]; females (n = 42): 4.83 [1.21–10.90]
- Relative weight (% of body weight × 100): Males (n = 39): 5.15 [2.23–9.42]; females (n = 42): 4.85 [1.49–11.93].

For the evaluation of the thymus weights of the male treated groups, the following facts were considered: 1) there was no corresponding effect on thymus weights in the 13-week dog study at comparable or even higher dose levels; 2) for the thymus weights at the middle and high doses, there

was no dose dependency, although dose levels were 5-fold different; and 3) the lower thymus weights were not correlated with plausible histopathological changes, especially with a depletion of lymphocytic tissue. Therefore, the lower group mean thymus weights in male dogs were not considered to be a treatment-related adverse effect.

The NOAEL in males was 3200 ppm (equal to 111 mg/kg bw per day), based on a reduction in body weight gain at 16 000 ppm (equal to 559 mg/kg bw per day). The NOAEL in females was 640 ppm (equal to 22 mg/kg bw per day), based on a reduction in feed consumption, body weight and body weight gain at 3200 ppm (equal to 108 mg/kg bw per day) and above (Weiler, 1999c; Gale 2005).

(b) Dermal application

Rats

In a range-finding dermal toxicity study, groups of five male and five female Crl:CD®(SD)IGS BR rats were treated with dinotefuran (purity 93.0%) as a suspension in 0.5% aqueous carboxymethylcellulose by dermal application at a nominal dose level of 0, 40, 200 or 1000 mg/kg bw per day for 14 days. Applications were made to clipped, intact dorsal skin sites (approximately 10% of body surface area) for 6–7 hours/day under semi-occluded dressings. The dressings were removed each day at the end of the exposure period, and the application sites were wiped with water to remove remaining dinotefuran formulation. The animals were observed twice daily for morbidity/mortality, and detailed clinical examinations outside the home cage were performed on days 1, 8 and 15. Dermal irritation reactions were scored immediately before application on days 1, 3, 5, 7, 9, 11, 13, 14 and 15. Body weights were recorded weekly starting on the 1st day of treatment. All animals were killed on day 15 and subjected to necropsy and postmortem examination of major organs and tissues. Samples of treated and untreated skin and gross lesions were preserved from all animals and, after histological preparation, examined microscopically.

There were no deaths or clinical signs of systemic toxicity at any dose level during the study. Slight to moderate skin atonia occurred on occasional days in all animals of both sexes treated at 1000 mg/kg bw per day and in all females treated at 200 mg/kg bw per day. Two males at 200 mg/kg bw per day, one control female and a female at 40 mg/kg bw per day also showed slight skin atonia. There was no other indication of dermal irritation at any dose level. In the absence of any dermal irritation (such as erythema or oedema) or any pathological changes in the skin, and due to the transient nature of this observation, the skin atonia was not considered adverse.

There were no treatment-related or statistically significant effects on body weight at any dose level. There were no treatment-related macroscopic or histopathological findings at any dose level. All histopathological findings in treated skin samples were considered to be incidental to treatment.

The NOAEL for systemic toxicity was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for local toxicity was 1000 mg/kg bw per day, the highest dose tested (Henwood, 2001a).

In a study of dermal toxicity conducted in compliance with OECD Test Guideline No. 410, groups of 10 male and 10 female Crl:CD®(SD)IGS BR rats were treated with dinotefuran (purity 93.0%) as a suspension in 0.5% aqueous carboxymethylcellulose by dermal application at a nominal dose level of 0, 40, 200 or 1000 mg/kg bw per day for 29 days. Applications were made to clipped, intact dorsal skin sites (approximately 10% of body surface area) for 6–7 hours/day under semi-occluded dressings. The animals were fitted with flexible collars during exposure. The dressings and collars were removed each day at the end of the exposure period, and the application sites were wiped with water to remove remaining dinotefuran formulation. The animals were observed twice daily for morbidity/mortality, and detailed clinical examinations outside the home cage were performed on days 1, 8, 15, 22 and 29. Changes in posture, reactivity to handling, tonic/clonic movements, stereotypical/bizarre behaviour patterns and gait abnormalities were also assessed weekly. On day 24/25, expanded clinical observations were performed on all animals. Motor activity of all animals

was quantitatively assessed on day 24/25. An ophthalmoscopic examination was performed on all animals predosing and on day 26. Dermal irritation reactions were scored immediately before application on days 1, 8, 15, 22 and 29 and on the day of necropsy (day 30). Body weights were recorded predosing and weekly thereafter, starting on the 1st day of treatment. Feed consumption was recorded weekly. Haematology and clinical chemistry analyses were performed on blood samples collected from all animals (feed deprived) on day 30. All survivors were killed on day 30 and subjected to necropsy, postmortem examination of major organs and tissues and organ weight recording. The decedent (see below) was also subjected to necropsy. A full range of tissues was preserved from all animals, and stained sections from the animals treated at 0 or 1000 mg/kg bw per day and the decedent were examined microscopically. Gross lesions from all animals were examined microscopically.

The death of a male animal treated at 40 mg/kg bw per day was considered incidental to treatment. All other animals survived the scheduled treatment period, and there were no clinical signs of systemic toxicity at any dose level. There were no treatment-related effects on the expanded clinical observation parameters evaluated and no statistically significant effects on quantitative motor activity. No ocular lesions were evident in any animal after 25 days of treatment. One male treated at 40 mg/kg bw per day and two females at 1000 mg/kg bw per day showed slight (grade 1) skin atonia at the application site on one or two occasions during the treatment period. There were no other signs of dermal irritation at any dose level. There were no treatment-related effects at any dose level on the feed consumption or body weight gains of either sex. There were no treatment-related effects in either sex at any dose level on the haematological or clinical chemistry parameters examined or on absolute or relative organ weights or macroscopic findings at necropsy. Treatment-related histopathological alterations were confined to an increase in the incidence and severity of acanthosis/hyperkeratosis in the treated skin of females at 1000 mg/kg bw per day (five animals, severity grade 1; three animals, severity grade 2), whereas this finding occurred in two control females (both animals severity grade 1). In males, all control and 1000 mg/kg bw per day animals showed this skin alteration (severity grades 1 and 2).

The NOAEL for systemic toxicity was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for local toxicity in males was 1000 mg/kg bw per day, the highest dose tested. In females, the LOAEL for local toxicity was 1000 mg/kg bw per day, based on an increase in the incidence and severity of acanthosis/hyperkeratosis in the treated skin; a NOAEL was not determined, as lower doses were not evaluated histopathologically (Henwood, 2001b).

(c) Exposure by inhalation

In a study of inhalation toxicity conducted in compliance with OECD Test Guideline No. 412, groups of 10 male and 10 female Crl:WI(GlxBRL/Han)BR rats were exposed for 6 hours/day for 29 or 30 days, by inhalation in nose-only chambers, to an atmosphere of dinotefuran (purity 99.1%) as a dust in air at a nominal concentration of 0 (air only), 2.89, 16.02 or 61.24 mg/l (equivalent to gravimetrically determined concentrations of 0, 0.22, 0.66 and 2.08 mg/l, respectively). The calculated MMAD ± GSD values were 2.03 ± 3.31 µm, 1.80 ± 3.60 µm and 1.55 ± 2.96 µm, respectively. The animals were observed twice daily for morbidity and mortality. All animals were observed daily for clinical signs immediately after the end of exposure. In addition, each animal was given a detailed physical examination at weekly intervals. Body weights were recorded before exposure on day 1, at weekly intervals and at necropsy. Feed consumption was determined weekly. Ophthalmoscopic examinations were performed on all animals pre-exposure and on all animals treated at 0 or 2.08 mg/l during week 4. Haematology, clinical chemistry and urine analysis were performed on all animals during week 4. Blood and urine samples were collected after overnight deprivation of feed and feed and water, respectively. All animals were subjected to necropsy after overnight deprivation of feed, and a full internal and external postmortem examination was performed. Selected organs were weighed. The tissues from the animals treated at 0 or 2.08 mg/l and gross lesions and respiratory tract tissues from all groups were examined microscopically.

There were no deaths or treatment-related clinical signs in any exposure group. Post-exposure clinical observations were predominantly localized thinning hair, attributable to the restraint procedure. The body weight gains of all male treated groups were significantly lower than the control gain during week 1. Thereafter, weight gains were slightly lower than the control values, but not statistically significant at any exposure concentration. The terminal body weights of the male treated groups were 5.2%, 3.8% and 6.4% lower than the control value, whereas the overall body weight gains were 19%, 21% and 25% lower than the control value, at 0.22, 0.66 and 2.08 mg/l, respectively (Table 33). The weight gain of all female treated groups was comparable to the control values throughout the study. The mean weekly feed consumption of all male treated groups in weeks 1 and 2 and in the high-dose group in week 3 was 4.3–10.7% lower than the control consumption. However, the differences were not statistically significant. The feed consumption of all female treated groups was unaffected by exposure to dinotefuran.

Table 33. Summary of selected findings in the 4-week rat inhalation study

	Males				Females			
	0 mg/l	0.22 mg/l	0.66 mg/l	2.08 mg/l	0 mg/l	0.22 mg/l	0.66 mg/l	2.08 mg/l
Body weight (g)								
- week 0	207.0	205.3	210.6	206.3	163.3	166.6	169.0	168.0
- week 4	271.9	257.8	261.7	254.6	189.3	193.7	190.2	197.6
Body weight gain (g)								
- weeks 0–1	26.7	17.1*	16.0**	14.3**	8.2	7.2	7.1	7.0
- weeks 1–4	38.2	35.4	35.2	34.0	17.8	19.8	14.1	22.5
- weeks 0–4	64.8	52.5	51.1*	48.3**	26.0	27.0	21.2	29.5
Food consumption (g)								
- week 1	146.3	137.8	138.9	130.7	109.5	110.1	109.0	109.7
- week 2	155.1	148.4	147.0	140.0	113.5	117.6	114.2	117.9
- week 3	155.3	153.9	151.3	145.7	117.2	122.8	119.2	120.2
- week 4	134.7	137.3	138.9	135.9	110.7	113.1	114.4	119.8
ALT (IU/l)	31	39	38	43**	41	42	38	41

From Shepherd (2002)
ALT, alanine aminotransferase; IU, international units; * $P < 0.05$; ** $P < 0.01$

There were no treatment-related ophthalmological findings at the highest exposure concentration. There were no treatment-related effects on the haematological or plasma and urine clinical chemistry profiles in either sex at any exposure concentration. There was a minimal, but statistically significant, difference from the controls in the ratio of neutrophils to lymphocytes in males exposed to 2.08 mg/l, but this was considered to reflect the biological variation inherent in small groups of animals. The group mean plasma ALT activity of the male group exposed to 2.08 mg/l was significantly higher than that of the male control group by 38.7%, but as the absolute value was within the range of historical control values, and in the absence of correlating histopathological alterations in the liver, it is not considered to be an adverse effect. All other haematological and clinical chemistry values at 2.08 mg/l were comparable to, and not significantly different from, the control values.

There were no treatment-related macroscopic findings or organ weight changes at any exposure concentration. There were no treatment-related histopathological alterations in the tissues of rats of either sex in the group exposed to 2.08 mg/l. Histopathological alterations were infrequent, minor in nature and consistent with the normal pattern of findings in rats of the strain and age used.

The nature and incidence of all microscopic findings were comparable in the treated and control groups.

The no-observed-adverse-effect concentration (NOAEC) in males was 0.22 mg/l, based on a reduction in body weight gain at 0.66 mg/l and above. The NOAEC in females was 2.08 mg/l, the maximum technically achievable aerosol concentration (Shepherd, 2002).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity conducted according to OECD Test Guideline No. 451, groups of 60 male and 60 female Crl:CD-1®(ICR)BR VAF/Plus® mice were given diets containing dinotefuran (purity 93.0%) at a concentration of 0, 25, 250, 2500 or 25 000 ppm (equal to 0, 3, 34, 345 and 3694 mg/kg bw per day for males and 0, 4, 45, 441 and 4728 mg/kg bw per day for females, respectively) for at least 78 weeks. An additional 10 animals of each sex per group, similarly treated, were killed after at least 52 weeks of treatment for interim evaluation. Animals were observed twice daily for morbidity or mortality, and detailed clinical examinations were performed weekly. Body weights and feed consumption were recorded weekly for 13 weeks and monthly thereafter. The water consumption of 12 animals of each sex per group was measured for 2 days/week each week for 13 weeks and monthly thereafter. Haematological investigations were performed in week 53 on 10 animals of each sex per group and in week 79 on all survivors. Ten animals of each sex per group were killed in week 53, and all remaining animals in week 79. All survivors and decedents were subjected to detailed necropsy and postmortem examination, but no organ weights were recorded in decedents. Organ weights were recorded in 10 animals of each sex per group killed after 52 and 78 weeks of treatment. Tissue/organ samples from all animals were preserved for possible light microscopy. Samples of liver, kidney and adrenal gland from two animals of each sex per group were also preserved for possible electron microscopy. All tissues from decedents and animals treated at 0 or 25 000 ppm and gross lesions, lungs, liver and kidneys from all animals were examined by light microscopy.

Survival was unaffected by treatment at all dose levels. Control male survival in week 79 was 73% compared with 72–83% in the dinotefuran-treated groups. Female control survival in week 79 was 76% compared with 67–80% in the dinotefuran-treated groups. There were no treatment-related clinical signs of toxicity, including the incidences of palpable tissue masses. Overall body weight gains of both sexes were significantly reduced by treatment at 25 000 ppm but were unaffected by treatment at lower dose levels. At 25 000 ppm, overall body weight gains were reduced by 17.9% and 25.2% in males and females, respectively, and group mean body weights at week 78 were 95.3% and 90.9% of control values in males and females, respectively (Table 34). Feed consumption and water consumption were unaffected by treatment at all dose levels. Treatment-related haematological effects were confined to slightly lower platelet counts in both sexes after 78 weeks of treatment at 25 000 ppm. The group mean counts were 19.9% and 17.4% lower than control values in males and females, respectively, but were statistically significant in males only. The effect was not apparent at lower dose levels after 78 weeks or in any group after 52 weeks of treatment.

There were no treatment-related macroscopic pathological findings or organ weight changes in either sex at any dose level after 52 and 78 weeks of treatment. The nature and incidence of non-neoplastic histopathological findings were similar in decedent animals and those surviving to termination, and there were no treatment-related differences between the control and treated groups. The nature and incidence of neoplastic changes were not influenced by treatment with dinotefuran at any dose level. There were no statistically significant trends or group differences in the incidences of any common tumours ($P > 0.01$) or rare tumours ($P > 0.05$), and all differences between the groups are considered to be normal biological variation.

Table 34. Summary of selected findings in the 78-week mouse study

	Males					Females				
	0 ppm	25 ppm	250 ppm	2500 ppm	25 000 ppm	0 ppm	25 ppm	250 ppm	2500 ppm	25 000 ppm
Body weight (g)										
- week 1	31.8	31.3	31.6	32.0	31.8	24.6	24.2	24.1	24.5	24.5
- week 13	39.9	39.8	39.9	39.2	37.9*	31.0	31.1	29.7*	30.1	29.3*
- week 50	46.1	46.7	45.9	45.7	41.6*	37.5	37.1	35.6	35.9	33.8*
- week 78	45.0	45.7	45.1	44.5	42.9	38.6	38.9	37.3	38.2	35.1*
Body weight gain (g), weeks 1–78	13.4	14.6	13.5	12.6	11.0*	13.9	14.8	13.4	13.6	10.4*
Platelet count (10^9/l)										
- week 53	1125	1138	1241	1329	1147	1028	1139	988	1063	1035
- week 79	1405	1309	1545	1245	1125*	1038	1011	1021	966	857
Spleen weight										
- absolute (g)	0.287	0.168	0.129	0.200	0.150	0.317	0.305	0.196	0.179	0.146
- relative (% of body weight)	0.69	0.37	0.31	0.47	0.37	0.87	0.80	0.55	0.50	0.45
Lung tumours										
- adenoma	4	5	6	3	6	5	4	5	5	3
- carcinoma	3	0	0	1	0	0	0	0	1	0
- adenoma + carcinoma	7	5	6	4	6	5	4	5	6	3
Liver tumours										
- adenoma	9	13	8	6	5	0	0	1	1	0
- carcinoma	4	3	1	1	2	0	0	0	0	0
- adenoma + carcinoma	13	16	9	7	7	0	0	1	1	0

From Weiler (2000d)
* $P < 0.05$

The NOAEL for carcinogenicity was 25 000 ppm (equal to 3694 mg/kg bw per day for males and 4728 mg/kg bw per day for females), the highest dose tested. The NOAEL for toxicity was 2500 ppm (equal to 345 mg/kg bw per day for males and 441 mg/kg bw per day for females), based on reduced body weight and body weight gain at 25 000 ppm (Weiler, 2000d,e).

Rats

In a combined study of chronic toxicity and carcinogenicity conducted according to OECD Test Guideline No. 453, groups of 60 male and 60 female Crl:CD®(SD)BR VAF/Plus® rats were given diets containing dinotefuran (purity 93.0%) at a concentration of 0, 60, 200, 2000 or 20 000 ppm (equal to 0, 3, 10, 100 and 991 mg/kg bw per day for males and 0, 4, 13, 127 and 1332 mg/kg bw per day for females, respectively) for at least 104 weeks. Additional groups of 10 animals of each sex per group were similarly treated for at least 26, 52 and 78 weeks, and further groups of 10 animals of each sex treated at 0 and 20 000 ppm were treated for 26 weeks and then maintained untreated for 6 weeks before necropsy. Morbidity and mortality checks were performed twice daily, and a detailed clinical examination of all animals was performed weekly. Body weights and feed consumption were recorded weekly for 13 weeks and every 4 weeks thereafter. Ophthalmic examinations were performed on all animals pretest and after 26, 32, 78 and 104 weeks. Haematology, serum clinical chemistry and urine analysis were performed on 10 animals of each sex per group after 26, 52, 78 and 104 weeks and after 26 weeks of treatment for the animals treated at 0 and 20 000 ppm followed by 6

weeks treatment free. After 26, 52 and 78 weeks of treatment, 10 animals of each sex per group were killed. The additional 10 animals of each sex per group treated at 0 and 20 000 ppm for 26 weeks were killed after a 6-week treatment-free period. All other survivors were killed after at least 104 weeks of treatment. All decedents and scheduled kill animals were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed from 10 animals of each sex per group, and samples of major organs and tissues were preserved from all animals. Samples of liver, kidney and adrenal were retained from two animals of each sex per group at each scheduled sacrifice for possible electron microscopic examination. All preserved tissues from the animals treated at 0 or 20 000 ppm and from decedents in all groups were examined by light microscopy. Gross lesions, endocrine glands, tissue masses, kidneys, liver, lungs and reproductive organs of both sexes were also examined from all animals in the intermediate-dose group.

Survival of both sexes to 104 weeks was unaffected by treatment at all dose levels. Control male survival in week 104 was 47% compared with 25–38% in the intermediate-dose groups and 53% in the high-dose dinotefuran group. Control female survival after 104 weeks was 33% compared with 30–43% in the dinotefuran-treated groups. There were no treatment-related clinical signs of toxicity, including the incidences of palpable tissue masses. There were no treatment-related ophthalmological findings at any dose level at any of the examination intervals. The body weight gains of both sexes were reduced by treatment at 20 000 ppm from week 2, but the effect was more severe in the females, with overall (weeks 1–104) body weight gains reduced by 5.5% and 44.1% in males and females, respectively. Body weight gain was unaffected by treatment at lower dose levels (Table 35). The mean weekly feed consumption of animals treated at 20 000 ppm was reduced by up to 10.0% during the first 77 weeks of treatment, but water intake was unaffected by treatment at all dose levels. Feed consumption was not affected by treatment at dose levels up to 2000 ppm (Table 35).

Table 35. Summary of selected findings in the 104-week rat study

	Males					Females				
	0 ppm	60 ppm	200 ppm	2000 ppm	20 000 ppm	0 ppm	60 ppm	200 ppm	2000 ppm	20 000 ppm
Body weight (g)										
- week 1	228	232	229	231	228	178	177	174	175	176
- week 26	685	694	692	702	638*	344	352	362*	350	314*
- week 50	794	802	815	822	722*	411	418	439*	418	347*
- week 78	836	841	865	867	777*	482	490	529*	497	377*
- week 105	758	792	808	800	729	553	608	565	545	417*
Body weight gain (g), weeks 1–104	531	565	585	574	504	376	433	395	370	242*
Feed consumption (g)										
- week 1	185	180	179*	181	167*	133	130	132	133	120*
- week 25	200	200	200	204	184*	145	145	146	146	133*
- week 49	205	201	204	213	189*	152	150	155	148	140*
- week 77	204	189	205	204	195	163	166	167	167	145*
- week 101	180	183	167	183	168	156	161	150	160	142
Kidney										
No. examined	*100*	*90*	*89*	*89*	*100*	*100*	*90*	*89*	*90*	*100*
- lymphohistiocytic infiltrate	42	51*	39	49*	65**	43	43	35	41	40
- tubular epithelial basophilia	42	47	37	51*	57*	48	42	34	40	26**

Table 35 (continued)

	Males					Females				
	0 ppm	60 ppm	200 ppm	2000 ppm	20 000 ppm	0 ppm	60 ppm	200 ppm	2000 ppm	20 000 ppm
- basement membrane thickened	30	35	32	44**	44*	23	24	19	19	15
- pelvic mineralization	5	5	4	7	27**	42	42	42	44	47
- chronic progressive nephropathy	35	26	36	24	14**	5	8	12*	8	0*
Thymus										
No. examined	*96*	*39*	*38*	*40*	*99*	*100*	*42*	*44*	*41*	*98*
- lymphocytic depletion	5	3	3	3	13*	9	4	2	2	11
Thyroid										
No. examined	*99*	*89*	*90*	*88*	*100*	*100*	*90*	*90*	*89*	*100*
- C-cell adenoma	8	12	10	12	17*	12	11	12	5	13
- C-cell carcinoma	1	0	0	0	0	0	0	1	1	1
- C-cell adenoma + carcinoma	9	12	10	12	17	12	11	13	6	14
Testes										
No. examined	*100*	*89*	*90*	*89*	*99*	—	—	—	—	—
- interstitial cell tumour	2	1	3	1	5	—	—	—	—	—
Uterus										
No. examined	—	—	—	—	—	*100*	*90*	*90*	*90*	*100*
- endometrial stromal polyps	—	—	—	—	—	1	0	3	3	6
Cervix										
No. examined	—	—	—	—	—	*100*	*40*	*43*	*40*	*100*
- endometrial stromal polyps	—	—	—	—	—	1	1	1	1	1
Vagina										
No. examined	—	—	—	—	—	*100*	*89*	*90*	*90*	*100*
- endometrial stromal polyps	—	—	—	—	—	0	0	1	1	0
Total (uterus + cervix + vagina)	—	—	—	—	—	2	1	5	5	7*
Mammary gland										
No. examined	—	—	—	—	—	*99*	*90*	*90*	*89*	*100*
- adenoma	—	—	—	—	—	11	11	7	9	9
- carcinoma	—	—	—	—	—	13	15	17	18	22
- adenoma + carcinoma	—	—	—	—	—	24	26	24	27	31

From Weiler (2000c)
* $P < 0.05$; ** $P < 0.01$

There were no treatment-related effects on haematology, serum clinical chemistry or urine analysis parameters at any dose level at any sampling interval. There were no treatment-related effects on the incidence of macroscopic findings at any necropsy interval. However, the mean number of uterine masses was higher than in the controls in the groups treated at 2000 and 20 000 ppm. There were 3, 1, 5 and 8 masses in the groups treated at 60, 200, 2000 and 20 000 ppm, respectively,

compared with 2 masses in the control group. The masses were commonly endometrial stromal polyps, but the incidence of the lesion and other uterine neoplasms was not significantly different from the controls ($P > 0.05$). There were no other notable differences in the incidence of macroscopic lesions between the treated and control groups. There were no treatment-related organ weight changes in either sex at any dose level after 26, 52, 78 and 104 weeks of treatment other than in females treated at 20 000 ppm, in which liver weight at week 79 was reduced as a consequence of growth retardation. There were no treatment-related effects on the nature and incidence of adverse non-neoplastic histopathological findings at any dose level. However, males treated at 20 000 ppm showed higher incidences of the renal changes pelvic mineralization, lymphohistiocytic infiltrate, tubular epithelial basophilia and thickening of the basement membrane. None of these renal changes was considered to be a treatment-related effect, as they either are common findings in rats or can be correlated with the lower incidence of chronic progressive nephropathy in males at 20 000 ppm. Similarly, the increased incidence of thymic lymphocyte depletion in males at 20 000 ppm was considered not to be a treatment-related effect, as these changes occur commonly in ageing rats.

There were no treatment-related effects at any dose level on the nature and incidence of tumours. However, pooled data from all animals showed differences between the control and 20 000 ppm group in the incidence of four tumour types. The incidence of thyroid C-cell adenomas was significantly higher in males treated at 20 000 ppm (17%) than in the controls (8%), but was within the historical control range of 1.7–24%. As this neoplasm is a common finding in the rat and because the total number of C-cell neoplasms (adenomas plus carcinomas) was not significantly higher than in the controls, this finding was considered not biologically relevant. Benign testicular interstitial cell tumours occurred at an incidence of 5.6% in animals treated at 20 000 ppm compared with a control incidence of 2.0%. As the difference from the controls was not statistically significant and the incidence was within the historical control range of 1.3–6.7%, the higher incidence is considered not to be treatment related. Benign endometrial stromal polyps occurred at a higher incidence in the uterus of animals treated at 20 000 ppm than in the controls, but the incidence in the uterus alone was not statistically significant. The combined incidence of this lesion in uterus, cervix and vagina at 20 000 ppm (7.0%) was significantly higher than the control incidence of 2.0%, but remained within the historical control range of 1.0–14%. As endometrial stromal polyps are considered a common spontaneous neoplasm, the slightly increased incidence at 20 000 ppm was considered not to be treatment related. Also, the incidence of mammary gland carcinomas was higher at 20 000 ppm (22%) than in the controls (13%), but was considered not to be treatment related, as it was not statistically significant and remained within the historical control range of 10.0–26.7%. With the exception of significantly lower incidences of pituitary adenomas in both sexes ($P < 0.05$) and adrenal phaeochromocytomas in males ($P < 0.01$), the incidences of all other tumours in the group treated at 20 000 ppm were not significantly different from the controls.

The NOAEL for carcinogenicity was 20 000 ppm (equal to 991 mg/kg bw per day for males and 1332 mg/kg bw per day for females), the highest dose tested. The NOAEL for toxicity was 2000 ppm (equal to 100 mg/kg bw per day for males and 127 mg/kg bw per day for females), based on a reduction in body weight, body weight gain and feed consumption at 20 000 ppm (Weiler, 2000c).

2.4 Genotoxicity

The results of the genotoxicity studies with dinotefuran are summarized in Table 36.

In a reverse gene mutation assay in bacteria conducted according to OECD Test Guideline No. 471, *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvrA*) were exposed to dinotefuran (purity 96.5%) dissolved in dimethylsulfoxide (DMSO) in the presence and absence of S9 metabolic activation, using the preincubation method. Doses were selected based on the results of a range-finding study. For the main assay, doses of up to 5000 µg/plate were used, with three plates for each strain, condition and dose. Vehicle and positive controls were included in each experiment. In the main assay, dinotefuran did not influence the growth of any strain tested at dose levels of up to 5000 µg/plate. No appreciable increase in the reversion frequencies occurred in any strain tested in the dose range 313–5000 µg/plate. In contrast,

the positive control substances produced marked increases in the number of revertant colonies in all strains tested. Under the conditions of this study, dinotefuran did not induce gene mutations in the strains of *S. typhimurium* and *E. coli* used at doses of up to 5000 µg/plate (Takeda, 1996).

Table 36. Summary of genotoxicity studies with dinotefuran

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537)	± S9 mix: 0–5000 µg/plate	96.5	Negative	Takeda (1996)
	Escherichia coli (WP2*uvrA*)				
DNA repair (rec-assay)	*Bacillus subtilis* (M45 *rec*$^-$, H17 *rec*$^+$)	± S9 mix: 0–16 000 µg/disc	96.5	Negative	Oguma (1999)
Gene mutation, *TK* locus	Mouse lymphoma L5178Y cells	± S9 mix: 0–2022 µg/ml	99.1	Negative	Lloyd (2002)
Chromosomal aberration	Chinese hamster lung (CHL) fibroblast cells	± S9 mix: 0–2000 µg/ml	96.5	Negative	Satou (1996)
In vivo					
Micronucleus induction	Male BDF1 (C57BL/6 × DBA/2) mice, bone marrow erythroblasts	0, 270, 540 and 1080 mg/kg bw; twice (24 h apart); oral administration	99	Negative	Nakajima (1995)

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

In a DNA repair assay (rec-assay) in bacteria conducted according to JMAFF test guideline 59 NohSan No. 4200 (1985), *Bacillus subtilis* strains M45 (*rec*$^-$) and H17 (*rec*$^+$) were exposed to dinotefuran (purity 96.5%) dissolved in DMSO in the presence and absence of S9 metabolic activation. Doses were selected based on the results of a range-finding study. For the main assay, doses of up to 16 000 µg/plate were used, with two plates for each strain, condition and dose. Vehicle (DMSO), negative controls (kanamycin or streptomycin) and positive controls (mitomycin C or 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole acetate) were included in each experiment. In the main assay, DMSO solvent alone and all doses of dinotefuran employed, up to and including the highest dose, 16 000 µg/plate, produced no growth inhibition of either strain of *B. subtilis* with or without S9 metabolic activation. The negative control substances without and with S9 produced differences in the inhibition zone diameters of 1.7 and 1.1 mm, respectively, whereas the positive control substances without and with S9 produced differences of 7.2 and 6.0 mm, respectively. Under the conditions of the study, dinotefuran did not exhibit DNA-damaging activity in *B. subtilis* at doses of up to 16 000 µg/plate (Oguma, 1999).

In an in vitro mammalian cell gene mutation test conducted according to OECD Test Guideline No. 476, dinotefuran (purity 99.1%) was tested for its ability to induce forward mutations at the *TK* locus in mouse lymphoma L5178Y cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. The highest dose levels for the main assay, equivalent to 10 mmol/l, were determined on the basis of two preliminary cytotoxicity tests. In experiment 1, duplicate cultures were exposed for 3 hours to dinotefuran in saline at a final concentration of 0 (saline), 400, 800, 1200, 1600 or 2022 µg/ml, both with and without metabolic activation. In experiment 2, the same concentrations were assayed without metabolic activation for a 24-hour exposure period or with metabolic activation for a 3-hour exposure period. Concurrent positive controls with and without S9 were also tested. After the exposure period, cells were washed

and cultures were transferred to flasks for a 2-day mutation expression period. Then, cultures were incubated for 12 days in selection medium containing trifluorothymidine at 3 µg/ml. Wells containing large and small colonies were enumerated for the negative and positive controls. In both experiments, relative survival at the highest concentration tested was at least 76% with and without metabolic activation. No statistically significant increases in mutation frequency occurred at any concentration in the absence or presence of metabolic activation in either independent experiment. Marked increases in the numbers of both small and large colony mutants occurred in response to both positive control materials. Under the conditions of the study, dinotefuran did not induce mutation at the *TK* locus of L5178Y mouse lymphoma cells at concentrations of up to 10 mmol/l in the presence and absence of S9 metabolic activation (Lloyd, 2002).

In an in vitro mammalian chromosomal aberration test conducted according to OECD Test Guideline No. 473, the clastogenic potential of dinotefuran (purity 96.5%) was tested in Chinese hamster lung (CHL/IU) cells. Concentrations for the main assay were determined following a preliminary cytotoxicity test, and the high concentration selected was 2000 µg/ml (10 mmol/l). A solvent control (saline) and a series of three concentrations (500, 1000 and 2000 µg/ml) were used for each of the four treatment regimens: 6-hour exposure without S9, 6-hour exposure with S9 and 24-hour or 48-hour exposure without S9. All dose levels were evaluated for clastogenicity by evaluating 200 metaphase cells per concentration. Cell survival was determined at harvest. Mitomycin C (0.03 µg/ml) was the positive control for the 24-hour and 48-hour exposures without activation, and cyclophosphamide (12 µg/ml) was the positive control for the 6-hour exposures with and without activation. Structural or numerical aberrations were not induced by dinotefuran after either 24 or 48 hours of exposure to concentrations up to 2000 µg/ml. However, slight growth inhibition occurred at 2000 µg/ml, 28% after the 24-hour exposure and 38% after the 48-hour exposure. Similarly, structural or numerical aberrations were not induced after 6 hours of exposure to dinotefuran in either the presence or absence of metabolic activation. Cell survival was unaffected at all concentrations after 6 hours of exposure. Both mitomycin C and cyclophosphamide produced marked increases in the incidences of structural aberrations, notably chromatid breaks and exchanges. Under the conditions of this study, dinotefuran did not induce structural or numerical chromosomal aberrations in Chinese hamster lung cells at concentrations of up to 2000 µg/ml (Satou, 1996).

In a mammalian erythrocyte micronucleus test conducted according to JMAFF test guideline 59 NohSan No. 4200 (1985), groups of six male BDF1 (C57BL/6 × DBA/2) mice received two oral (gavage) doses (24 hours apart) of dinotefuran (purity 99%) at 0 (0.5% aqueous carboxymethylcellulose), 270, 540 or 1080 mg/kg bw in a volume of 20 ml/kg bw. The dose levels were based on the results of a range-finding study in which groups of six male mice were treated once daily, by oral gavage, for 2 days with dinotefuran at a dose level of 648, 1080, 1800, 3000 or 5000 mg/kg bw. The vehicle served as negative control, and mitomycin C (2 mg/kg bw, intraperitoneally) as positive control. The animals were terminated 24 hours after the last administration, the bone marrow of the femur was prepared, and 1000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The ratio of polychromatic erythrocytes to normochromatic erythrocytes was determined in 1000 erythrocytes. In the range-finding study, all mice treated at 3000 or 5000 mg/kg bw and four of the six mice treated at 1800 mg/kg bw died, and the mean incidences of micronucleated polychromatic erythrocytes were similar between treated groups and between the different sampling time points (24, 48 and 72 hours after dosing). In the main study, there were no deaths during the treatment period, and no signs of toxicity, including body weight change, were evident at any dose level. The micronucleated polychromatic erythrocyte frequencies and polychromatic erythrocyte/total erythrocyte ratios of all dinotefuran-treated groups were comparable to and not significantly different from control values. In contrast, mitomycin C produced a statistically significant increase in the micronucleated polychromatic erythrocyte frequency and a statistically significant decrease in the polychromatic erythrocyte/total erythrocyte ratio. Under the conditions of this study, dinotefuran did not induce the formation of micronuclei in bone marrow erythroblasts of mice after two oral doses of up to 1080 mg/kg bw (Nakajima, 1995).

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a dose range–finding reproductive toxicity study, groups of six male and six female Hanlbm: WIST (SPF) rats were given diets containing dinotefuran (purity 98.9%) at a concentration of 0, 10 000 or 20 000 ppm for 2 weeks before mating and during the mating period. The substance intakes at 10 000 and 20 000 ppm during the premating period were equal to 700 and 1340 mg/kg bw per day for males and 779 and 1507 mg/kg bw per day for females, respectively. P generation males continued to be treated until necropsy, shortly before weaning of the F_1 litters, whereas P generation females continued to be treated throughout gestation and lactation until necropsy shortly after weaning of the F_1 litters. Pregnant females were allowed to litter normally and rear their young to weaning. Litters were culled on day 4 to four pups of each sex, where possible. After weaning (day 21), six F_1 animals of each sex per group were treated at the same dietary concentrations as the P generation for a further 2 weeks. The substance intakes at 10 000 and 20 000 ppm from weaning were at least 745 and 1585 mg/kg bw per day for males and 770 and 1796 mg/kg bw per day for females, respectively.

P generation animals were checked at least twice daily for morbidity and mortality and for clinical signs of a reaction to treatment. Body weights were recorded weekly except during pairing. After mating, females were weighed on days 0, 7, 14 and 21 of gestation and days 1, 4, 7, 14 and 21 postpartum. Feed consumption was measured weekly throughout the study until day 14 postpartum, except during mating. Towards the end of gestation, females were examined twice daily for evidence of parturition. Day 0 of lactation was the day of completion of parturition. P generation animals were subjected to necropsy and postmortem examination. Implantation sites were counted in dams with litters, and uteri were placed in ammonium sulfide solution to aid the visualization of implantation sites. Major organs, including testes/ovaries, were preserved from P generation animals, weaned F_1 pups and F_1 pups selected for further study. An epididymal sperm sample was obtained from all P generation males, and one epididymis and one testis were retained for the determination of homogenization-resistant spermatids and caudal epididymal sperm reserve. Ovarian histopathology comprising quantitative primordial follicle counts in 10 levels per ovary and a comparison with secondary/tertiary follicles was performed on all females treated at 0 or 20 000 ppm.

Litters were examined as soon as possible after birth for litter size, live births, stillbirths and gross abnormalities. The sex ratio was determined on days 0, 4 and 21 of lactation. Pups were weighed on days 0/1, 4, 7, 14 and 21 of lactation. Dams and pups were observed daily for survival and abnormalities in nesting or nursing behaviour. F_1 animals selected for 2 weeks of further treatment were weighed on days 24, 28 and 35 postpartum, and feed consumption was recorded at 4- or 6-day intervals. Dead pups, except where excessively cannibalized, were subjected to necropsy. All F_1 pups were culled on day 4 postpartum, and pups not selected for further study were culled on day 21; pups selected for 2 weeks of further treatment were subjected to necropsy and examined for gross abnormalities.

There were no deaths in the P generation at any dose level, and treatment-related clinical signs were confined to soft faeces in females at 20 000 ppm during lactation. There was a treatment- and dose-related decrease in feed consumption and body weight gain throughout the treatment period in females treated at 10 000 and 20 000 ppm. Thus, the group mean body weights were significantly lower than those of the control group at the end of the prepairing, gestation and lactation periods. Males treated at 20 000 ppm also showed reduced body weight gain and significantly reduced feed consumption during the 1st week of treatment (Table 37).

Table 37. Summary of selected findings in the dose range–finding reproductive toxicity study in rats

	Males			Females		
	0 ppm	10 000 ppm	20 000 ppm	0 ppm	10 000 ppm	20 000 ppm
P generation						
Body weight gain (g)						
- weeks 1–2	36	25	14	16	11	6
- weeks 3–7	54	45	41	—	—	—
- gestation	—	—	—	122	102	82
- lactation	—	—	—	52	26	7
Feed consumption (g/day)						
- week 1	24.4	22.6	20.3*	18.6	15.4**	14.6**
- week 2	24.8	23.6	21.6	18.4	16.7**	16.1*
- weeks 3–7	24.1	24.2	22.3	—	—	—
- gestation	—	—	—	21.3	18.9	19.6
- lactation	—	—	—	43.8	40.4	32.9
F_1 generation (after weaning)						
Body weight (g)						
- day 1	48	35**	31**	46	34**	29**
- day 14	118	95*	76**	99	84*	70**
Body weight gain (g), days 1–14	70	60	45	53	50	41
Feed consumption (g/day)						
- days 1–4	7.0	3.2**	2.9**	6.3	3.1**	2.8**
- days 4–8	11.9	10.2	8.3**	10.6	9.6	8.2**
- days 8–14	15.8	12.6*	11.0**	13.3	12.0	10.2**

From Edwards, Knuppe & Weber (2001)
* $P < 0.05$; ** $P < 0.01$

Treatment-related gross findings at necropsy in the P generation were confined to an increased incidence of small thymus in females at 20 000 ppm (incidences were 2/6, 3/6 and 6/6 in the groups treated at 0, 10 000 and 20 000 ppm, respectively). There were no treatment-related gross findings at necropsy in the surplus F_1 generation pups culled on day 4 or 21 postpartum or in animals selected for 2 weeks of treatment after weaning, although one male animal at 20 000 ppm died in the 1st week after selection. There were no treatment-related effects at either dose level in P generation males on sperm motility (% progressively motile sperm), sperm morphology or sperm counts (homogenization-resistant spermatids and caudal epididymal sperm counts). There was no treatment-related effect on the number and distribution of ovarian follicle types in females treated at 20 000 ppm. Although the chi-squared test revealed a significant ($P < 0.001$) difference between the control and treated groups in the distribution of follicle types, the difference was due to a slightly higher number of primordial follicles in the treated group, which is considered to be within the normal range of variation. Moreover, the *t*-test revealed negative results only. Therefore, dinotefuran was considered not to have altered ovarian anatomy at a dose level of 20 000 ppm (Table 38).

Table 38. Summary of selected reproductive findings in the dose range–finding reproductive toxicity study in rats

	0 ppm	10 000 ppm	20 000 ppm
Ovary			
- no. of primordial follicles	749	NE	877
- no. of growing primordial follicles	616	NE	665
- no. of secondary/tertiary follicles	1151	NE	1149
Mean duration of gestation (days)	21.5	22.0	22.0
Total no. of litters born	6	6	6
Mean no. of implantations/dam	14.5	12.0	11.5*
Birth index[a]	95.4	95.8	79.7**
Post-implantation loss, total (% implantations)	4 (4.6)	3 (4.2)	14** (20.3)
Live pups at birth (mean/dam)	13.8	11.5	9.2*
Dead pups at birth (mean/dam)	0.0	0.0	0.0
Mean postnatal loss, days 0–4 (mean/dam)	0.3	0.0	0.2
Mean no. of live pups on day 4	8.0	8.0	7.5
Viability index[b]	97.6	100.0	98.2
Postnatal loss, days 5–21 (mean/dam)	0.2	0.0	0.5
Mean no. of live pups on day 21	7.8	8.0	7.0
Weaning index[c]	97.9	100.0	93.3
Sex ratio (% males)	49	54	48
Pup body weights (g), males + females			
- day 0	5.1	5.3	5.3
- day 4	7.7	7.9	8.1
- day 7	12.9	12.3	11.5
- day 14	29.5	22.4*	19.4**
- day 21	46.8	35.0*	29.0**

From Edwards, Knuppe & Weber (2001)
NE, not evaluated; * $P < 0.05$; ** $P < 0.01$
[a] No. of liveborn pups × 100 / no. of implantations.
[b] No. of pups alive on day 4 × 100 / no. of liveborn pups.
[c] No. of pups alive on day 21 × 100 / no. of pups alive on day 4.

There was no effect of treatment on fertility or mating performance. All females in all experimental groups mated successfully and reared pups to weaning. The median precoital time was comparable in the treated and control groups. There was a treatment-related decrease in the number of implantations and increased post-implantation loss, leading to a significantly reduced mean litter size at birth, at 20 000 ppm. Although the mean number of implantations and litter size at birth at 10 000 ppm were lower than control values, a treatment effect was not indicated, because neither parameter was statistically significant or the control values were higher than normal (Table 38).

Neonatal and preweaning viability, the weaning indices and sex ratios at 10 000 and 20 000 ppm were not significantly different from those of the control group. There was a treatment- and dose-related decrease in the preweaning body weight gain of male and female pups at 10 000 and 20 000 ppm. The effect was statistically significant from day 14 postpartum, and at weaning on day 21, the group mean pup weights for the sexes combined were reduced by 25.2% and 38.0%, respectively (Table 38).

The weight gain of F_1 generation animals treated for 2 weeks after weaning continued to be depressed in both sexes at both dose levels. The feed consumption of both sexes at both dose levels was also markedly depressed, particularly during the first 4 days of treatment.

No NOAEL for parental toxicity and offspring toxicity could be identified, because reduced body weight, body weight gain and feed consumption were observed at all dose levels. The LOAEL was 10 000 ppm (equal to 700 mg/kg bw per day). The NOAEL for reproductive toxicity was 10 000 ppm (equal to 700 mg/kg bw per day), based on a reduced number of implantations, an increase in post-implantation loss and a reduced litter size at birth at 20 000 ppm (equal to 1340 mg/kg bw per day) (Edwards, Knuppe & Weber, 2001).

In a two-generation reproductive toxicity study conducted according to OECD Test Guideline No. 416, groups of 25 male and 25 female Hanlbm: WIST (SPF) rats were given diets containing dinotefuran (purity 98.9%) at a concentration of 0, 300, 1000, 3000 or 10 000 ppm (equal to 0, 24.1, 79.9, 241 and 822 mg/kg bw per day for P generation males [premating period] and 0, 26.8, 90.1, 268 and 907 mg/kg bw per day for P generation females [premating period] for 10 weeks prior to mating through to weaning of the F_1 offspring. Groups of 25 male and 25 female F_1 generation offspring were then similarly treated; the mean substance intakes at 0, 300, 1000, 3000 and 10 000 ppm were equal to 0, 27.2, 90.5, 269 and 935 mg/kg bw per day for F_1 generation males (premating period) and 0, 29.6, 96.5, 293 and 1005 mg/kg bw per day for F_1 generation females (premating period). In P and F_1 parental animals, clinical signs were recorded daily, body weight and feed consumption were recorded approximately weekly, estrous cyclicity was monitored, the duration of gestation was recorded and the F_1 animals were examined for sexual development landmarks. Pregnant females were allowed to litter normally, and the litters were examined for live births, stillbirths and external abnormalities. The sexes and body weights of pups were recorded. Litters were not standardized by culling. Anogenital distance was measured in all F_2 generation pups on day 1 of lactation. At 6 weeks of age, F_1 animals selected for functional investigations (20 animals of each sex per group) were subjected to assessment of behavioural function, grip strength and locomotor activity.

All surviving and decedent P and F_1 parental animals were subjected to necropsy and gross pathological examination. Analyses for sperm motility, morphology and epididymal counts and quantitative primordial ovarian follicle counts were performed. Major organs, including reproductive organs, were weighed. Histopathological examination of reproductive organs, pituitary and adrenal glands was performed on all P and F_1 generation parental animals treated at 0 or 10 000 ppm. Additional testicular histopathology, qualitative sperm staging, was performed. Histopathology was also performed on the reproductive organs of animals treated at 300, 1000 or 3000 ppm that failed to mate.

Unselected F_1 weanlings, decedent F_1 offspring and weaned F_2 offspring were subjected to gross necropsy. One male and one female weanling per litter per generation were subjected to organ weight analysis of brain, spleen, thymus and uterus. Reduced-weight thymus and spleen of F_1/F_2 weanlings were examined microscopically.

P/F_1 parental animals: One P generation female at 10 000 ppm died prematurely on day 21 postpartum after showing clinical signs of soft faeces, ruffled fur and blood-stained urine during lactation. Macroscopic and histopathological examination showed renal changes that were considered causal of death. Although death may have been incidental to treatment with dinotefuran, a treatment-related etiology cannot be precluded because of its occurrence at the highest dose level at a time of greatly increased dosage. All other P and F_1 generation parental animals survived the scheduled treatment period. Treatment-related clinical signs were confined to soft faeces during lactation in all P generation females at 10 000 ppm and one F_1 generation female treated at 10 000 ppm. The observation was considered to be treatment related, as its occurrence was confined to the lactation period, at which time high dose levels were ingested. No treatment-related clinical signs occurred in males at 10 000 ppm or in either sex at lower dose levels in either parental generation.

Minor and transient effects on feed consumption occurred during the prepairing period, which were suggestive of reduced diet palatability. P and F_1 generation parental animals of both sexes showed significantly reduced feed consumption during weeks 1 and/or 2 of treatment. P generation males at 300 and 1000 ppm also showed slightly lower feed consumption compared with the controls during the 1st week of treatment only. Thereafter, there was no clear treatment-related effect on the feed consumption of either sex at any dose level during the prepairing period. The feed consumption of the P generation females during gestation was not affected by treatment at any dose level, but was significantly reduced by 11.3% during the 1st week of gestation in F_1 generation females at 10 000 ppm. During lactation, the feed consumption in P and F_1 generation females treated at 3000 and 10 000 ppm was slightly reduced, but as the effect at 3000 ppm was not accompanied by an effect on body weight gain, the effect was considered not to be adverse at 3000 ppm (Table 39).

The group mean body weight gains during the prepairing period were reduced in P generation males and females at 10 000 ppm. Thus, group mean body weights at the start of mating were 5.8% and 4.0% lower than those of the controls, respectively. The treatment-related reduction in preweaning body weight gains of F_1 generation males and females at 10 000 ppm persisted during the prepairing treatment period. Thus, group mean body weights at the start of mating were 9.3% and 4.3% lower than the control values, respectively. The body weight gains of P and F_1 generation animals of both sexes at 300–3000 ppm were unaffected by treatment with dinotefuran (Table 39).

The slightly lower body weights of P and F_1 generation females at 10 000 ppm persisted during the gestation and lactation periods. The group mean body weights at 21 days postpartum were 6.4% and 7.9% lower, respectively, than control values. The body weights of P and F_1 generation females treated at 300–3000 ppm were comparable to control values throughout gestation and lactation (Table 39).

There was no effect of treatment at any dose level in either generation on the duration of the estrous cycle. There were no treatment-related effects at any dose level in either generation on fertility and mating performance, duration of gestation, number of implantations, post-implantation loss, litter size at birth, pup mortality, litter size at weaning or sex ratio. With the exception of two non-dose-related occurrences of statistical significance in the F_1 generation (higher neonatal pup mortality at 300 ppm and higher number of empty implantation sites at 3000 ppm), all reproductive data in the treated groups were comparable to, and not significantly different from, the control group values (Table 40).

There were no treatment-related effects on sperm motility, morphology or counts in either P or F_1 generation males at any dose level. The proportions of non-motile, stationary and progressively motile sperm in all treated P generation groups were similar to, and not significantly different from, the control group values. In the F_1 generation, statistically significant variation from the control values was observed for progressively motile and stationary sperm in the group treated at 10 000 ppm and for progressively motile sperm in the group treated at 1000 ppm. Males at 10 000 ppm showed 40% stationary and 50% progressively motile sperm compared with the control group, which showed 32% stationary and 58% progressively motile sperm. The proportion of non-motile sperm in both groups was 10%. The group treated at 1000 ppm also showed a significantly lower proportion of progressively motile sperm. As the differences in the mean values were numerically small and showed no clear dose dependency, the small differences recorded are considered to be incidental to treatment. P generation males at 10 000 ppm showed a slightly, but significantly, higher incidence of sperm abnormality type D (normal head but abnormally curved hook). However, the finding occurred in association with a high percentage incidence of normal sperm (95.0% versus 96.2% in the control), and the numerical difference from the control for this abnormality (type D) was small (2% affected versus 1.1% in the control). Furthermore, the F_1 generation control incidence of type D abnormality was 1.7%. Therefore, the higher incidence in the P generation males at 10 000 ppm was considered to be incidental to treatment. In the F_1 generation, there were minor differences from the control in the sperm morphology data, but the differences occurred in association with a high percentage incidence of normal sperm (95.4% versus 96.4% in the control), and the differences were considered to be incidental to treatment. There was no significant effect on epididymal sperm count for the P or F_1 generation males, but the testicular sperm counts of the P generation males showed a slight reduction

at 10 000 ppm, which was statistically significant. However, as the magnitude of the difference was small, occurred in the absence of a significant effect on epididymal sperm count and was not repeated in the F_1 generation males, the difference was considered incidental to treatment.

Table 39. Summary of selected parental findings in the two-generation rat study

	Males					Females				
	0 ppm	300 ppm	1000 ppm	3000 ppm	10 000 ppm	0 ppm	300 ppm	1000 ppm	3000 ppm	10 000 ppm
P generation										
Body weight (g)										
- week 1	145	145	143	145	144	112	113	109	110	109
- week 10	397	391	384	380	374*	225	227	226	223	216
- week 14	450	443	436	431	424*	—	—	—	—	—
- GD 0	—	—	—	—	—	224	225	225	222	217
- GD 7	—	—	—	—	—	245	243	244	240	233*
- GD 21	—	—	—	—	—	338	332	331	327	317**
- LD 1	—	—	—	—	—	245	244	246	243	230*
- LD 21	—	—	—	—	—	282	281	282	274	264**
Feed consumption (g/day)										
- week 1	22.9	21.9	21.0**	21.7*	20.6**	15.7	16.2	15.3	15.5	14.6**
- week 2	23.3	22.9	22.4	22.9	22.5	16.3	16.4	16.1	15.9	15.3*
- weeks 1–10	24.4	24.1	23.4	23.7	23.8	17.0	16.8	16.7	16.5	16.4
- gestation	—	—	—	—	—	21.3	20.2	20.9	20.7	20.4
- lactation	—	—	—	—	—	44.7	43.3	44.2	41.7	41.2
F_1 generation										
Body weight (g)										
- week 1	61	62	64	65	52*	58	56	60	59	52*
- week 10	344	347	351	344	312*	207	207	211	209	198
- week 18	421	423	427	422	387*	—	—	—	—	—
- GD 0	—	—	—	—	—	211	208	216	211	203
- GD 7	—	—	—	—	—	229	227	234	230	220
- GD 21	—	—	—	—	—	319	319	322	315	307
- LD 1	—	—	—	—	—	233	230	235	232	225
- LD 21	—	—	—	—	—	280	275	279	269	258**
Feed consumption (g/day)										
- week 1	12.8	13.0	13.3	13.6	11.3	12.2	11.7	12.2	12.2	11.2
- week 2	17.4	17.8	18.0	17.2	14.6**	15.1	14.7	15.2	14.0*	13.3**
- weeks 1–10	20.3	21.3	21.4	21.2	19.4	14.8	15.5	15.5	15.4	15.0
- gestation	—	—	—	—	—	19.6	18.5	18.9	18.6	18.5
- lactation	—	—	—	—	—	41.2	40.4	42.2	40.6	40.0

From Becker (2002)
GD, gestation day; LD, lactation day; * $P < 0.05$; ** $P < 0.01$

Table 40. Summary of selected reproductive data in the two-generation rat study

	0 ppm	300 ppm	1000 ppm	3000 ppm	10 000 ppm
P generation					
No. paired / no. mated	25 / 25	25 / 25	25 / 25	25 / 25	25 / 25
No. pregnant	24	25	25	25	24
No. with viable litters	23	25	22	25	23
Duration of estrous cycle (days)	5.3	5.6	4.8	5.1	4.9
Mean precoital time (days)	2.8	2.8	2.8	2.6	2.6
Duration of gestation (days)	21.5	21.4	21.5	21.5	21.8
No. of implantations/dam	13.1	13.2	12.6	12.5	12.9
Post-implantation loss (%)	7.3	7.9	9.7	9.3	7.1
Live litter size on day 0/1	12.2	12.2	11.4	11.3	12.0
Pup mortality (group total) on days 0–4 postpartum	5	17*	6	4	7
Litter size on day 4 postpartum	12.0	11.5	11.1	11.2	11.6
Pup mortality (group total) on days 5–21 postpartum	6	4	2	0	7
Litter size on day 21 postpartum	11.7	11.4	11.0	11.2	11.3
Sex ratio (% males)	51	49	47	47	45
F_1 generation					
No. paired / no. mated	25 / 25	25 / 25	25 / 25	25 / 25	25 / 25
No. pregnant	25	24	24	25	25
No. with viable litters	25	24	24	25	25
Duration of estrous cycle (days)	4.9	4.9	4.9	5.0	5.0
Mean precoital time (days)	2.7	2.8	2.5	2.6	2.4
Duration of gestation (days)	21.5	21.4	21.5	21.5	21.5
No. of implantations/dam	11.8	11.8	11.5	11.4	12.3
Post-implantation loss (%)	6.4	3.5	4.0	10.9	5.2
Live litter size on day 0/1	11.1	11.4	11.0	10.2	11.6
Pup mortality (group total) on days 0–4 postpartum	5	6	1	1	5
Litter size on day 4 postpartum	10.9	11.1	11.0	10.1	11.4
Pup mortality (group total) on days 5–21 postpartum	2	2	1	2	0
Litter size on day 21 postpartum	10.8	11.0	11.0	10.0	11.4
Sex ratio (% males)	52	46	49	55	46

From Becker (2002)
* $P < 0.05$; ** $P < 0.01$

There were no treatment-related gross findings at necropsy in the male and female P and F_1 generation parental animals at any dose level, but significantly reduced spleen weights (absolute and brain weight ratios, 10.0–16.0% reduced) occurred in both sexes of the P generation treated at 10 000 ppm. The effect was not evident at lower dose levels or in F_1 generation parental animals at any dose level. Female F_1 generation parental animals at 10 000 ppm showed significantly reduced thyroid weights (absolute, body weight and brain weight ratios, 20.0–25.0% reduced). Other minor, but statistically significant, differences in organ weights at 10 000 ppm were considered to be secondary

to lower terminal body weights. All histopathological findings recorded in the reproductive organs, pituitary and adrenal glands of P and F_1 generation males and females were considered to be within the range of background lesions commonly observed in rats of this strain and age. The incidences of all individual findings at 10 000 ppm did not indicate an effect of treatment. There were no treatment-related findings during staging analysis of the testes. All cycles were complete, and there were no indicators for maturation arrest. There were no treatment-related, biologically relevant effects on the quantitative evaluation of ovarian follicular stages. Although ovary staging revealed a significantly lower number of primordial follicles in 10 000 ppm animals, the finding was deemed to have no biological significance, because the numbers of antral follicles were markedly higher at 10 000 ppm, and the numbers of corpora lutea were also slightly higher than the control values. The numbers of pre-antral follicles were comparable in the treated and control groups.

F_1/F_2 offspring: There were no treatment-related effects at any dose level in either generation on the nature and incidence of pup abnormalities during the preweaning period. The anogenital distance of F_2 progeny of both sexes was unaffected by treatment at all dose levels. The significantly greater anogenital distances of F_2 pups at 1000 ppm are considered incidental to treatment, as a dose–response relationship was not evident. Preweaning pup growth in both the F_1 and F_2 generations was retarded at 10 000 ppm. Group mean male and female pup weights were significantly reduced from day 14 postpartum, except for female F_1 pups, for which weights were significantly reduced on day 21 postpartum only. Thus, at weaning, pup weights were 11.6–15.1% lower than control values. Pup weights were unaffected by treatment at lower dose levels (Table 41).

Sexual maturation of F_1 generation pups, based on preputial separation or vaginal patency, was unaffected by treatment at all dose levels. The group mean age at which these events occurred was 27.9, 28.0, 27.8, 28.2 and 28.2 days (males) and 34.3, 34.1, 34.5, 33.9 and 35.5 days (females), in order of ascending dose level. Quantitative locomotor activity of F_1 progeny at 6 weeks of age was not affected by treatment at any dose level. There were no statistically significant differences in the low beam counts recorded for control and treated groups of either sex. There were no treatment-related effects at any dose level on motor capability as assessed by grip strength. Quantitative measurement of grip strength showed significantly lower absolute values at 10 000 ppm for male forelimb grip strength and female hindlimb grip strength. However, grip strength to body weight ratios were not significantly different from those of the controls, suggesting that the differences were due to lower body weight or smaller size at 10 000 ppm, rather than a specific effect of dinotefuran on motor capability. Absolute grip strength at 3000 ppm and below was not affected by treatment. None of the animals at any dose level showed any behavioural, postural, motor, respiratory or reflex anomalies in the modified Irwin screen, and all animals were of normal appearance (Table 41).

The types and frequencies of gross lesions at necropsy in F_1 and F_2 pups shortly after weaning gave no indication of treatment-related effects. The most common finding in both generations was renal pelvic dilatation, but the group incidences did not indicate an effect of treatment. The overall incidences of renal pelvic dilatation were 11.0%, 7.7%, 11.5%, 14.3% and 10.0% (F_1 generation) and 8.0%, 2.1%, 17.0%, 12.5% and 4.1% (F_2 generation), in order of ascending dose level. All other gross lesions occurred at very low incidences, and their distribution did not suggest an effect of treatment. Direct treatment-related effects on organ weights were confined to the spleen in both the F_1 and F_2 generations. The mean absolute spleen weight (both sexes) and mean spleen weight relative to body weight (females only) were significantly reduced by up to 25.6% in F_1 generation pups treated at 10 000 ppm. The mean brain weight relative to body weight was also significantly elevated in these animals, but this is considered to be due to the lower body weights of the group. There was no effect on thymus weights at any dose level. Absolute and relative spleen weights were significantly reduced to a similar extent in both sexes of the F_2 generation at 10 000 ppm. Absolute brain and thymus weights were significantly reduced and relative brain weights were significantly increased in F_2 animals exposed to 10 000 ppm. However, the pattern of response was indicative of a body weight effect rather than a specific effect of dinotefuran on these organs. There were no effects on any of the measured organ weights in F_1 and F_2 generation male and female pups at 300–3000 ppm (Table 41).

Table 41. Summary of selected offspring findings in the two-generation rat study

	Males					Females				
	0 ppm	300 ppm	1000 ppm	3000 ppm	10 000 ppm	0 ppm	300 ppm	1000 ppm	3000 ppm	10 000 ppm
F_1 generation										
Body weight (g)										
- day 0/1	5.7	5.4	5.7	6.0	5.5	5.4	5.1	5.8	5.6	5.5
- day 4	8.3	8.1	8.5	8.7	8.2	8.0	7.6	8.3	8.2	8.0
- day 7	12.3	12.0	12.8	12.9	11.7	11.9	11.4	12.6	12.3	11.4
- day 14	23.9	24.0	25.0	24.7	21.2**	23.4	22.7	24.5	23.7	21.3
- day 21	37.4	37.3	38.8	38.1	32.0**	36.2	35.3	38.2	36.6	32.0*
Spleen weight, day 21										
- absolute (g)	0.15	0.14	0.17	0.16	0.12*	0.16	0.15	0.18	0.16	0.12**
- relative (% of body weight)	0.41	0.38	0.44	0.43	0.37	0.44	0.42	0.46	0.44	0.37**
Forelimb grip strength, week 6										
- absolute (g)	618	576	557	554	505*	536	513	504	521	449
- grip strength/ body weight ratio	3.37	3.21	3.05	3.05	3.23	3.93	3.85	3.62	3.88	3.64
Hindlimb grip strength, week 6										
- absolute (g)	387	416	350	379	343	428	401	364*	385	336*
- grip strength/ body weight ratio	2.12	2.30	1.94	2.09	2.21	3.15	3.03	2.60*	2.88	2.73
F_2 generation										
Body weight (g)										
- day 1	5.9	5.9	6.2	6.2	5.9	5.6	5.6	5.8	5.8	5.7
- day 4	8.7	8.6	9.0	9.2	8.5	8.3	8.4	8.6	8.7	8.3
- day 7	12.7	12.4	13.2	13.5	12.1	12.2	12.2	12.7	12.8	12.0
- day 14	24.7	24.9	25.6	25.8	21.5**	24.2	24.4	24.7	24.7	21.2**
- day 21	39.7	39.5	41.7	41.4	33.7**	38.7	38.8	40.1	39.6	33.1**
Anogenital distance (mm), day 1	2.12	2.17	2.24**	2.16	2.12	1.00	1.00	1.04*	1.02	1.01
Spleen weight, day 21										
- absolute (g)	0.17	0.17	0.19	0.19	0.13**	0.17	0.16	0.19	0.18	0.13**
- relative (% of body weight)	0.43	0.43	0.45	0.45	0.37**	0.45	0.41	0.47	0.45	0.39*

From Becker (2002)
* $P < 0.05$; ** $P < 0.01$

The NOAEL for reproductive toxicity was 10 000 ppm (equal to 822 mg/kg bw per day for males and 907 mg/kg bw per day for females), the highest dose tested. The NOAEL for parental toxicity was 3000 ppm (equal to 241 mg/kg bw per day for males and 268 mg/kg bw per day for females), based on a reduction in feed consumption, body weight and spleen weight at 10 000 ppm.

The NOAEL for offspring toxicity was 3000 ppm (equal to 241 mg/kg bw per day for males and 268 mg/kg bw per day for females), based on reduced pup weight gain during lactation and reduced spleen weight at 10 000 ppm (Becker, 2002).

(b) Developmental toxicity

Rats

In a range-finding prenatal developmental toxicity study, groups of six mated female Crj:CD(SD) IGS rats were administered dinotefuran (purity 92.9%) in 0.5% aqueous carboxymethylcellulose at a dose volume of 10 ml/kg bw by oral gavage at a dose level of 0, 30, 100, 300 or 1000 mg/kg bw per day from gestation day (GD) 6 to GD 15. Clinical signs, body weight and feed consumption were recorded. On GD 20, dams were sacrificed and the fetuses delivered by caesarean section. The uterine tract and ovaries were removed, and pregnancy was confirmed. If implantations were not visible macroscopically, the uterus was immersed in ammonium sulfate to aid visualization. Maternal organs of the cranial, thoracic and abdominal cavities and ovaries (including corpora lutea count) and uteri (implantation site count) were examined macroscopically. The uterine contents were classified as live fetuses, embryo/fetal deaths, placental remnants, early or late resorptions or macerated fetuses. Live fetuses were sexed, examined for external malformations and weighed.

There were no deaths and no clinical signs of toxicity at any dose level. At 1000 mg/kg bw per day, body weight gain from GD 6 was significantly reduced on GDs 7–8, whereas feed consumption was lower on GDs 6–11. There were no effects on water intake, the numbers of corpora lutea and implantations, the preimplantation and post-implantation loss rates, the number of live fetuses, sex ratio or live fetal body weights. Also, there were no external abnormalities of fetuses in any group.

The NOAEL for maternal toxicity was 300 mg/kg bw per day, based on reduced feed consumption and a transient decrease in body weight gain at 1000 mg/kg bw per day. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Sakurai, 1998a).

In a prenatal developmental toxicity study conducted according to OECD Test Guideline No. 414, groups of 24 mated female Crj:CD(SD) IGS (SPF) rats were administered dinotefuran (purity 92.9%) in 0.5% aqueous carboxymethylcellulose at a dose volume of 10 ml/kg bw by oral gavage at a dose level of 0, 100, 300 or 1000 mg/kg bw per day from GD 6 to GD 15. Mortality checks were performed and clinical signs recorded at least once daily on non-treatment days and at least twice daily during the treatment period. Body weights were recorded on GDs 0 and 3 and then daily from GD 5 until sacrifice. Feed consumption and water consumption were recorded on GDs 0 and 3 and then daily from GD 6 until sacrifice. Dams were sacrificed on GD 20 and the fetuses delivered by caesarean section. The uterine tract and ovaries were removed, and pregnancy was confirmed. If implantations were not visible macroscopically, the uterus was immersed in ammonium sulfate to aid visualization. Maternal organs of the cranial, thoracic and abdominal cavities and ovaries (including corpora lutea count) and uteri (implantation site count) were examined macroscopically. The uterine contents were classified as live fetuses, embryo/fetal deaths, placental remnants, early or late resorptions or macerated fetuses. Fetuses were sexed, examined for external malformations and weighed. Approximately one half of the fetuses (alternate) from each litter were examined for soft tissue malformations and variations by fixation in Bouin's solution and subsequent microdissection of the cranial and abdominal cavities by Wilson's method and of the thoracic cavity by the method of Nishimura. The remaining fetuses were subjected to skeletal evaluation using a dual staining technique for cartilage and bone and examined for skeletal malformations and variations, including counting the number of ossification centres in vertebrae, metacarpals, metatarsals, and proximal and medial phalanges.

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

The body weight gain from day 6 to day 11 of the group treated at 1000 mg/kg bw per day was significantly reduced by 21%. Thereafter, weight gain was not significantly different from the controls, and on GD 20, the group mean body weights of all treated groups were not significantly different from control values (Table 42). The mean feed consumption of the group treated at 1000 mg/kg bw per day was significantly reduced by 10.5–13.0% on GDs 7, 8 and 10. The mean water consumption of this group was significantly increased by 19.8–23.8% on GDs 11–13. On other occasions during the treatment period, the feed consumption and water consumption at 1000 mg/kg bw per day were comparable to control values. There were no treatment-related effects on feed or water consumption in the groups treated at 100 or 300 mg/kg bw per day. There were no treatment-related macroscopic findings in the maternal animals at any dose level.

Table 42. Summary of selected maternal findings in the prenatal developmental toxicity study in rats

	0 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day
Body weight (g)				
- day 6	280	281	281	277
- day 20	386	390	390	373
Body weight gain (g)				
- days 6–11	21.9	22.0	22.2	17.3*
- days 6–15	41.2	40.3	43.0	37.3
- days 6–20	107	110	109	97
Feed consumption (g/day)				
- day 7	24.0	24.2	24.1	21.1**
- day 8	24.3	23.8	24.4	21.1**
- day 10	24.2	23.9	24.5	21.7*
Water intake (ml/day)				
- day 11	36.0	41.1	37.3	43.1*
- day 12	36.3	40.2	37.8	44.7*
- day 13	34.9	40.7	37.2	43.3*

From Sakurai (1998b)
* $P < 0.05$; ** $P < 0.01$

Litter parameters as assessed by pregnancy incidence, numbers of corpora lutea, implantations and live fetuses, post-implantation loss, external anomalies, fetal weights and sex ratio were unaffected by treatment at all dose levels. Although preimplantation loss in the group treated at 1000 mg/kg bw per day was high (24.0%) in relation to the control group (9.2%), it was not significantly different from the control value and is considered incidental to treatment with dinotefuran, as implantation was complete at the initiation of treatment. The mean number of implantations in the 1000 mg/kg bw per day group was slightly lower than, but not significantly different from, the control group value as a consequence of higher preimplantation loss (Table 43).

Table 43. Summary of selected reproductive data in the prenatal developmental toxicity study in rats

	0 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day
No. pregnant / no. mated	22/24	22/24	20/24	20/24
No. of corpora lutea (mean/dam)	15.6	16.2	15.3	15.7
No. of implantations (mean/dam)	14.2	14.8	14.1	12.2
Preimplantation loss (%)	9.2	8.3	10.1	24.0
Total embryo/fetal loss (%)	5.1	5.1	3.4	3.6
Implant remnant (%)	0.0	0.0	0.0	0.0
Retained placenta (%)	4.3	3.7	3.4	2.7
Early death (%)	0.0	1.1	0.0	0.9
Late death (%)	0.8	0.0	0.0	0.0
Macerated fetuses (%)	0.0	0.3	0.0	0.0
No. of live fetuses (mean/dam)	13.5	14.0	13.6	11.8
Sex ratio (% males)	53.0	55.7	50.4	42.8
Mean body weight (g), males	3.73	3.72	3.83	3.71
Mean body weight (g), females	3.55	3.51	3.65	3.47
Total no. live fetuses	298	309	272	236
External abnormalities (no. fetuses)	0	0	0	0
Skeletal examination				
- no. of fetuses examined	143	150	132	114
- skeletal abnormalities (no. of fetuses/litters)	0/0	0/0	0/0	0/0
- skeletal variations (no. of fetuses/litters)	26/14	12/8	14/9	15/8
- skeletal variations (% of fetuses)	18.4	7.6	10.5	12.1
Visceral examination				
- no. of fetuses examined	155	159	140	122
- visceral abnormalities (no. of fetuses/litters)	8/6	4/3	6/5	5/5
- visceral abnormalities (% of fetuses)	5.0	3.5	8.4	5.1

From Sakurai (1998b)

There were no external fetal abnormalities in any of the treated or control groups. There were no treatment-related or statistically significant differences between treated and control groups in the incidence or nature of skeletal and visceral abnormalities and variations. No skeletal abnormalities occurred in any group, and the incidences of visceral abnormalities, thymic remnant, microphthalmia, ectopic ovary, pyeloectasia, ureteroectasia and left umbilical artery were similar in all groups. Delayed ossification, as assessed by the number of vertebral and phalangeal ossification centres, was not apparent at any dose level.

The NOAEL for maternal toxicity was 300 mg/kg bw per day, based on decreased weight gain and feed consumption and increased water consumption at 1000 mg/kg bw per day. The NOAEL for prenatal developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Sakurai, 1998b).

Rabbits

A single-dose study and a 2-week range-finding study were conducted in non-mated female New Zealand White rabbits in order to select the dose levels for the prenatal developmental toxicity study. Dinotefuran (purity 92.9%) in 0.5% aqueous carboxymethylcellulose was administered at a dose volume of 10 ml/kg bw by oral gavage to groups of two rabbits at a dose level of 0, 100, 300, 1000 or 2000 mg/kg bw in the single-dose study or to groups of three rabbits at a dose level of 0, 100, 300 or 1000 mg/kg bw per day in the 2-week study.

In the single-dose study, clinical signs (hypoactivity, sedation and flush of the nose and ears at 300 mg/kg bw, as well as panting, abdominal position, tremor, ptosis and side position at 1000 mg/kg bw and above) were observed on the day of dosing, whereas decreased feed consumption was observed at 1000 mg/kg bw and above from days 1 to 4 after dosing.

In the 2-week study, clinical signs were observed from the start of treatment and included hypoactivity, sedation, flush of the nose and ears and panting at 300 mg/kg bw per day, whereas at 1000 mg/kg bw per day, additionally abdominal position, tremor, ptosis, side position and bradypnoea were observed. Clinical signs ceased by day 6 of treatment, with the exception of hypoactivity at the top dose.

The NOAEL was 100 mg/kg bw per day in both the single-dose and 2-week studies, based on treatment-related clinical signs (hypoactivity, sedation, flush, panting) at 300 mg/kg bw per day and above (Sakurai, 1998c).

In a range-finding prenatal developmental toxicity study, groups of six mated female New Zealand White rabbits were administered dinotefuran (purity 92.9%) in 0.5% aqueous carboxymethylcellulose at a dose volume of 10 ml/kg bw by oral gavage at a dose level of 0, 100, 300 or 1000 mg/kg bw per day from GD 6 to GD 18. Clinical signs, body weight and feed consumption were recorded. On GD 28, dams were sacrificed and the fetuses delivered by caesarean section. The uterine tract and ovaries were removed, and pregnancy was confirmed. If implantations were not visible macroscopically, the uterus was immersed in 2% potassium hydroxide to aid visualization. Maternal organs of the cranial, thoracic and abdominal cavities and ovaries (including corpora lutea count) and uteri (implantation site count) were examined macroscopically. The uterine contents were classified as live fetuses, embryo/fetal deaths, placental remnants, early or late resorptions or macerated fetuses. Live fetuses were sexed, examined for external malformations and weighed.

There were no deaths at any dose level. Abortion occurred in four dams at 1000 mg/kg bw per day and in one control dam, and one control dam was non-pregnant. At 1000 mg/kg bw per day, dams showed hypoactivity, flush of auricle and nose, panting, prone position, tremors and sedation on GDs 6–9, whereas only hypoactivity was observed from GD 10 to GD 18. At 300 mg/kg bw per day, dams showed hypoactivity and panting on GDs 6–9, and two dams showed tremors on GD 8 or 9. There were no clinical signs at 100 mg/kg bw per day. Decreases in body weight gain and feed consumption were observed at 1000 mg/kg bw per day, whereas water intake was not affected. At necropsy, grey-white plaques in the fundic region of the stomach were seen in dams at all dose levels, whereas pale brown discoloration of the liver was observed in one, three or four dams of the low-, mid- and high-dose groups, respectively. There were no significant differences in the numbers of corpora lutea and implantations, the preimplantation and post-implantation loss rates, the number of live fetuses, sex ratio or live fetal body weights. Also, there were no treatment-related external abnormalities of fetuses in any group.

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on treatment-related clinical signs (hypoactivity, panting, tremor) at 300 mg/kg bw per day and above. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Sakurai, 1998d).

In a prenatal developmental toxicity study conducted according to OECD Test Guideline No. 414, groups of 22 mated female New Zealand White rabbits were administered dinotefuran (purity

92.9%) in 0.5% aqueous carboxymethylcellulose at a dose volume of 10 ml/kg bw by oral gavage at a dose level of 0, 52, 125 or 300 mg/kg bw per day from GD 6 to GD 18. Mortality checks were performed and clinical signs recorded at least once daily on non-treatment days and at least twice daily during the treatment period. Body weights were recorded on GD 0, daily from GD 6 to GD 19, on GDs 21, 23, 25 and 27 and on the day of necropsy. Feed consumption and water consumption were recorded daily from GD 6 until necropsy. Dams were sacrificed on GD 28 and the fetuses delivered by caesarean section. The uterine tract and ovaries were removed, and pregnancy was confirmed. If implantations were not visible macroscopically, the uterus was immersed in 2% potassium hydroxide to aid visualization. Maternal organs of the cranial, thoracic and abdominal cavities and ovaries (including corpora lutea count) and uteri (implantation site count) were examined macroscopically. Gross lesions were preserved for subsequent histological examination. The uterine contents were classified as live or dead fetuses, placental remnants, early or late resorptions or macerated fetuses. Live fetuses were weighed and examined for external and oral cavity abnormalities. The thoracic and abdominal organs were examined macroscopically, and sexes were recorded by examination of the internal reproductive organs. The heads of approximately one half of the fetuses from each litter and the thoracic viscera from all fetuses were examined for soft tissue malformations and variations by fixation in Bouin's solution and subsequent microdissection of the cranial cavities and of the thoracic cavity. The carcasses of all fetuses were subjected to skeletal evaluation using a dual staining technique for cartilage and bone and examined for skeletal malformations and variations, including enumeration of ossification centres in vertebrae, metacarpals, metatarsals, and right limb proximal and medial phalanges.

There were no treatment-related deaths or abortions during the study, but a control animal died on GD 7 due to incorrect dosing. One animal to be treated at 300 mg/kg bw per day was excluded from the study on GD 6 due to body weight loss prior to the start of treatment. Clinical signs of toxicity were confined to the group treated at 300 mg/kg bw per day. This group showed hypoactivity, prone position, panting, erythema of the nose and ears and tremor from the start of treatment until GD 13.

The mean body weight gain of the group treated at 300 mg/kg bw per day was significantly reduced by 50% during the treatment period (Table 44). After that, the group gained weight at a greater rate than the controls, and body weights were similar to those of the control group at termination. The mean body weight gain of the group treated at 125 mg/kg bw per day was slightly, but significantly, reduced on GD 8 only. The mean weight gain of the group treated at 52 mg/kg bw per day was unaffected by treatment with dinotefuran. The mean feed consumption during treatment of the group treated at 300 mg/kg bw per day was reduced by 22.7%, and water consumption was significantly reduced by up to 34.2% on GDs 14–16. The feed consumption and water consumption of the groups treated at 125 or 52 mg/kg bw per day were unaffected by treatment with dinotefuran.

Treatment-related macroscopic findings in maternal animals occurred at 125 and 300 mg/kg bw per day (Table 44). Pale brown discoloration of the liver and grey-white plaque formation in the fundic region of the stomach occurred in most animals treated at 300 mg/kg bw per day and in a smaller proportion of animals treated at 125 mg/kg bw per day. One animal at 300 mg/kg bw per day also showed liver enlargement. Histological examination of representative maternal liver and stomach lesions revealed no correlative histopathological alterations. No macroscopic changes occurred at 52 mg/kg bw per day.

There were no treatment-related effects on pregnancy incidence, numbers of corpora lutea, implantations and live fetuses, post-implantation loss, external anomalies, fetal weights or sex ratio at all dose levels (Table 45).

There were no treatment-related effects on the incidence or nature of skeletal and visceral abnormalities and variants at any dose level. The incidences of skeletal and visceral abnormalities and skeletal variants were not significantly different from those of the control group. Although one litter from a dam treated at 300 mg/kg bw per day had three fetuses with hydrocephalus, this abnormality occurs spontaneously in rabbits of the strain and source employed. Delayed ossification, measured by the number of vertebral and phalangeal ossification centres, was not apparent at any dose level.

Table 44. Summary of selected maternal findings in the prenatal developmental toxicity study in rabbits

	0 mg/kg bw per day	52 mg/kg bw per day	125 mg/kg bw per day	300 mg/kg bw per day
No. of animals mated	22	22	22	22
No. of animals died or killed in extremis	1	0	0	1
No. of animals with clinical signs of toxicity	0	0	0	21
Body weight (kg)				
- day 6	3.31	3.27	3.42	3.37
- day 8	3.33	3.28	3.39	3.33
- day 19	3.53	3.51	3.61	3.48
- day 28	3.71	3.67	3.78	3.73
Body weight gain (g)				
- days 6–8	0.017	0.016	−0.037*	−0.039*
- days 6–10	0.050	0.046	−0.006	−0.009
- days 6–14	0.145	0.182	0.105	0.019**
- days 6–19	0.224	0.246	0.183	0.107*
Feed consumption (g/day)				
- day 6	155	154	159	152
- day 8	154	161	138	118*
- day 14	153	162	157	77**
- day 16	152	156	156	107**
Water intake (ml/day)				
- day 7	439	401	411	399
- day 14	482	506	488	317*
- day 16	503	505	507	380*
No. of animals examined at necropsy	22	22	22	22
Pale brown discoloration of liver	0	0	8	20
Liver enlargement	0	0	0	1
Grey-white discoloration of gastric mucosa	0	0	0	1
Grey-white plaque in fundus of stomach	0	0	15	20
Thickening of gastric mucosa	0	0	0	2

From Sakurai (1998e)
* $P < 0.05$; ** $P < 0.01$

The NOAEL for maternal toxicity was 52 mg/kg bw per day, based on a reduction in body weight gain at 125 mg/kg bw per day and above. The NOAEL for acute clinical signs in dams was 125 mg/kg bw per day, based on the manifestation of hypoactivity, prone position, panting, erythema and tremor from the start of treatment until GD 13. The NOAEL for developmental toxicity was 300 mg/kg bw per day, the highest dose tested (Sakurai, 1998e).

Table 45. Summary of selected reproductive data in the prenatal developmental toxicity study in rabbits

	0 mg/kg bw per day	52 mg/kg bw per day	125 mg/kg bw per day	300 mg/kg bw per day
No. mated / no. pregnant	22 / 20	22 / 19	22 / 21	22 / 19
No. of corpora lutea (mean/dam)	8.6	8.5	9.0	8.8
No. of implantations (mean/dam)	7.9	8.3	8.7	8.4
Preimplantation loss (%)	7.7	3.2	5.0	4.4
Total embryo/fetal loss (%)	5.1	2.1	6.9	6.9
Implant remnant (%)	0	0	0	0
Retained placenta (%)	4.1	2.1	2.9	1.8
Early death (%)	0	0	1.3	0.5
Late death (%)	0.5	0	2.1	3.6
Macerated fetuses (%)	0.5	0	0.6	1.0
No. of live fetuses (mean/dam)	7.6	8.1	8.1	7.7
Sex ratio (% males)	59.2	43.9	52.0	51.6
Mean body weight (g), males	42.7	40.6	39.7	40.4
Mean body weight (g), females	42.1	39.9	38.7	39.9
Total no. of live fetuses	151	153	170	147
External abnormalities (no. of fetuses/litters)	0/0	1/1	2/2	1/1
Skeletal examination				
- no. of fetuses examined	151	153	170	147
- skeletal abnormalities (no. of fetuses/litters)	2/2	0/0	1/1	0/0
- skeletal variations (no. of fetuses/litters)	92/20	106/19	126/21	101/19
- skeletal variations (% of fetuses)	58.9	70.2	60.7	67.6
Visceral examination				
- no. of fetuses examined	151	153	170	138
- visceral abnormalities (no. of fetuses/litters)	2/2	1/1	3/3	4/2
- visceral abnormalities (% of fetuses)	1.6	0.7	2.9	3.0

From Sakurai (1998e)

2.6 Special studies

(a) Neurotoxicity

In an acute neurotoxicity study conducted in accordance with OECD Test Guideline No. 424, groups of 10 male and 10 female Crl:CD®(SD) IGS BR rats received dinotefuran (purity 93.0%) in 0.5% aqueous carboxymethylcellulose at a dose volume of 20 ml/kg bw by oral gavage at a single dose of 0, 325, 750 or 1500 mg/kg bw and were then maintained for a 14-day observation period. The animals were observed twice daily for morbidity/mortality and daily for clinical signs. Body weights were recorded predosing and on days 1, 8 and 15, and individual feed consumption was measured weekly. All animals were subjected to a functional observational battery predosing, 3 hours after treatment on day 1 (the estimated time of peak effect) and on days 8 and 15. The tests and observations were performed without knowledge of the treatment of each animal. The functional observational battery comprised a series of qualitative and semiquantitative observations made in the home cage, during handling, in an open arena and during manipulations to assess reflex responses and physiological parameters. The assessments included evaluation of posture, activity, gait, locomotor

activity, unusual behaviour, reactivity to handling, vocalization, palpebral closure, exophthalmos, lacrimation, salivation, respiration, appearance of fur, piloerection, muscle tone, pupillary status, latency to first step in an open field, grooming and rearing activity, defecation, micturition, auditory reactivity, proprioceptive positioning, pinna response, approach response, righting reflex, corneal touch response, nociceptive reflexes and hindlimb foot splay. Quantitative measurements were made of rectal temperature, forelimb and hindlimb grip strength, and motor activity counts for 2-minute intervals for 40 minutes.

During week 3, the animals were deprived of feed overnight and subjected to necropsy and postmortem examination of major organs and tissues. The tissues of all animals were perfusion-fixed in situ, and six animals of each sex from the groups treated at 0 or 1500 mg/kg bw that were suitably perfusion-fixed were designated for neuropathological evaluation. The brain, entire spinal cord, pituitary, cervical and lumbar dorsal root ganglia, trigeminal ganglia, eyes, fibular, optic, sciatic, tibial and sural nerves, anterior tibialis and gastrocnemius muscles and gross lesions were preserved from all animals. The olfactory bulb, forebrain, caudate nucleus, hypothalamus/thalamus, midbrain, cerebellum, medulla, pituitary, three levels of spinal cord, eye, skeletal muscles and gross lesions from the six animals of each sex in the groups treated at 0 or 1500 mg/kg bw were processed to paraffin blocks, and sections were stained with haematoxylin and eosin. Dorsal root and trigeminal ganglia and all peripheral nerves from the same animals were embedded in epoxy resin, sectioned and stained with toluidine blue. The stained sections were examined microscopically.

There were no deaths or treatment-related clinical signs during the study at any dose level. There were no effects on body weight gain or feed consumption at any dose level. The group mean body weights and feed consumption of each treated group of each sex varied by less than 5% and 6%, respectively, from the controls. There were some minor variations in the functional observational battery observations, but none was considered to be treatment related, because they were not dose related, occurred also during the predosing evaluation or occurred at very low incidence, or there were no other correlating behavioural changes. Therefore, there were no treatment-related effects in either sex at any dose level on the qualitative and semiquantitative evaluation of functional observational battery observations, reflexes and responses at any of the testing intervals. The motor activity of both sexes at 1500 mg/kg bw, as measured quantitatively, was reduced on day 1 relative to predosing values and to the controls. The effect was statistically significant in females for the first 10-minute interval, the last 10-minute interval and the total 40-minute interval, but in males for the first 10-minute interval only. The effect did not occur subsequently at 1500 mg/kg bw. Motor activity at lower dose levels was unaffected by treatment at all testing intervals (Table 46).

Table 46. Summary of motor activity findings in the acute neurotoxicity study in rats

Time point	Motor activity (activity counts)							
	Males				Females			
	0 mg/kg bw	325 mg/kg bw	750 mg/kg bw	1500 mg/kg bw	0 mg/kg bw	325 mg/kg bw	750 mg/kg bw	1500 mg/kg bw
Pre-exposure	1369	1365	1377	1323	1147	1016	1061	1151
Day 1	809	1074	837	567[#]	1006	940	682	531*
Day 8	1302	1529	1471	1435	1400	1105	1118	1175
Day 15	1389	1662	1650	1363	1375	1258	1157	1068

From Weiler (2001a)
* $P < 0.05$; [#] $P < 0.05$ for the first 10-minute interval only

There were no other treatment-related effects in either sex at any dose level on the quantitative evaluation of grooming and rearing activity, defecation, micturition, grip strength, nociceptive reflex, foot splay or rectal temperature.

Macroscopic examination at necropsy revealed no treatment-related lesions at any dose level. There were few histopathological findings in the central and peripheral nervous tissues and other tissues examined, and the nature and distribution between the groups did not indicate an effect of treatment at 1500 mg/kg bw. All histopathological findings were considered incidental to treatment and common to animals of the strain and age used.

The NOAEL in this study was 750 mg/kg bw, based on a transient decrease in motor activity at 1500 mg/kg bw. The NOAEL for neuropathological effects was 1500 mg/kg bw, the highest dose tested (Weiler, 2001a).

In a subchronic neurotoxicity study conducted in accordance with OECD Test Guideline No. 424, groups of 10 male and 10 female Crl:CD®(SD) IGS BR rats were fed diets containing dinotefuran (purity 93.0%) at a concentration of 0, 500, 5000 or 50 000 ppm (equal to 0, 33, 327 and 3413 mg/kg bw per day for males and 0, 40, 400 and 3806 mg/kg bw per day for females, respectively) for at least 13 weeks. The animals were observed twice daily for morbidity/mortality and daily for clinical signs. Body weights were recorded predosing, on day 1 and weekly thereafter. Body weights were also recorded on the days of functional observational battery testing. Individual feed consumption was measured weekly. All animals were subjected to a battery of behavioural tests and observations (functional observational battery) predosing and during weeks 2, 4, 8 and 13. The tests and observations were performed without knowledge of the treatment of each animal. The functional observational battery comprised a series of qualitative and semiquantitative observations made in the home cage, during handling, in an open arena and during manipulations to assess reflex responses and physiological parameters. The assessments included evaluation of posture, activity, gait, locomotor activity, unusual behaviour, reactivity to handling, vocalization, palpebral closure, exophthalmos, lacrimation, salivation, respiration, appearance of fur, piloerection, muscle tone, pupillary status, latency to first step in an open field, grooming and rearing activity, defecation, micturition, auditory reactivity, proprioceptive positioning, pinna response, approach response, righting reflex, corneal touch response, nociceptive reflexes and hindlimb foot splay. Quantitative measurements were made of rectal temperature, forelimb and hindlimb grip strength, and motor activity counts for 2-minute intervals for 40 minutes. During week 14, the animals were deprived of feed overnight and subjected to necropsy and postmortem examination of major organs and tissues. The tissues of all animals were perfusion-fixed in situ, and six animals of each sex from the groups treated at 0 or 50 000 ppm that were suitably perfusion-fixed were designated for neuropathological evaluation. The brain, entire spinal cord, pituitary, cervical and lumbar dorsal root ganglia, trigeminal ganglia, eyes, fibular, optic, sciatic, tibial and sural nerves, anterior tibialis and gastrocnemius muscles and gross lesions were preserved from all animals. The olfactory bulb, forebrain, caudate nucleus, hypothalamus/thalamus, midbrain, cerebellum, medulla, pituitary, three levels of spinal cord, eye, skeletal muscles and gross lesions from the six animals of each sex in the groups treated at 0 or 50 000 ppm were processed to paraffin blocks, and sections were stained with haematoxylin and eosin. Dorsal root and trigeminal ganglia and all peripheral nerves from the same animals were embedded in epoxy resin, sectioned and stained with toluidine blue. The stained sections were examined microscopically.

There were no deaths during the study and no treatment-related clinical signs at any dose level, but the overall body weight gain of both sexes treated at 50 000 ppm was significantly reduced. The group mean body weights at 50 000 ppm were significantly lower than those of the controls from week 2 of the study and at termination were 20.8% and 18.9% lower than control values in males and females, respectively. The effect on body weight at 50 000 ppm was accompanied by reduced feed consumption, which was frequently significantly lower than control values. The body weight gain and feed consumption of both sexes at lower dose levels were unaffected by treatment with dinotefuran.

There were no treatment-related effects in either sex at any dose level at any of the assessment intervals on the qualitative and semiquantitative observations made in the home cage, during handling, in an open arena and during manipulations to assess reflex responses and physiological parameters. Similarly, there were no treatment-related effects in either sex at any dose level at any of the assessment intervals on the quantitative parameters of grip strength, speed of

nociceptive reflex, foot splay and body temperature. Although minor statistically significant differences between the groups were apparent for rearing activity (50 000 ppm females in week 2), number of urine pools (5000 and 50 000 ppm males in week 4), hindlimb grip strength (500 ppm females in week 2), foot splay (50 000 ppm females in week 8) and body temperature (50 000 ppm females in week 2), none was considered to be treatment related, because they were not dose related, there was no consistency between testing intervals or the numerical differences were small. Motor activity in females at 50 000 ppm was significantly lower than that of the controls in week 2 during each 10-minute interval and during the entire 40-minute test period (Table 47). As a reduction in motor activity was also observed in the acute neurotoxicity study, a relationship to treatment was considered plausible. All other quantitative assessments in dinotefuran-treated groups were comparable to, and not significantly different from, control values.

Table 47. Summary of selected findings in the subchronic neurotoxicity study in rats

	Males				Females			
	0 ppm	500 ppm	5000 ppm	50 000 ppm	0 ppm	500 ppm	5000 ppm	50 000 ppm
Body weight (g)								
- week 1	256	251	257	249	174	183	173	180
- week 2	295	292	298	245*	197	199	189	165*
- week 4	370	358	363	300*	226	227	218	193*
- week 8	461	454	450	365*	257	262	251	217*
- week 14	514	511	508	407*	286	291	277	232*
Body weight gain (g), weeks 1–14	258	260	251	159*	112	107	103	52*
Feed consumption (g/week)								
- week 1	174	177	182	132*	138	141	124	87*
- week 2	182	176	181	156*	131	134	125	114*
- week 4	191	184	185	174	132	138	130	107*
- week 8	182	187	183	173	128	137	128	107*
- week 13	175	186	183	156	126	134	137	99*
Motor activity (activity counts)								
- pre-exposure	1115	1219	1098	1231	857	1177	1194	923
- week 2	1179	1437	1643	1050	1280	1346	1742	705*
- week 4	1321	1561	1686	1394	1462	1358	1577	1049
- week 8	1532	1462	1902	1630	1386	1502	1604	1165
- week 13	1298	1436	1559	1789	1585	1678	1648	1234

From Weiler (2001b)
* $P < 0.05$

There were no treatment-related gross lesions at necropsy at any dose level and no treatment-related microscopic lesions in the animals treated at 50 000 ppm in central and peripheral nervous tissues, skeletal muscle and other tissues examined. Histopathological alterations were infrequent and generally occurred in isolation, and the distribution between the control and dinotefuran-treated group did not indicate an effect of treatment.

The NOAEL for neurotoxicity in male rats was 50 000 ppm (equal to 3413 mg/kg bw per day), the highest dose tested. In female rats, a transient decrease in motor activity was observed at 50 000 ppm (equal to 3806 mg/kg bw per day), which is well above the limit dose. The NOAEL for systemic toxicity was 5000 ppm (equal to 327 mg/kg bw per day for males and 400 mg/kg bw per day

for females), based on reduced body weight gain and feed consumption at 50 000 ppm (Weiler, 2001b).

(b) Developmental neurotoxicity and immunotoxicity

In a dose range–finding developmental neurotoxicity and immunotoxicity study, groups of 10 mated female Crl:CD(SD) rats were given diets containing dinotefuran (purity 95.6%) at a concentration of 0, 1000, 3000 or 10 000 ppm from GD 6 through lactation day (LD) 21. F_1 generation rats were treated continuously after weaning until sacrifice. The mean test substance intake for the P generation females at 1000, 3000 and 10 000 ppm was equal to 69.5, 212 and 670 mg/kg bw per day during gestation and 141, 424 and 1401 mg/kg bw per day during lactation, respectively. For the F_1 generation, the mean test substance intake at 1000, 3000 and 10 000 ppm was equal to 100, 311 and 1043 mg/kg bw per day for males and 112, 316 and 1120 mg/kg bw per day for females, respectively. In P generation females, the day on which a vaginal plug, or sperm in a smear, was detected was designated day 0 of gestation (GD 0); the day of birth was designated postnatal day 0 (PND 0)/day 0 of lactation (LD 0).

P generation females were observed twice daily for viability and for clinical signs and general appearance weekly at approximately the same time each week during the pre-exposure period and on GD 0. The dams were also examined for clinical observations, abortions, premature deliveries and deaths on GDs 6, 9, 12, 15, 18, 20 and 25 (rats that did not deliver a litter) and LDs 0, 4, 7, 13 and 21. Body weights were recorded weekly during the pre-exposure period and on GDs 0, 6, 9, 12, 15, 18, 20 and 25 (if necessary) and LDs 0, 4, 7, 13 and 21. Feed consumption was recorded on GDs 0, 6, 9, 12, 15, 18, 20 and 25 (if necessary) and LDs 0, 4, 7 and 13. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes, live litter size and pup viability at birth. Maternal behaviour was evaluated on LDs 0, 4, 7, 13 and 21, and variations from expected maternal behaviour were recorded on all other days of the postpartum period. On LD 21, dams were sacrificed, and a gross necropsy of thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites were recorded. Rats that did not deliver a litter were sacrificed on GD 25 and examined for gross lesions. Uteri were examined to confirm the absence of implantation sites.

All pups in a litter were individually weighed on the day of birth and on PNDs 4, 7, 11, 13, 17 and 21. Each litter was evaluated for viability at least twice daily. The number of pups in each litter and clinical observations were recorded once daily during the preweaning period. Body weights were recorded weekly during the post-weaning period and prior to sacrifice. Feed consumption was measured weekly during the post-weaning period. On PND 4, the litter size was reduced, by random selection, to six pups of each sex, when possible. On PND 21, 20 pups of each sex per group (up to 3 pups of each sex per litter) were selected randomly for each of the two immunological assays, when possible. The first one or two pups of each sex per litter were selected for assay 1, and the last one or two pups of each sex per litter were assigned to assay 2.

Pups that died before initial examination of the litter for pup viability were evaluated for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sank were considered stillborn; pups with lungs that floated were considered liveborn and to have died shortly after birth. Pups found dead were examined for gross lesions and for the cause of death. On PND 4, all offspring selected for standardization were sacrificed and necropsied. On PND 21, all F_1 generation pups not selected for assays 1 and 2 were sacrificed and examined for gross lesions.

In immunological assay 1, rats were sacrificed 4 days following sensitization with the injection of sheep red blood cells and completion of the exposure period (PNDs 36–40 for male rats and PNDs 43–47 for female rats). Rats were examined for gross lesions, and spleens were harvested aseptically. The primary immunoglobulin M (IgM) response to sheep red blood cells was measured using a modified haemolytic plaque assay. Spleen cell number, following lysis of red blood cells, was determined. The cells per spleen, antibody-forming cells (AFC) per 10^6 spleen cells (specific activity), AFC/spleen (total spleen activity) and plaques were determined.

In immunological assay 2, rats were sacrificed following completion of the exposure period (PNDs 36–42 for male rats and PNDs 45–49 for female rats). Rats were examined for gross lesions, and spleens were harvested aseptically. The spleen cell phenotypes were evaluated using the following antibodies: CD45RA for total B cells, CD5 for total T cells, CD4 for T helper cells and CD8 for cytotoxic T cells. Natural killer (NK) cells were enumerated as cells that were NKP-P1A positive and CD8a negative. The samples were processed by flow cytometry for evaluation and enumeration. In the NK cell assay, the NK cell was used as the effector cell, and mouse lymphoma YAC-1 cells, introduced by inoculation of the Moloney leukaemia virus into a newborn A/Sn mouse, was used as the target cell. The mean per cent cytotoxicity at each effector concentration was determined.

All P generation female rats survived until scheduled sacrifice. There were no treatment-related clinical signs or gross lesions at necropsy. Body weights and body weight gains during the gestation and lactation periods were unaffected by exposure to the test substance at all dose levels. For absolute and relative feed consumption, all values were comparable among the dose groups and did not significantly differ, with the exception of absolute and relative feed consumption values for GDs 6–9, which were significantly reduced (about 10%) in the 10 000 ppm group (Table 48). Pregnancy occurred in 9, 9, 10 and 10 of the 10 mated female rats in the 0, 1000, 3000 and 10 000 ppm groups, respectively. All pregnant dams delivered litters. There were no effects of treatment on the numbers of dams delivering litters, the duration of gestation, the numbers of implantation sites per litter, the gestation index, the numbers of dams with stillborn pups and of dams with all pups dying, litter sizes, viability and lactation indices, surviving pups per litter, sex ratio or live litter size at weighing.

Pup weights in the 10 000 ppm group were significantly reduced on PNDs 13, 17 and 21. These reductions were probably due to direct exposure of the pups to the test substance in the diet as well as exposure through the milk, as pups start to eat feed at approximately PND 13. No deaths and no treatment-related clinical signs or gross lesions at necropsy were observed at any dose level.

In F_1 pups designated for the immunological assays, there were no treatment-related clinical signs, deaths or gross lesions at any dose level. However, one female rat in the 1000 ppm exposure group was found dead on PND 25, but death was considered not to be treatment related. Body weights of male and female rats were significantly lower than control values in the 1000 ppm and higher exposure groups on PND 22. However, significantly lower body weights continued only in the 10 000 ppm exposure group, through PND 57 for male rats and for PNDs 29, 36, 57 and 64 for female rats (Table 49). Significantly lower body weights in male rats occurred in the 1000 ppm exposure group on PND 29, but this reduction was not dose dependent and not considered related to the test substance. Body weight gains of male and female rats were unaffected by exposures to the test substance at all dose levels. No significant differences occurred among the groups with the exception of PNDs 57–64 in the 3000 and 10 000 ppm female exposure groups, where a non-dose-dependent but statistically significant reduction occurred. The general lack of statistically significant reductions in body weight gains indicates that the lower body weights that occurred on PND 22 were due to the high dose of test substance consumed at or near weaning. Feed consumption during the post-weaning period was unaffected by exposure to the test substance at concentrations as high as 10 000 ppm.

In immunological assay 1, exposure to dinotefuran did not produce dose-dependent decreases in the IgM AFC responses to sheep red blood cells when evaluated either as specific activity or as total spleen activity for both male and female rats (Table 49).

In immunological assay 2, terminal body weights in male rats and spleen weights (absolute and relative) in male and female rats did not differ significantly among the groups compared with the control group values. Terminal body weights in female rats were significantly reduced at 3000 and 10 000 ppm. Overall, the phenotypic analysis of splenic subpopulations demonstrated a lack of effect at all dose levels on the numbers of total B cells ($CD45^+$), total T cells ($CD5^+$), helper/delayed-type sensitivity (DTH) T cells ($CD4^+CD5^+$), cytotoxic T cells ($CD8^+CD5^+$) and NK cells ($NKR\text{-}PIA^+CD8^-$). Although males at 10 000 ppm showed a significantly lower group mean absolute number of NK cells, this was due to a lower absolute number of spleen cells, as the relative (per cent)

value for NK cells was comparable to the control value. In the NK cell assay, there was no effect on NK cell activity after exposures as high as 10 000 ppm in either male or female rats (Table 49).

Table 48. Selected maternal and litter data in the dose range–finding developmental neurotoxicity and immunotoxicity study in rats

	0 ppm	1000 ppm	3000 ppm	10 000 ppm
Maternal body weight (g)				
- GD 0	229	229	230	230
- GD 9	270	271	276	270
- GD 20	362	366	378	363
- LD 0	279	279	289	274
- LD 21	292	287	296	289
Maternal feed consumption (g/day)				
- GDs 0–6	18.6	19.0	19.7	19.2
- GDs 6–9	21.2	21.0	21.2	19.2**
- GDs 9–12	21.4	21.8	21.8	20.7
- GDs 15–18	21.8	21.7	23.4	21.9
Litters with liveborn pups/no. of pups delivered	9/118	9/126	10/139	10/139
Live litter size				
- PND 0	12.9	14.0	13.9	13.9
- PND 21	11.4	12.0	11.9	12.0
Pup weight/litter (g)				
- PND 0	6.7	6.2	6.5	6.6
- PND 4, pre-cull	10.6	9.9	10.5	10.3
- PND 4, post-cull	10.7	10.0	10.6	10.4
- PND 7	15.8	14.9	15.6	15.1
- PND 11	22.6	21.1	21.3	20.7
- PND 13	26.0	23.9	23.9	22.6**
- PND 17	33.0	30.0*	30.6	28.1**
- PND 21	46.7	41.5*	42.3	38.2**

From Hoberman (2009)
* $P < 0.05$; ** $P < 0.01$

The NOAEL for maternal toxicity was 10 000 ppm (equal to 670 mg/kg bw per day), the highest dose tested. The NOAEL for toxicity in offspring was 3000 ppm (equal to 311 mg/kg bw per day for males and 316 mg/kg bw per day for females), based on reduced body weight and body weight gain at 10 000 ppm. The NOAEL for developmental neurotoxicity and immunotoxicity was 10 000 ppm (equal to 1043 mg/kg bw per day for males and 1120 mg/kg bw per day for females), the highest dose tested (Hoberman, 2009).

In a developmental neurotoxicity study conducted in accordance with OECD Test Guideline No. 426, groups of 25 bred female Crl:CD(SD) rats were given diets containing dinotefuran (purity 99.5%) at a concentration of 0, 1000, 3000 or 10 000 ppm from GD 6 through LD 21. The mean test substance intakes at 1000, 3000 and 10 000 ppm were equal to 79.4, 237 and 784 mg/kg bw per day through gestation and 158, 501 and 1643 mg/kg bw per day through lactation, respectively. The day on which a vaginal plug, or sperm in a smear, was detected was designated GD 0; the day of birth was

designated LD 0 or PND 0. Direct dosing of F_1 generation pups during the lactation period was not performed, as the transfer of dinotefuran into the milk of lactating females has been confirmed (Hassler, 2006). F_1 generation offspring selected for study beyond PND 21 were not treated.

Table 49. Selected F_1 generation data in the dose range–finding developmental neurotoxicity and immunotoxicity study in rats

	Males				Females			
	0 ppm	1000 ppm	3000 ppm	10 000 ppm	0 ppm	1000 ppm	3000 ppm	10 000 ppm
Body weight (g)								
- PND 22	53.4	47.7**	48.8*	41.8**	51.8	44.4**	46.8*	42.1**
- PND 29	96.3	90.0*	93.6	83.3**	88.9	82.8	86.2	79.2**
- PND 36	152.4	146.2	151.6	135.8**	131.7	124.4	128.4	119.6**
- PND 43	214.0	204.8	212.1	193.4**	163.8	156.7	159.2	151.6
- PND 50	274.0	266.7	269.6	250.5**	189.4	184.8	184.6	176.1
- PND 57	332.0	327.1	330.0	308.1**	214.2	206.5	208.8	198.6*
- PND 64	—	—	—	—	235.8	225.4	223.4	214.3**
Body weight change (g)								
- PNDs 22–29	42.8	42.3	44.8	41.5	37.2	37.6	39.3	37.0
- PNDs 29–36	56.2	56.2	58.0	52.5	42.8	41.6	42.2	40.4
- PNDs 36–43	61.6	58.6	60.5	57.6	32.0	32.3	30.8	32.0
- PNDs 43–50	59.9	62.0	57.4	57.0	25.7	28.1	25.4	24.6
- PNDs 50–57	58.1	60.4	60.4	57.6	24.8	21.7	24.2	22.4
- PNDs 57–64	—	—	—	—	21.6	18.9	14.6**	15.8**
- PNDs 22–57	278.6	279.4	281.2	266.3	—	—	—	—
- PNDs 22–64	—	—	—	—	184.0	180.3	176.6	172.2
Immunological assay 1								
Body weight (g)	344	354	356	340	239	238	234	230
Spleen weight (mg)	619	846**	803*	776	521	546	549	557
Spleen cells ($\times 10^7$)	78.5	82.8	102	85.4	61.8	60.1	61.9	59.0
IgM AFC/10^6 spleen cells	1172	670	1027	909	2198	1673	1496	1668
IgM AFC/spleen ($\times 10^3$)	912	611	1022	788	1400	960	918	929
Immunological assay 2								
Body weight (g)	369	370	374	341	254	246	235*	218**
Spleen weight (mg)	749	774	733	671	498	512	476	482
Spleen cells ($\times 10^7$)	88.0	88.6	74.4	69.7*	57.3	62.8	59.6	55.7
Total B cells	485.2	503.2	433.6	386.3	310.6	323.8	348.5	316.0
Total T cells	251.4	246.0	195.9	209.5	167.6	203.7	162.9	163.1
Helper/DTH T cells	139.9	131.0	112.2	124.5	99.4	117.9	97.0	92.3
Cytotoxic T cells	109.8	111.2	83.3	83.8	74.8	90.8	68.8	72.8
NK cells	140.3	142.4	110.9	109.8*	97.8	114.6	90.7	95.8

From Hoberman (2009)
AFC, antibody-forming cell; DTH, delayed-type hypersensitivity; IgM, immunoglobulin M; NK, natural killer; * $P < 0.05$; ** $P < 0.01$

Parental generation females were observed twice daily for viability and for clinical signs and general appearance weekly at approximately the same time each week during the pre-exposure period and on GD 0. The dams were also examined for detailed clinical observations daily starting on GD 6 by an observer unaware of the dose groups. Body weights were recorded weekly during the pre-exposure period, on GD 0 and daily thereafter. Feed consumption was recorded on GD 0 and daily during all other periods. Dams were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes, live litter size and pup viability at birth. Maternal behaviour was evaluated on LDs 0, 4, 7, 13 and 21. Litters were standardized to five pups of each sex (where possible) on LD 4, and litters of fewer than nine pups were not assigned to study, but retained until needed. Parental generation females delivering a litter were sacrificed, and a gross necropsy of thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites were recorded. Female rats that did not deliver a litter were sacrificed on presumed GD 25 and examined for gross lesions. Uteri were examined to confirm the absence of implantation sites.

All pups in a litter were individually weighed on PNDs 0, 4, 7, 11, 13, 17 and 21. Each litter was evaluated for viability at least twice daily. The number of pups in each litter and clinical observations were recorded once daily during the preweaning period. Offspring were observed for viability at least twice daily, and clinical observations were recorded daily during the preweaning period. Body weights were recorded on PNDs 0, 4, 7, 11, 13, 17 and 21 and weekly during the post-weaning period and prior to sacrifice for pups selected for further study (subsets 1–5). Feed consumption was measured weekly during the post-weaning period. Pups that died before scheduled termination and not selected for further study were examined for gross lesions and the cause of death as soon as possible. Pups found dead on days 1–4 were preserved for possible future evaluation. All offspring not selected for continued evaluation were necropsied prior to weaning, and gross lesions were subjected to histological evaluation. Further evaluations were performed on the following subsets using, where possible, one pup of each sex per litter:

- *Subset 1 (up to 25 rats of each sex per group)*: Ten rats of each sex per group were sacrificed on PND 21 for recording of brain weight/gross dimensions and microscopic brain measurements (all treated groups) and neurohistopathological examination (0 and 10 000 ppm only). Pups not selected for neurohistopathology were sacrificed and evaluated for gross lesions.

- *Subset 2 (up to 25 rats of each sex per group)*: Pups were evaluated for day of preputial separation or vaginal patency and for passive avoidance response on PND 22. Water maze testing, swimming ability, learning and memory were measured on PNDs 58–62. In the passive avoidance and water maze trials, each rat was tested twice, with test sessions separated by a 1-week interval. After completion of the behavioural evaluations, pups were sacrificed and examined for gross lesions.

- *Subset 3 (up to 24 rats of each sex per group)*: Pups were evaluated for day of preputial separation or vaginal patency and motor activity on PNDs 13, 17, 21 and 60–62. The acoustic startle habituation response was measured on PNDs 22 and 59–62. After completion of the behavioural evaluations, pups were sacrificed and examined for gross lesions.

- *Subset 4 (up to 25 rats of each sex per group)*: Pups were evaluated for detailed clinical observations outside the home cage on LDs 4 and 11 and the day of preputial separation or vaginal patency. Ten rats of each sex per group were killed on PND 69 for recording of brain weight/gross dimensions and microscopic brain measurements (all groups) and neurohistopathology examinations (0 and 10 000 ppm only); pups not selected for these procedures were sacrificed and examined for gross lesions.

- *Subset 5*: Pups selected for subset 5 were not individually identified; they were killed on PND 21 and examined for gross lesions. Gross lesions were subjected to histological evaluation.

All P generation female rats survived to scheduled sacrifice, and there were no treatment-related adverse clinical observations. Mean body weight gain at 10 000 ppm was reduced by 11.2% from GD 6 to GD 20, but mean body weights were generally comparable among the dose groups

(Table 50). Body weight gains during the lactation period did not differ significantly among the exposure groups. Treatment at up to 3000 ppm during gestation and lactation did not affect body weight gains or body weights. Feed consumption was generally comparable among the treated and control groups throughout gestation and lactation. Pregnancy incidences were 92%, 100%, 92% and 96% at 0, 1000, 3000 and 10 000 ppm, respectively, and all pregnant dams delivered litters. All natural delivery observations and litter parameters were comparable in all treated and control groups. Although a statistically significantly lower viability index and significantly higher pup mortality from LD 1 to LD 4 occurred at 3000 ppm, they were considered unrelated to treatment, as the values at 10 000 ppm were comparable to the control values. No clinical or necropsy observations in the F_1 generation pups were attributed to maternal exposure to the test substance at any dose level. No maternal gross lesions related to treatment were evident at necropsy.

Table 50. Selected maternal and litter data in the developmental neurotoxicity study in rats

	0 ppm	1000 ppm	3000 ppm	10 000 ppm
Maternal body weight (g)				
- GD 0	235	235	235	236
- GD 20	391	385	390	378
- LD 0	298	293	297	294
- LD 11	337	324	335	325*
- LD 16	350	340	349	335*
- LD 21	331	323	331	325
Maternal body weight gain (g)				
- GDs 0–6	34.9	34.5	37.6	36.0
- GDs 6–9	14.2	14.8	12.0	7.7**
- GDs 9–12	17.5	17.4	18.3	17.2
- GDs 12–15	20.9	17.7	21.4	19.5
- GDs 15–18	37.6	36.6	36.4	34.1
- GDs 18–20	30.1	30.0	28.3	28.3
- GDs 6–20	120.3	116.4	116.4	106.8**
- LDs 0–21	31.8	28.8	34.2	33.0
No. of rats pregnant/delivering a litter	23/23	25/25	23/23	24/24
Duration of gestation (days)	22.6	22.7	22.5	22.5
Mean no. of implantation sites/litter	14.9	14.5	15.0	14.5
Dams with stillborn pups	1	3	3	2
Litters with 1 or more liveborn pups	23	25	23	24
Mean no. of liveborn pups/litter	14.3	13.7	13.8	13.3
Mean no. of stillborn pups/litter	0.0	0.1	0.1	0.1
Mean litter size, LD 0	14.3	13.7	13.7	13.3
Viability index (%)	99.4	98.5	95.9**	99.1
Lactation index (%)	100	100	100	99.0
Surviving pups/litter, LD 21	10.0	10.0	9.5	9.9
Sex ratio (% males), LD 21	48.1	50.0	49.0	49.5
Mean pup weight/litter (g), LD 0	6.6	6.6	6.5	6.6
Mean pup weight/litter (g), LD 21	50.2	48.6	51.3	50.2

From Hoberman (2010)
* $P < 0.05$; ** $P < 0.01$

In F_1 generation offspring retained beyond weaning for further investigation, there was no treatment-related mortality at any dose level. Two females at 10 000 ppm were found dead, and two control females were missing or killed due to poor clinical condition. All other animals survived to scheduled sacrifice. All clinical observations, including the detailed clinical observations outside the home cage on PNDs 4 and 11, in the F_1 offspring from all treated groups were considered unrelated to treatment, and evaluation of autonomic functions did not reveal any treatment-related effects. Statistically significant differences between the treated and control groups were confined to a higher incidence of umbilical hernia in females at 3000 ppm and a higher incidence of red- or black-coloured tail/tip of tail. There were no treatment-related necropsy observations at any dose level.

In F_1 generation rats, there was no effect of treatment at any dose level on brain weight or cerebral and cerebellar lengths evaluated in subsets 1 and 4 at PNDs 21 and 69, respectively. All group mean values were comparable to, and not significantly different from, control values, with the exception of 1000 ppm males on PND 21, which showed significantly lower mean brain weight compared with controls. Mean brain weights in males at 3000 or 10 000 ppm were comparable to the control value.

In F_1 generation rats, body weights and body weight gains were unaffected by treatment at all dose levels. A single significant increase in body weight gain of subset 4 male rats at 10 000 ppm occurred on PNDs 28–35 but was considered unrelated to treatment, because the increase did not persist and a similar increase was not evident in the other subsets evaluated. Feed consumption was not affected by treatment at any dose level. Statistically significant differences that occurred were considered not to be treatment related because the differences were not dose dependent and/or did not persist.

There was no effect of treatment on the day of vaginal patency or preputial separation in subsets 2, 3 and 4. The mean day of occurrence for each sex was comparable between the treated and control groups, and there were no statistically significant differences. For each sex, group mean body weights at these events were comparable in all treated and control groups.

In subset 2, there were no treatment-related effects on passive avoidance evaluated at PND 22 and again 1 week later or in a water maze test at PNDs 58–62 and again 1 week later. In the passive avoidance test, the mean number of trials to response, the latency of response and the numbers of animals failing to learn were comparable in all treated and control groups, and none of the values for the treated groups was significantly different from the control values. In the water maze performance assessment, the number of trials to achieve criterion, errors per trial, the numbers failing to learn and the latency periods were comparable in all treated and control groups, and none of the values for the treated groups was significantly different from the control values.

In subset 3, motor activity evaluated on PNDs 13, 17, 21 and 58–62 was not affected by treatment. All group mean values for the individual 10-minute blocks and totals (1 hour) for the number of movements and time spent in movement were comparable between the groups, and there were no statistically significant differences. Also, acoustic startle habituation evaluated on PNDs 22 and 58–62 was not affected by treatment.

There were no statistically significant intergroup differences at any dose level for brain weights or for gross cerebral and cerebellar measures in subsets 1 and 4 at PNDs 21 and 69, respectively. In addition, none of the microscopic brain measurements (frontal cortex, parietal cortex, striatum, corpus callosum, hippocampus and cerebellum) were significantly different from the control values at any dose level on PNDs 22 and 69. No treatment-related microscopic lesions were present in any of the tissues examined in the central and peripheral nervous systems in 22-day-old and adult (PND 69) offspring at 10 000 ppm.

The NOAEL for maternal toxicity was 3000 ppm (equal to 237 mg/kg bw per day), based on reduced body weight gain at 10 000 ppm (equal to 784 mg/kg bw per day). The NOAEL for developmental neurotoxicity was 10 000 ppm, the highest dose tested (Hoberman, 2009).

(c) Immunotoxicity

Mice

In an immunotoxicity study conducted in accordance with USEPA test guideline OPPTS 870.7800, groups of 10 male and 10 female Crl:CD1 (ICR) mice were fed diets containing dinotefuran (purity 97.9%) at a concentration of 0, 1120, 2800 or 7000 ppm (groups 1–4) (equal to 0, 153, 405 and 1053 mg/kg bw per day for males and 0, 223, 581 and 1438 mg/kg bw per day for females, respectively) for 4 weeks. A group of eight mice of each sex (group 5), given five daily oral (gavage) doses of cyclophosphamide at 20 mg/kg bw per day on days 22–26, acted as a positive control group. All animals received a sensitizing intravenous dose of sheep red blood cells on day 25 of the study. Animals were examined twice daily for morbidity or mortality, and a detailed clinical examination was performed weekly. Body weights of groups 1–4 were recorded twice during the week before treatment (day −7 and −4), on the 1st day of test (day 1), twice weekly during the treatment period and at necropsy. Group 5 animals were weighed on the 1st day of cyclophosphamide treatment only (day 22). Feed consumption was recorded weekly, and water consumption was recorded for a 3-day period each week throughout the treatment period for groups 1–4. The spleen from each animal in all groups was used as the source of splenocytes for conducting a plaque-forming cell (PFC) assay using a modification of the Jerne PFC assay, which had been fully validated at the conducting laboratory in addition to the concurrent positive controls. All animals were killed after 4 weeks of treatment and subjected to detailed necropsy. Spleen and thymus were weighed and were also adjusted for terminal body weight. Spleen tissues required for immunotoxicology investigations were retained from groups 1–5 for assessment of the acquired or adaptive immune response using a modification of the Jerne PFC assay.

The whole spleen was transferred to individual containers of Hank's balanced salt solution and held on (water) ice until processed for analysis. Splenocyte suspensions were prepared by mechanical dissociation and used for the PFC assay. All samples were coded and randomized for the assay. Duplicate samples per test per animal were enumerated using a dissection microscope with substage illumination. Viability testing was conducted on spleen cell preparations using a trypan blue dye exclusion method. The numbers of viable and non-viable cells were recorded to enable calculation of both the proportion and number of viable spleen cells per millilitre. The number of lytic plaques for each animal was determined, and group mean responses were calculated as PFCs per spleen and PFCs per 10^6 cells. The number of mononuclear cells per spleen was also calculated.

There were no treatment-related clinical signs at any dose level or in the cyclophosphamide-treated group, and no animals died prematurely. There were no treatment-related effects on body weight gain in either sex at any dose level. Although the overall mean body weight gain of males at 7000 ppm (2.3 g) was lower than the mean control gain (3.8 g), the difference was not statistically significant. Some males from all treated and control groups lost weight following the administration of antigen on day 25, but the loss in males at 7000 ppm was greater than in other groups and largely accounted for the observed difference.

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

Absolute and body weight–adjusted spleen and thymus weights were not significantly different from the control values for all dinotefuran-treated groups of both sexes and were therefore considered not to have been affected by treatment.

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

There was no effect of treatment at any dose level on the humoral T lymphocyte–dependent antibody response to sheep red blood cells, as measured using the modified PFC assay. There were no statistically significant differences in the dinotefuran-treated groups at any dose level in the number of

cells per spleen, PFCs per 10^6 viable cells and PFCs per spleen, when compared with the vehicle control group.

Treatment with five daily oral doses of cyclophosphamide at 20 mg/kg bw per day on days 22–26 resulted in a marked and statistically significant reduction of the PFC response. The number of cells per spleen, PFCs per 10^6 viable cells and PFCs per spleen were all statistically significantly reduced for males and females when compared with the controls, demonstrating the sensitivity of the PFC assay (Table 51).

Table 51. Summary of selected findings in the immunotoxicity study in mice

	Males					Females				
	0 ppm	1120 ppm	2800 ppm	7000 ppm	CP[a]	0 ppm	1120 ppm	2800 ppm	7000 ppm	CP[a]
Body weight (g)										
- day 1	35.7	36.0	36.0	33.8	—	27.3	26.4	27.4	26.7	—
- day 8	37.1	37.5	37.6	34.8	—	28.5	27.9	28.3	28.1	—
- day 22	39.1	40.6	40.1	36.4	—	30.4	29.8	31.2	28.7	—
- day 29	39.5	39.3	40.4	36.1	—	31.0	30.2	31.3	30.1	—
Body weight gain (g), days 1–29	3.8	3.3	4.4	2.3	—	3.7	3.8	3.9	3.5	—
Final body weight (g)	39.8	39.1	40.3	36.2*	—	30.9	30.0	31.1	29.9	—
Spleen weight (mg)	148	128	151	144	—	162	174	178	170	—
Thymus weight (mg)	41.6	36.5	36.4	31.0	—	54.0	49.6	51.1	51.9	—
Cells/spleen ($\times 10^7$)	9.29	6.40	7.74	7.65	4.84*	7.01	8.35	8.20	7.72	2.91**
PFCs/10^6 viable cells	1456	1729	1547	1519	17***	1197	1588	1135	1603	196***
PFCs/spleen	138 221	114 871	125 781	114 221	839***	96 605	140 864	106 564	129 998	7091***

From Bottomley (2011)
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$
[a] Cyclophosphamide, 20 mg/kg bw per day, by oral gavage, on days 22–26.

The NOAEL for immunotoxicity was 7000 ppm (equal to 1053 mg/kg bw per day for males and 1438 mg/kg bw per day for females), the highest dose tested. The NOAEL for systemic toxicity in males was 2800 ppm (equal to 405 mg/kg bw per day), based on decreased body weight at 7000 ppm. In females, the NOAEL for systemic toxicity was 7000 ppm (equal to 1438 mg/kg bw per day), the highest dose tested (Bottomley, 2011).

Rats

In an immunotoxicity study conducted in accordance with USEPA test guideline OPPTS 870.7800, groups of 10 male and 10 female Crl:CD (SD) rats were fed diets containing dinotefuran (purity 97.9%) at a concentration of 0, 2240, 5600 or 14 000 ppm (groups 1–4) (equal to 0, 164, 425 and 992 mg/kg bw per day for males and 0, 179, 430 and 1018 mg/kg bw per day for females) for 4 weeks. A group of eight rats of each sex (group 5), given a single intraperitoneal dose of

cyclophosphamide at 50 mg/kg bw on day 27, acted as a positive control group. All animals received a sensitizing intravenous dose of sheep red blood cells on day 25 of the study. Animals were examined twice daily for morbidity or mortality, and a detailed clinical examination was performed weekly. Body weights of groups 1–4 were recorded twice during the week before treatment (days −7 and −4), on the 1st day of test (day 1), twice weekly during the treatment period and at necropsy. Group 5 animals were weighed on the day of cyclophosphamide treatment only (day 27). Feed consumption was recorded weekly, and water consumption was recorded for a 3-day period each week throughout the treatment period for groups 1–4. The spleen from each animal in all groups was used as the source of splenocytes for conducting a PFC assay using a modification of the Jerne PFC assay, which had been fully validated at the conducting laboratory in addition to the concurrent positive controls. All animals were killed after 4 weeks of treatment and subjected to detailed necropsy. Spleen and thymus were weighed and were also adjusted for terminal body weight. Spleen tissues required for immunotoxicology investigations were retained from groups 1–5 for assessment of the acquired or adaptive immune response using a modification of the Jerne PFC assay.

The whole spleen was transferred to individual containers of Hank's balanced salt solution and held on (water) ice until processed for analysis. Splenocyte suspensions were prepared by mechanical dissociation and used for the PFC assay. All samples were coded and randomized for the assay. Duplicate samples per test per animal were enumerated using a dissection microscope with substage illumination. Viability testing was conducted on spleen cell preparations using a trypan blue dye exclusion method. The numbers of viable and non-viable cells were recorded to enable calculation of both the proportion and number of viable spleen cells per millilitre. The number of lytic plaques for each animal was determined, and group mean responses were calculated as PFCs per spleen and PFCs per 10^6 cells. The number of mononuclear cells per spleen was also calculated.

There were no treatment-related clinical signs at any dose level or in the cyclophosphamide-treated group, and no animals died prematurely. The body weight gain of males at 14 000 ppm was persistently lower than control values throughout the treatment period, resulting in an overall (days 1–29) weight gain that was statistically significantly lower than the control value by approximately 28%. On day 29, the group mean body weight of males at 14 000 ppm was 89.7% of the control value. The body weights for both sexes at 2240 and 5600 ppm and females at 14 000 ppm were considered to have been unaffected by treatment. Although the overall weight gain among females at 14 000 ppm was 10% lower than the control value, it did not attain statistical significance and was due to the lower weight gain that occurred between days 1 and 4 of treatment, with the subsequent weight gain being similar to that of controls. Thus, the group mean body weight of females at 14 000 ppm on day 29 remained marginally higher than the control value.

Feed consumption was persistently lower than that of controls for both sexes at 14 000 ppm, resulting in an overall reduction, compared with controls, of 12% in males and 10% in females. The feed consumption of both sexes at 2240 and 5600 ppm was considered to be unaffected by treatment. The slightly low feed consumption of females at 5600 ppm represented a trend that was present before treatment commenced and was therefore considered not to be due to treatment with dinotefuran. Water consumption among all treated groups of both sexes, although variable, was similar to control or pretreatment values and was therefore considered to be unaffected by treatment.

The macroscopic examination performed after 4 weeks of treatment revealed no lesions attributable to treatment with dinotefuran. The nature and incidence of all the findings were consistent with the commonly seen background of macroscopic changes in Crl: CD (SD) strain rats. Absolute and body weight–adjusted spleen and thymus weights were similar to, and not significantly different from, the control values for all dinotefuran-treated groups of both sexes and were therefore not affected by treatment.

There was no effect of treatment at any dose level on the humoral T lymphocyte–dependent antibody response to sheep red blood cells, as measured using the modified PFC assay. There were no statistically significant changes in the number of cells per spleen, PFCs per 10^6 viable cells or PFCs per spleen, when compared with the controls, for the treated groups that received dinotefuran at 2240, 5600 or 14 000 ppm.

Treatment with a single dose of cyclophosphamide at 50 mg/kg bw on day 27 resulted in a marked and statistically significant reduction of the PFC response. The number of cells per spleen, PFCs per 10^6 viable cells and PFCs per spleen were all statistically significantly reduced for males and females when compared with the controls, demonstrating the sensitivity of the PFC assay (Table 52).

Table 52. Summary of selected findings in the immunotoxicity study in rats

	Males					Females				
	0 ppm	2240 ppm	5600 ppm	14 000 ppm	CP[a]	0 ppm	2240 ppm	5600 ppm	14 000 ppm	CP[a]
Body weight (g)										
- day 1	295	298	303	292	—	210	210	208	219	—
- day 8	343	342	355	322	—	223	224	220	227	—
- day 22	412	408	421	375	—	244	248	237	248	—
- day 29	447	439	460	401	—	253	255	249	258	—
Body weight gain (g), days 1–29	152	141	157	109**	—	44	45	40	39	—
Feed consumption (g/week)										
- week −1	191	191	178	186	—	134	126	123	141	—
- week 1	193	188	203	171	—	133	128	124	116	—
- week 2	196	194	207	174	—	143	137	124	122	—
- week 3	188	183	197	166	—	129	132	120	123	—
- week 4	199	193	208	176	—	136	129	124	123	—
Final body weight (g)	447	439	458	399**	—	252	254	248	257	—
Spleen weight (mg)	872	901	925	772	—	614	599	528	611	—
Thymus weight (mg)	530	527	486	455	—	385	363	343	387	—
Cells/spleen ($\times 10^7$)	48.3	45.9	48.2	45.3	9.7***	37.0	40.4	29.8	34.0	8.0***
PFCs/10^6 viable cells	291	561	609	405	6.9***	814	757	763	626	29.4***
PFCs/spleen	147 252	256 969	290 493	186 292	672***	334 615	296 590	224 822	230 603	2339***

From Chambers (2011)
** $P < 0.01$; *** $P < 0.001$
[a] Cyclophosphamide, 50 mg/kg bw, by intraperitoneal injection, on day 27.

The NOAEL for immunotoxicity was 14 000 ppm (equal to 992 mg/kg bw per day for males and 1018 mg/kg bw per day for females), the highest dose tested. The NOAEL for systemic toxicity was 5600 ppm (equal to 425 mg/kg bw per day in males and 430 mg/kg bw per day in females), based on reduced body weight and body weight gain in males and reduced feed consumption in both sexes at 14 000 ppm (Chambers, 2011).

(d) Transfer into milk of lactating rats

In a study conducted to provide information on the transfer of ^{14}C-labelled dinotefuran and/or its radiolabelled metabolites into the milk of lactating rats, groups of six lactating Sprague-Dawley rats were administered ^{14}C-labelled dinotefuran (purity 97.3%; radiochemical purity 96.0%) as a single dose by oral gavage at a dose level of 50 or 500 mg/kg bw on LDs 2, 4, 8 and 12. The concentration of radioactivity in maternal milk, whole blood and plasma was determined in three rats of each group at each of two time points after each oral dose, 0.5 and 1.5 hours (50 mg/kg bw) and 2 and 4 hours (500 mg/kg bw). Only dams with at least eight viable pups were selected for study, and the number of pups in each litter was reduced to 12 by random selection on LD 1 (the day after completion of parturition). Clinical signs and unusual behaviour of animals were visually checked each working day. Individual body weights were recorded at the start of acclimatization, just before the administration on LDs 2, 4, 8 and 12 and prior to sacrifice. The total weight of the litters was determined on LDs 2, 4, 8 and 16. Three hours prior to milking, the dams were separated from the pups, and milk injection into the mammary glands was stimulated by an intraperitoneal injection of oxytocin (4 international units [IU]/kg bw) about 5 minutes prior to milking. The milk specimen was obtained by a vacuum-driven milking pump at the specified time point from three animals each, and then dams were placed back with their pups. After milking, a 0.5 ml blood sample was withdrawn from the sublingual vein from each animal at the selected time point. Radioactivity was measured by LSC equipped for computing quench-corrected disintegrations per minute.

The range of the average dose administered to the six lactating dams at a nominal dose level of 50 or 500 mg/kg bw was 48.1–51.7 mg/kg bw and 491–509 mg/kg bw, respectively. No unusual appearance or behaviour of pups and dams was observed during the experimental period. Parturition in all pregnant females occurred on day 22 or 23 of pregnancy. The weight gains of the lactating females and their litters were considered to be within the normal range.

In the low-dose group (Table 53), the test substance was rapidly absorbed from the gastrointestinal tract into the systemic circulation. The concentrations in blood, plasma and milk 0.5 hour after administration showed very little variation between sampling occasions from LD 2 to LD 12. The mean values for whole blood and plasma were within the range 30.1–35.2 ppm dinotefuran equivalents, but the concentrations in milk, which were within the range 55.2–62.9 ppm, were approximately 2-fold higher than the whole blood/plasma concentrations. Within 1.5 hours after administration, the concentrations in blood and plasma had declined to about half the levels determined after 0.5 hour and were within the range 13.9–17.5 ppm dinotefuran equivalents. The concentrations in milk were within the range 26.4–36.9 ppm dinotefuran equivalents and remained approximately 2-fold higher than blood and plasma concentrations at all sampling occasions. The achieved concentrations in blood and plasma were very similar at all sampling time points during the observed lactation period, whereas the concentrations in milk showed slightly increasing values.

In the high-dose group (Table 53), the absorption and depletion profiles resembled those observed for the low-dose group. However, the measured concentrations in blood and plasma were only approximately 4 times higher for a 10-fold increase in dose level. Thus, 2 hours after administration, the concentrations in blood and plasma were within the range 104–144 ppm dinotefuran equivalents, and the concentrations in milk were 160–199 ppm dinotefuran equivalents, on all sampling occasions from LD 2 to LD 12. The concentrations in blood and plasma were very similar, and milk concentrations were 40–68% higher than the average concentration in blood and plasma on all sampling occasions. Four hours after administration, the concentrations in blood and plasma remained very similar but had declined to 70–96 ppm dinotefuran equivalents. Milk concentrations had declined to 114–196 ppm and were 50–161% higher than blood/plasma concentrations on all sampling occasions from LD 2 to LD 12. The levels of radioactivity in blood and plasma were not influenced by the period of lactation, but concentrations in milk at 4 hours tended to increase as lactation progressed. However, at this time on LD 12, the measured value showed a high interindividual variation, and the correlation to the blood concentration was significantly different from that at all other time points. Therefore, it was considered likely that the high concentration in milk was partially caused by contamination with urine, as at this time point the posterior-most teats were used for milk sampling.

Table 53. Concentration of total radioactivity in blood, plasma and milk in the transfer-into-milk study in rats

	Concentration of total radioactivity (ppm dinotefuran equivalents)							
	Dosed on LD 2		Dosed on LD 4		Dosed on LD 8		Dosed on LD 12	
Low-dose group								
Dose (mg/kg bw)	*51.7*	*51.3*	*49.8*	*50.7*	*48.1*	*48.5*	*50.5*	*50.8*
Sampling time (h)	*0.5*	*1.5*	*0.5*	*1.5*	*0.5*	*1.5*	*0.5*	*1.5*
Blood	33.1	16.6	30.5	15.3	30.1	13.9	31.2	14.8
Plasma	35.2	17.5	32.5	16.1	32.2	14.8	33.7	15.8
Milk	60.0	26.4	55.2	27.8	58.7	30.7	62.9	36.9
High-dose group								
Dose (mg/kg bw)	*491*	*495*	*509*	*492*	*492*	*492*	*496*	*492*
Sampling time (h)	*2*	*4*	*2*	*4*	*2*	*4*	*2*	*4*
Blood	136	90	104	74	108	70	106	72
Plasma	144	96	109	77	114	75	112	77
Milk	199	141	160	114	187	136	178	196

From Hassler (2006)

In conclusion, the study provides evidence of neonatal exposure of suckling pups to dinotefuran and/or metabolites via the maternal milk (Hassler, 2006).

3. Observations in humans

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with dinotefuran.

Comments

Biochemical aspects

In rats given (^{14}C-tetrahydrofuran)-labelled or (^{14}C-guanidine)-labelled dinotefuran orally by gavage, absorption was rapid and accounted for at least 88% of the total administered radioactivity after a single low dose (50 mg/kg bw) or high dose (1000 mg/kg bw). The maximum plasma concentrations of radioactivity were reached after approximately 0.5 and 2 hours after administration of the low and high doses, respectively, whereas the half-lives in plasma ranged from 4 to 15 hours for the low and high doses, respectively. Radioactivity was widely distributed throughout the body. Elimination of the radioactivity was mainly via urine (≥ 88% of the administered dose), whereas elimination via faeces accounted for 1–3% after oral administration and 1% after intravenous administration. Residues in tissues 168 hours after a single oral or intravenous dose as well as after repeated oral dosing accounted for less than 0.5% of the administered radioactivity, and the concentrations in most tissues were below the limit of detection (0.001 ppm).

Metabolism of dinotefuran in rats was limited, with more than 90% of the dose being eliminated as unchanged parent molecule, which was also the major component in plasma, milk, bile and most tissues collected 4–8 hours after administration. About 20 metabolites were identified; the metabolic routes included hydroxylation on the tetrahydrofuran ring, followed by further oxidation, reduction and acetylation. Other routes of metabolism involved desmethylation, nitro-reduction and hydrolysis.

Toxicological data

The LD_{50} in rats treated orally with dinotefuran was 2450 mg/kg bw. The dermal LD_{50} in rats was greater than 2000 mg/kg bw, and the inhalation LC_{50} in rats was greater than 4.09 mg/l. Dinotefuran was not a skin irritant in rabbits, was slightly irritating to the eye of rabbits and was not a skin sensitizer in the maximization test in guinea-pigs.

Although dinotefuran is neurotoxic in insects, neurotoxicity in mammals was not a critical effect after repeated exposure. No specific target organs were clearly identified in any species following short-term or long-term oral exposure, despite the administration of very high doses of up to 10 635, 3156 and 862 mg/kg bw per day for 13 weeks in mice, rats and dogs, respectively. In all species, the NOAELs were based on decreases in body weight and/or body weight gain as the critical effect. At higher dose levels, a number of minor effects on clinical chemistry parameters, without histopathological correlates, occurred in all species and comprised increased serum albumin concentration and reduced urinary pH in mice, increased serum cholesterol and urea nitrogen concentrations and reduced serum glucose and protein concentrations in rats, and reduced urinary pH in dogs.

In a 4-week study in mice, the NOAEL was 5000 ppm (equal to 901 mg/kg bw per day), based on reduced body weight gain at 25 000 ppm (equal to 4612 mg/kg bw per day) and above. In a 13-week study in mice, the NOAEL was 25 000 ppm (equal to 4442 mg/kg bw per day), based on reduced body weight and body weight gain at 50 000 ppm (equal to 10 635 mg/kg bw per day).

In a 4-week study in rats, the NOAEL was 5000 ppm (equal to 390 mg/kg bw per day), based on reduced body weight gain and increased serum cholesterol in males at 25 000 ppm (equal to 1814 mg/kg bw per day) and above. In a 13-week study in rats, the NOAEL was 500 ppm (equal to 38 mg/kg bw per day), based on reduced body weight and body weight gain at 5000 ppm (equal to 384 mg/kg bw per day) and above in females.

In a 13-week feeding study in dogs, a NOAEL could not be identified in females; the LOAEL was 1600 ppm (equal to 58 mg/kg bw per day) in females, based on a reduction in feed consumption, body weight and body weight gain at all doses administered. In males, the NOAEL was 8000 ppm (equal to 307 mg/kg bw per day), based on a reduction in feed and water consumption, body weight and body weight gain at 24 000–40 000 ppm (equal to an average dose of 862 mg/kg bw per day). In a 1-year feeding study in dogs, the NOAEL was 640 ppm (equal to 22 mg/kg bw per day) in females, based on a reduction in feed consumption, body weight and body weight gain at 3200 ppm (equal to 108 mg/kg bw per day) and above. In males, the NOAEL was 3200 ppm (equal to 111 mg/kg bw per day), based on a reduction in body weight gain at 16 000 ppm (equal to 559 mg/kg bw per day).

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In a 78-week study of carcinogenicity in mice, there was no evidence for carcinogenicity up to the highest dose tested (25 000 ppm, equal to 3694 mg/kg bw per day). The NOAEL for toxicity was 2500 ppm (equal to 345 mg/kg bw per day), based on reduced body weight and body weight gain at 25 000 ppm.

In a 104-week study of toxicity and carcinogenicity in rats, there was no evidence for carcinogenicity up to the highest dose tested (20 000 ppm, equal to 991 mg/kg bw per day). The NOAEL for toxicity was 2000 ppm (equal to 100 mg/kg bw per day), based on a reduction in body weight, body weight gain and feed consumption at 20 000 ppm.

The Meeting concluded that dinotefuran is not carcinogenic in mice or rats.

Dinotefuran was tested for genotoxicity in vitro and in vivo in an adequate range of assays. It was not found to be genotoxic.

The Meeting concluded that dinotefuran is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in rats and mice, the Meeting concluded that dinotefuran is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, the NOAEL for reproductive toxicity was 10 000 ppm (equal to 822 mg/kg bw per day), the highest dose tested, whereas a reduced

number of implantations, increased post-implantation loss and reduced litter size were observed in a range-finding study at 20 000 ppm (equal to 1340 mg/kg bw per day). The NOAEL for parental toxicity was 3000 ppm (equal to 241 mg/kg bw per day), based on a reduction in feed consumption, body weight and spleen weight at 10 000 ppm. The NOAEL for offspring toxicity was 3000 ppm, based on reduced pup weight gain during lactation and reduced spleen weight at 10 000 ppm.

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 300 mg/kg bw per day, based on decreased weight gain and feed consumption and increased water consumption at 1000 mg/kg bw per day. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits, acute clinical signs (hypoactivity, prone position, panting, erythema, tremor) were observed in dams at 300 mg/kg bw per day from the start of treatment at GD 6 until GD 13; the NOAEL was 125 mg/kg bw per day. Also in dams, a reduction in body weight gain was noted at 125 mg/kg bw per day and above, with a NOAEL of 52 mg/kg bw per day. The NOAEL for developmental toxicity was 300 mg/kg bw per day, the highest dose tested.

The Meeting concluded that dinotefuran was not teratogenic in rats or rabbits.

In an acute neurotoxicity study in rats, the NOAEL was 750 mg/kg bw, based on a transient decrease in motor activity at 1500 mg/kg bw. There was no evidence for neuropathological effects up to the highest dose tested (1500 mg/kg bw).

In a 13-week neurotoxicity study in rats, a transient decrease in motor activity was observed in females at 50 000 ppm (equal to 3806 mg/kg bw per day), which is well above the limit dose. The NOAEL for the study was 5000 ppm (equal to 327 mg/kg bw per day), based on reduced body weight gain and feed consumption at 50 000 ppm (equal to 3413 mg/kg bw per day).

In a dose range–finding developmental neurotoxicity and immunotoxicity study in rats, there was no evidence for developmental neurotoxicity or immunotoxicity up to the highest dose tested (10 000 ppm, equal to 1043 mg/kg bw per day). The NOAEL for maternal toxicity was 10 000 ppm (equal to 670 mg/kg bw per day), the highest dose tested, whereas the NOAEL for offspring toxicity was 3000 ppm (equal to 311 mg/kg bw per day), based on reduced body weight and body weight gain at 10 000 ppm.

In a developmental neurotoxicity study in rats, there was no evidence for developmental neurotoxicity up to the highest dose tested (10 000 ppm, equal to 784 mg/kg bw per day). The NOAEL for maternal toxicity was 3000 ppm (equal to 237 mg/kg bw per day), based on reduced body weight gain at 10 000 ppm.

In 4-week immunotoxicity studies in mice and rats, there was no evidence for immunotoxicity up to the highest dose tested (7000 ppm in mice, equal to 1053 mg/kg bw per day; 14 000 ppm in rats, equal to 992 mg/kg bw per day). The NOAELs for systemic toxicity were 2800 and 5600 ppm (equal to 405 and 425 mg/kg bw per day, respectively) in mice and rats, respectively, based on decreased body weight gain at 7000 and 14 000 ppm, respectively.

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with dinotefuran.

The Meeting concluded that the existing database on dinotefuran was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for dinotefuran of 0–0.2 mg/kg bw, based on the NOAEL of 22 mg/kg bw per day for reduced body weight/body weight gain in female dogs in a 1-year toxicity study and application of a safety factor of 100.

The Meeting established an acute reference dose (ARfD) for dinotefuran of 1 mg/kg bw, based on the NOAEL of 125 mg/kg bw for acute clinical signs observed in dams after a single dose of 300 mg/kg bw in a developmental toxicity study in rabbits. A 100-fold safety factor was applied.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	2500 ppm, equal to 345 mg/kg bw per day	25 000 ppm, equal to 3694 mg/kg bw per day
		Carcinogenicity	25 000 ppm, equal to 3694 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	2000 ppm, equal to 100 mg/kg bw per day	20 000 ppm, equal to 991 mg/kg bw per day
		Carcinogenicity	20 000 ppm, equal to 991 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	10 000 ppm, equal to 822 mg/kg bw per day[b]	—
		Parental toxicity	3000 ppm, equal to 241 mg/kg bw per day	10 000 ppm, equal to 822 mg/kg bw per day
		Offspring toxicity	3000 ppm, equal to 241 mg/kg bw per day	10 000 ppm, equal to 822 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	300 mg/kg bw per day	1000 mg/kg bw per day
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity study[c]	Maternal toxicity	125 mg/kg bw per day[d] 52 mg/kg bw per day[e]	300 mg/kg bw per day 125 mg/kg bw per day
		Embryo and fetal toxicity	300 mg/kg bw per day[b]	—
Dog	Thirteen-week and 1-year studies of toxicity[a,f]	Toxicity	640 ppm, equal to 22 mg/kg bw per day	1600 ppm, equal to 58 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] NOAEL for acute clinical signs.
[e] NOAEL for maternal toxicity.
[f] Two studies combined.

Estimate of acceptable daily intake for humans

0–0.2 mg/kg bw

Estimate of acute reference dose

1 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to dinotefuran

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; ≥ 88%
Dermal absorption	No data

Distribution	Widely distributed; highest concentrations in kidney and urine
Potential for accumulation	None
Rate and extent of excretion	≥ 93% within 168 h (≥ 88% in urine; 1–3% in faeces; 1–6% in cage rinse)
Metabolism in animals	Limited (> 90% eliminated as parent); hydroxylation on the tetrahydrofuran ring, followed by oxidation, reduction and acetylation; other routes include desmethylation, nitro-reduction and hydrolysis
Toxicologically significant compounds in animals, plants and the environment	Dinotefuran
Acute toxicity	
Rat, LD_{50}, oral	2450 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.09 mg/l (4 h, nose-only exposure)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slightly irritating
Dermal sensitization	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Reduced body weight gain
Lowest relevant oral NOAEL	22 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (28-day study in rats)
Lowest relevant inhalation NOAEC	0.22 mg/l (28-day study in rats)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weight gain
Lowest relevant NOAEL	100 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic in mice or rats
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive toxicity; reduced pup weight gain and reduced spleen weight at parentally toxic dose
Lowest relevant reproductive NOAEL	822 mg/kg bw per day (highest dose tested)
Lowest relevant parental NOAEL	241 mg/kg bw per day
Lowest relevant offspring NOAEL	241 mg/kg bw per day
Developmental toxicity	
Developmental target/critical effect	No evidence for developmental toxicity (rats and rabbits)
Lowest relevant maternal NOAEL	52 mg/kg bw per day (rabbits)
Lowest relevant embryo/fetal NOAEL	300 mg/kg bw per day (rabbits) (highest dose tested)
Neurotoxicity	
Acute and subchronic neurotoxicity	No specific signs of acute or subchronic neurotoxicity at highest dose tested (acute: 1500 mg/kg bw; subchronic: 3806 mg/kg bw per day)
Developmental neurotoxicity	No evidence for developmental neurotoxicity at highest dose tested (784 mg/kg bw per day)

Other toxicological studies	
Immunotoxicity	No evidence for immunotoxicity at highest dose tested (1053 mg/kg bw per day in mice; 992 mg/kg bw per day in rats)
Developmental immunotoxicity	No evidence for developmental immunotoxicity at highest dose tested (1043 mg/kg bw per day)
Medical data	
	No adverse health effects reported in manufacturing plant personnel

Summary

	Value	Study	Safety factor
ADI	0–0.2 mg/kg bw	One-year study of toxicity in dogs	100
ARfD	1 mg/kg bw	Developmental toxicity study in rabbits (acute clinical signs in dams)	100

References

Becker H (2002). MTI-446 two-generation reproduction study in the Han Wistar rat by oral (dietary) administration. Unpublished report no. 775192. RCC Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Bottomley AL (2011). Dinotefuran: 4-week dietary immunotoxicity study in the CD-1 mouse. Unpublished report no. MCW0019. Huntington Life Sciences Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Chambers PR (2011). Dinotefuran: 4-week dietary immunotoxicity study in the CD rat. Unpublished report no. MCW0018. Huntington Life Sciences Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Cheng T (2000a). Metabolism of [^{14}C]-MTI-446 in rats. Unpublished report no. Covance 6648-136. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Cheng T (2000b). First amendment to report—Metabolism of [^{14}C]-MTI-446 in rats. Unpublished report no. Covance 6648-136. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Cheng T (2000c). Absorption, distribution, metabolism and excretion of [G-^{14}C]-MTI-446 following administration of a single oral dose to neonatal rats. Unpublished report no. Covance 6648-141. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Cheng T (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. Unpublished report no. Covance 6648-141. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Cheng T (2001). Second amendment to report—Metabolism of [^{14}C]-MTI-446 in rats. Unpublished report no. Covance 6648-136. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Edwards JA, Knuppe C, Weber K (2001). MTI-446 technical preliminary two generation study in the Han Wistar rat. Unpublished report no. 774990. RCC Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Gale EP (2005). Historical control data for 52-week dog studies. MRID No. 46621601. Unpublished report. Submitted to WHO by Mitsui Chemicals, Inc.

Glaza SM (1997a). Acute oral toxicity study of MTI-446 in rats. Unpublished report no. CHW 6648-118. Corning Hazleton Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Glaza SM (1997b). Acute oral toxicity study of MTI-446 in mice. Unpublished report no. CHW 6648-119. Corning Hazleton Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Glaza SM (1997c). Acute dermal toxicity study of MTI-446 in rats. Unpublished report no. CHW 6648-120. Corning Hazleton Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Glaza SM (1997d). Dermal sensitization study of MTI-446 in guinea pigs—maximisation test. Unpublished report no. Covance 6648-123. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Glaza SM (1998a). Primary dermal irritation study of MTI-446 in rabbits. Unpublished report no. Covance 6648-121. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Glaza SM (1998b). Primary eye irritation study of MTI-446 in rabbits. Unpublished report no. Covance 6648-122. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Hassler S (2006a). Transfer of [^{14}C]MTI-446 into milk of lactating rats after oral administration. Unpublished report no. A29136. RCC Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Henwood SM (2001a). 14-day range-finding dermal toxicity study with MTI-446 in rats. Unpublished report no. 6648-150. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Henwood SM (2001b). 28-day dermal toxicity study with MTI-446 in rat. Unpublished report no. 6648-149. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Hoberman AM (2009). Oral (diet) dosage-range finding developmental neurotoxicity and immunotoxicity study of MTI-446 (dinotefuran) in Crl:CD (SD) rats. Unpublished report no. SRY00001. Charles River Laboratories Preclinical Services. Submitted to WHO by Mitsui Chemicals, Inc.

Hoberman AM (2010). Oral (diet) developmental neurotoxicity study of MTI-446 (dinotefuran) in Crl:CD (SD) rats. Unpublished report no. SRY00002. Charles River Laboratories Preclinical Services. Submitted to WHO by Mitsui Chemicals, Inc.

Kay JH, Calandra JC (1962). Interpretation of eye irritation tests. *Journal of the Society of Cosmetic Chemists*, 13:281–289.

Kuhn JO (2004). LX-1434-04 (dinotefuran technical): acute eye irritation study in rabbits. Unpublished report no. 8394-04. Stillmeadow, Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Lloyd M (2002). MTI-446 technical material: mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the microtitre fluctuation technique. Unpublished report no. 719/15-D6173. Covance Laboratories Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Nakajima M (1995). Micronucleus test of EXP-316 with mice. Unpublished report no. 2498. Biosafety Research Center. Submitted to WHO by Mitsui Chemicals, Inc.

Oguma Y (1999). A DNA repair assay of *Bacillus subtilis* on MTI-446. Unpublished report no. 4731. BML Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Sakurai T (1998a). A dose finding teratogenicity study of MTI-446 given orally to rats. Unpublished report no. H-97162. Nippon Experimental Medical Research Institute Co. Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Sakurai T (1998b). Teratogenicity study of MTI-446 given orally to rats. Unpublished report no. H-97163. Nippon Experimental Medical Research Institute Co. Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Sakurai T (1998c). A single and 2-week repeated dose preliminary study of MTI-446 given orally to rabbits. Unpublished report no. H-97164. Nippon Experimental Medical Research Institute Co. Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Sakurai T (1998d). A dose finding teratogenicity study of MTI-446 given orally to rabbits. Unpublished report no. H-97165. Nippon Experimental Medical Research Institute Co. Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Sakurai T (1998e). Teratogenicity study of MTI-446 given orally to rabbits. Unpublished report no. H-97166. Nippon Experimental Medical Research Institute Co. Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Satou T (1996). MTI-446: in vitro mammalian cytogenetics test. Unpublished report no. CRC0076. Chromosome Research Center Olympus Optical Co. Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Shepherd N (1999). MTI-446: acute inhalation (nose only) toxicity study in the rat. Unpublished report no. 1300/3-D6154. Covance Laboratories Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Shepherd N (2000a). First amendment to report—MTI-446: acute inhalation (nose only) toxicity study in the rat. Unpublished report no. 1300/3-D6154. Covance Laboratories Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Shepherd N (2000b). Second amendment to report—MTI-446: acute inhalation (nose only) toxicity study in the rat. Unpublished report no. 1300/3-D6154. Covance Laboratories Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Shepherd NM (2002). MTI-446: 28-day inhalation (nose only) toxicity study in the rat. Unpublished report no. 719/16. Covance Laboratories Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Takeda C (1996). MTI-446: microbial reverse mutation assay. Unpublished report no. CRC3133. Chromosome Research Center, Olympus Optical Co. Ltd. Submitted to WHO by Mitsui Chemicals, Inc.

Teramoto I (1998a). Repeated dose toxicity test of MTI-446 mixed in the diet for one week using dogs. Unpublished report no. H-97327. Jitsuiken Medical Research Institute, Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Teramoto I (1998b). Repeated dose toxicity test of MTI-446 by forced oral administration for one week using dogs. Unpublished report no. H-97326. Jitsuiken Medical Research Institute, Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Weiler MS (1997a). 4-week dietary toxicity study with MTI-446 in rats. Unpublished report no. CHW 6648-125. Corning Hazleton Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Weiler MS (1997b). 4-week dietary toxicity study with MTI-446 in mice. Unpublished report no. CHW 6648-124. Corning Hazleton Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Weiler MS (1997c). 13-week dietary toxicity study with MTI-446 in rats. Unpublished report no. CHW 6648-127. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Weiler MS (1997d). 13-week dietary toxicity study with MTI-446 in mice. Unpublished report no. CHW 6648-126. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Weiler MS (1999a). 13-week dietary toxicity study with MTI-446 in dogs. Unpublished report no. CHW 6648-128. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Weiler MS (1999b). First amendment to report—13-week dietary toxicity study with MTI-446 in dogs. Unpublished report no. CHW 6648-128. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Weiler MS (1999c). 52-week dietary chronic toxicity study with MTI-446 in dogs. Unpublished report no. CHW 6648-129. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Weiler MS (2000a). First amendment to report—13-week dietary toxicity study with MTI-446 in rats. Unpublished report no. CHW 6648-127. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Weiler MS (2000b). First amendment to report—13-week dietary toxicity study with MTI-446 in mice. Unpublished report no. CHW 6648-126. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Weiler MS (2000c). 104-week dietary combined chronic toxicity and carcinogenicity study with MTI-446 in rats. Unpublished report no. CHW 6648-131. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

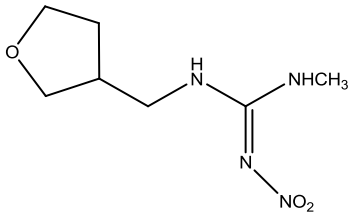
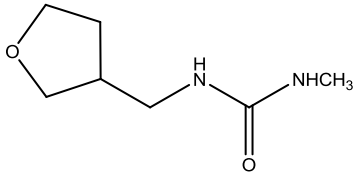
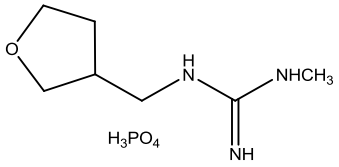
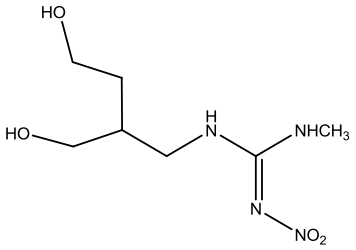
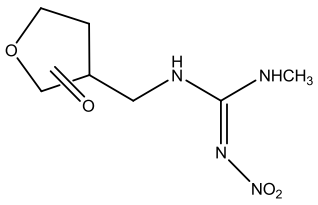
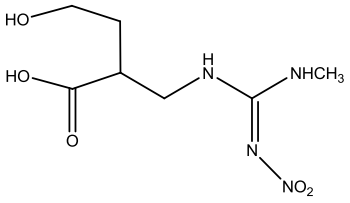
Weiler MS (2000d). 78-week dietary carcinogenicity study with MTI-446 in mice. Unpublished report no. CHW 6648-130. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

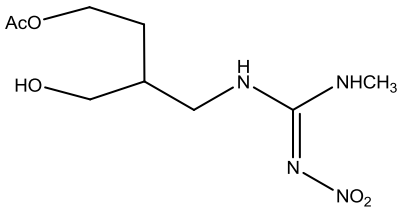
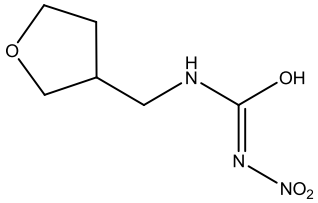
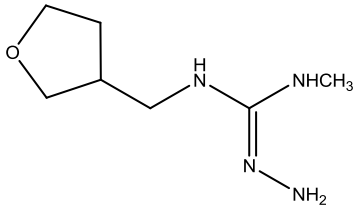
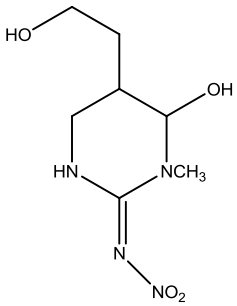
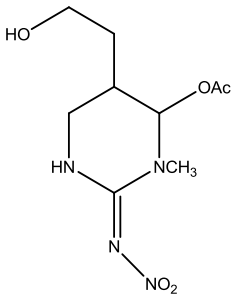
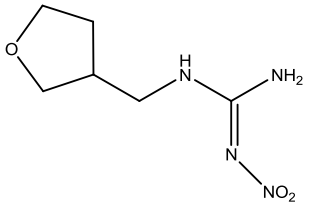
Weiler MS (2000e). First amendment to report—78-week dietary carcinogenicity study with MTI-446 in mice. Unpublished report no. CHW 6648-130. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

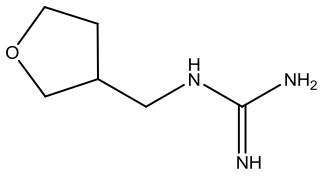
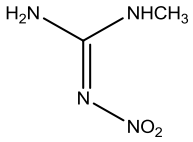
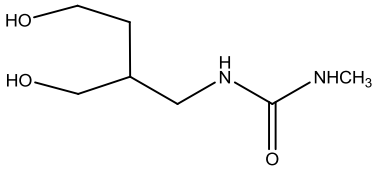
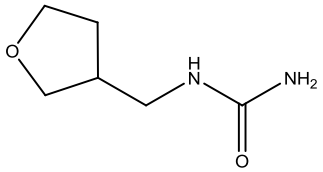
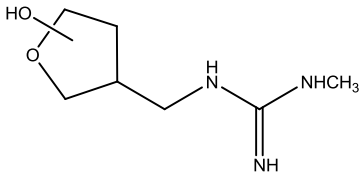
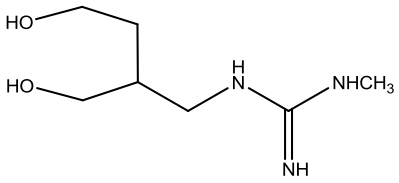
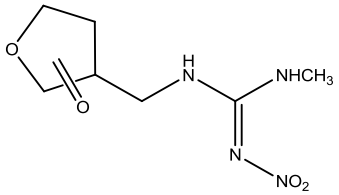
Weiler MS (2001a). Acute oral gavage neurotoxicity study with MTI-446 in rats. Unpublished report no. CHW 6648-147. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

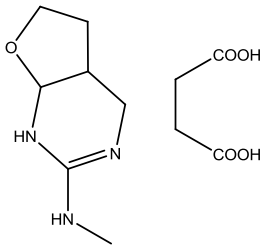
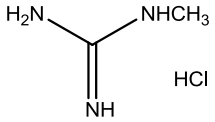
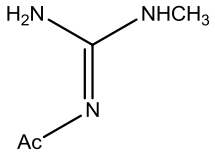
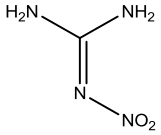
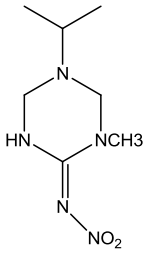
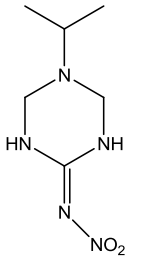
Weiler MS (2001b). 13-week dietary neurotoxicity study with MTI-446 in rats. Unpublished report no. CHW 6648-148. Covance Laboratories Inc. Submitted to WHO by Mitsui Chemicals, Inc.

Appendix 1. Dinotefuran and metabolite key

Code name	IUPAC name CAS No. Formula Molecular weight	Structure	Where found
Dinotefuran MTI-446	(*RS*)-1-Methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine CAS No.: 165252-70-0 Formula: $C_7H_{14}N_4O_3$ MW: 202.11		Parent molecule
UF	1-Methyl-3-(tetrahydro-3-furylmethyl)urea CAS No.: not given Formula: $C_7H_{14}N_2O_2$ MW: 158.20		Metabolism: apple, potato, rice, rape, lettuce, rat
DN phosphate	1-Methyl-3-(tetrahydro-3-furylmethyl)guanidinium dihydrogen phosphate CAS No.: not given Formula: $C_7H_{18}N_3O_5P$ MW: 255.10		Metabolism: apple, rice, rape, lettuce, rat
446-DO	1-[4-Hydroxy-2-(hydroxymethyl)butyl]-3-methyl-2-nitroguanidine CAS No.: not given Formula: $C_7H_{16}N_4O_4$ MW: 220.12		Metabolism: apple, potato, rice, lettuce, rat
446-CO	1-Methyl-2-nitro-3-(2-oxotetrahydro-3-furylmethyl)guanidine CAS No.: not given Formula: $C_7H_{12}N_4O_4$ MW: 216		Metabolism: rat
446-OH+COOH	2-(2-Hydroxyethyl)-3-(3-methyl-2-nitroguanidino)propionic acid CAS No.: not given Formula: $C_7H_{14}N_4O_5$ MW: 234		Metabolism: rat

Code name	IUPAC name CAS No. Formula Molecular weight	Structure	Where found
446-DO-Ac	1-[4-Hydroxy-2-(hydroxymethyl)butyl]-3-methyl-2-nitroguanidine acetyl CAS No.: not given Formula: $C_9H_{18}N_4O_5$ MW: 262		Metabolism: rat
446-NU	1-Nitro-2-(tetrahydro-3-furylmethyl)urea CAS No.: not given Formula: $C_6H_{11}N_3O_4$ MW: 189		Metabolism: rat
446-NH_2	2-Amino-1-methyl-3-(tetrahydro-3-furylmethyl)guanidine CAS No.: not given Formula: $C_7H_{16}N_4O$ MW: 172		Metabolism: rat
PHP	6-Hydroxy-5-(2-hydroxyethyl)-1-methyl-1,3-diazinane-2-ylidene-*N*-nitroamine CAS No.: not given Formula: $C_7H_{14}N_4O_4$ MW: 218.21		Metabolism: apple, potato, rice, rape, lettuce, rat
PHP-Ac	6-Hydroxy-5-(2-hydroxyethyl)-1-methyl-1,3-diazinane-2-ylidene-*N*-nitroamine acetyl conjugate CAS No.: not given Formula: $C_9H_{16}N_4O5$ MW: 260		Metabolism: rat
FNG	2-Nitro-1-(tetrahydro-3-furylmethyl)guanidine CAS No.: 168688-99-1 Formula: $C_6H_{12}N_4O_3$ MW: 188.18		Metabolism: apple, potato, rape, rat

Code name	IUPAC name CAS No. Formula Molecular weight	Structure	Where found
FNG-DN	1-(Tetrahydro-3-furylmethyl)guanidine CAS No.: not given Formula: $C_6H_{13}N_3O$ MW: 143		Metabolism: rat
MNG	1-Methyl-2-nitroguanidine CAS No.: not given Formula: $C_2H_6N_4O_2$ MW: 118.09		Metabolism: apple, potato, rice, rape, lettuce, rat
UF-DO	1-[Hydroxy-2-(hydroxymethyl)butyl]-3-methylurea CAS No.: not given Formula: $C_7H_{16}N_2O_3$ MW: 176.21		Metabolism: apple
UF-DM	1-(Tetrahydro-3-furylmethyl)urea CAS No.: not given Formula: $C_6H_{12}N_2O_2$ MW: 144.17		Metabolism: rat
DN-OH	1-[(5-Hydroxyoxolan-3-yl)methyl]-3-methylurea CAS No.: not given Formula: $C_7H_{15}N_3O_2$ MW: 173.12		Metabolism: rice, lettuce, rat
DN-DO	1-[4-Hydroxymethyl)butyl]-3-methylguanidine CAS No.: not given Formula: $C_7H_{18}N_3O_2$ MW: 175		Metabolism: rat
DN-CO	1-Methyl-3-(2-oxotetrahydro-3-furylmethyl)guanidine CAS No.: not given Formula: $C_7H_{13}N_3O_2$ MW: 171		Metabolism: rat

Code name	IUPAC name CAS No. Formula Molecular weight	Structure	Where found
BCDN succinate	3-(Methylamino)-9-oxa-2-aza-4-azoniabicyclo[4.3.0]non-3-ene hydrogen succinate CAS No.: none given Formula: $C_{11}H_{19}N_3O_5$ MW: 273.13		Metabolism: apple, rice, rape, lettuce, rat
MG hydrogen chloride	1-Methylguanidinium chloride CAS No.: 22661-87-6 Formula: $C_2H_8ClN_3$ MW: 109.04		Metabolism: apple, rice, rape, rat
MG-Ac	1-Methyl-2-acetylguanidine CAS No.: not given Formula: $C_4H_9N_3O$ MW: 115		Metabolism: rat
NG	Nitroguanidine CAS No.: 556-88-7 Formula: $CH_4N_4O_2$ MW: 104.07		Metabolism: apple, potato, lettuce
MPZ	1-Methyl-5-(1-methylethyl)-1,3,5-triazinane-2-ylidene-*N*-nitroamine CAS No.: none given Formula: $C_7H_{15}N_5O_2$ MW: 201.23		Reference substance
PZ	5-(1-Methylethyl)-1,3,5-triazinane-2-ylidene-*N*-nitroamine CAS No.: none given Formula: $C_6H_{13}N_5O_2$ MW: 187.20		Reference substance

CAS, Chemical Abstracts Service; IUPAC, International Union of Pure and Applied Chemistry; MW, molecular weight