SEDAXANE

First draft prepared by
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Explanation

Sedaxane is the common name that has been provisionally approved by the International Organization for Standardization (ISO) for mixtures of two *cis* isomers, 2′-[(1*RS*,2*RS*)-1,1′-bicycloprop-2-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxanilide, and two *trans* isomers, 2′-[(1*RS*,2*SR*)-1,1′-bicycloprop-2-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxanilide (International Union of Pure and Applied Chemistry), for which the Chemical Abstracts Service number is 874967-67-6. *Trans* and *cis* isomers of sedaxane show comparable toxicological profiles, and both are fungicidally active. The definition of the active ingredient is a mixture of the two isomers in ratios of

a minimum 810 g/kg *trans* isomer and a maximum 150 g/kg *cis* isomer. The nominal (typical) content is approximately 980 g/kg. Sedaxane contains approximately 81–85% of the *trans* isomers and approximately 10–15% of the *cis* isomers.

Sedaxane is a new broad-spectrum seed-applied fungicide belonging to the chemical class of pyrazole-carboxamides. The pesticidal mode of action of this group of fungicides is inhibition of succinate dehydrogenase, which is a functional part of the mitochondrial electron transport chain and oxidative phosphorylation involved in the tricarboxylic acid cycle. It is efficient in the control of a wide range of fungal pathogens, including *Microdochium nivale*, smut (*Ustilago tritici*), stink bunt (*Tilletia caries*), loose smut (*Ustilago nuda*), head smut (*Sphacelotheca reiliana*), Asian soya bean rust (*Phakopsora pachyrhizi*) and *Rhizoctonia* spp.

Sedaxane is being reviewed for the first time by the Joint FAO/WHO Expert Meeting on Pesticide Residues at the request of the Codex Committee on Pesticide Residues. All critical studies complied with good laboratory practice.

Evaluation for acceptable daily intake

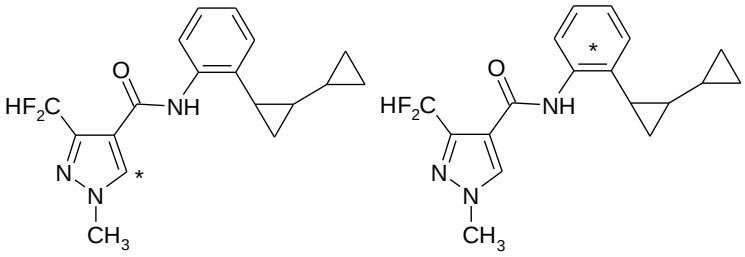
1. Biochemical aspects

1.1 Absorption, distribution and excretion

The mammalian metabolism of sedaxane has been assessed in studies investigating its absorption, distribution, metabolism and excretion in rats. In a biotransformation study, the nature of the metabolites formed was determined both qualitatively and quantitatively. The fate was investigated following administration of both single and multiple doses. Biliary elimination studies were conducted with [^{14}C-pyrazole]sedaxane and [^{14}C-phenyl]sedaxane. As evidence suggested that cleavage of the sedaxane molecule during biotransformation was a relatively minor process, a single radiolabelled form was used in the remaining metabolism studies. In these studies, radiolabelled material comprising an approximate 6:1 mixture of the *trans* and *cis* isomers was used, which is therefore similar to the definition ratio of 5.77.

The structure and positions of the ^{14}C radiolabel in the two radiolabelled forms of sedaxane used in the metabolism studies are shown in Figure 1.

Figure 1. Structures of the radiolabelled molecules



[Pyrazole-5-^{14}C]sedaxane [Phenyl-U-^{14}C]sedaxane

(a) Absorption

Oral

A single oral dose of [pyrazole-5-^{14}C]sedaxane (lot nos CL-LXII-15 for low dose, CL-LXII-14 for high dose; purity 97.0–99.2%; ratios of isomers 84.6% *trans* to 15.4% *cis* for low dose and 85.9% *trans* to 14.1% *cis* for high dose) was administered by gavage to eight male and eight female bile duct–cannulated Han Wistar rats at a dose level of 1 or 80 mg/kg body weight (bw). The excretion of radioactivity was measured over 2 days. After this period, the rats were killed, and

residual radioactivity was measured in blood, plasma, the gastrointestinal tract (and contents) and the remaining carcass (Shaw, 2009c). In addition, a single oral dose of [phenyl-U-^{14}C]sedaxane (lot nos RDR-III-25 for low dose, RDR-III-24 for high dose; purity 98.6–99.1%; ratios of isomers 86.0% *trans* to 14.0% *cis* for low dose and 85.6% *trans* to 14.4% *cis* for high dose) was administered in the same manner as for [pyrazole-5-^{14}C]sedaxane (Shaw, 2009d).

The major route of elimination of a single oral dose of 1 mg/kg bw of both [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane was via the bile, with means of 78.6–81.1% of the dose recovered in male and female bile over 2 days post-dosing. Urinary excretion accounted for means of 6.5–8.1% of the dose in males and females. Faecal excretion accounted for means of 4.7–8.6% in males and females by the end of the sampling period (Tables 1 and 2). Excretion of both [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane at the low dose was rapid, with most of the administered radioactivity excreted by 24 hours after dosing (for pyrazole-5-^{14}C, means of approximately 91.4% and 90.5% in males and females, respectively; for phenyl-U-^{14}C, means of 93.6% and 94.5% in males and females, respectively). Means of 87.4% and 87.9% of the administered dose of [pyrazole-5-^{14}C]sedaxane and 89.1% and 87.5% of the administered dose of [phenyl-U-^{14}C]sedaxane were absorbed in males and females, respectively, as calculated from the radioactivity eliminated in urine, daily cage wash and bile over 2 days, together with that present in the residual carcass (Table 1). The total mean per cent recoveries of administered radioactivity including excreta and residual carcasses following oral gavage dosing at 1 mg/kg bw were 94.1% for males and 92.6% for females treated with [pyrazole-5-^{14}C]sedaxane and 94.9% for males and 96.1% for females treated with [phenyl-U-^{14}C]sedaxane, respectively (Table 2).

Table 1. Absorption of radioactivity after oral administration of [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane

	Absorption (% of radioactive dose)							
	[Pyrazole-5-^{14}C]sedaxane				[Phenyl-U-^{14}C]sedaxane			
	1 mg/kg bw		80 mg/kg bw		1 mg/kg bw		80 mg/kg bw	
	Males (n = 4)	Females (n = 4)	Males (n = 4)	Females (n = 3)	Males (n = 4)	Females (n = 4)	Males (n = 4)	Females (n = 3)
Urine	6.7	6.9	5.9	10.2	6.5	8.1	6.7	5.3
Bile	79.0	79.4	81.8	81.2	81.1	78.6	85.3	81.0
Cage wash	1.4	1.5	1.3	0.9	1.2	0.7	1.6	0.7
Carcass	0.3	0.1	0.5	0.2	0.3	0.2	0.3	0.1
Total absorption	87.4	87.9	89.5	92.5	89.1	87.5	93.9	87.1

From Shaw (2009c,d)

Following a single oral dose of 80 mg/kg bw of [pyrazole-5-^{14}C]sedaxane or [phenyl-U-^{14}C]sedaxane, the major route of elimination was similarly via the bile in both sexes, with means of 81.0–85.3% of the administered radioactivity recovered in bile over 2 days post-dosing in males and females. Urinary excretion of [pyrazole-5-^{14}C]sedaxane accounted for means of 5.9% and 10.2% of the dose in males and females, respectively. Urinary excretion of [phenyl-U-^{14}C]sedaxane accounted for means of 6.7% and 5.3% of the dose in males and females, respectively. Faecal elimination of [pyrazole-5-^{14}C]sedaxane accounted for means of 7.1% and 3.3% of the administered dose in males and females, respectively. Faecal elimination of [phenyl-U-^{14}C]sedaxane accounted for means of 4.4% and 10.6% of the administered dose in males and females, respectively (Tables 1 and 2). Excretion of both [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane at the high dose was rapid, with the majority of the administered radioactivity excreted by 24 hours post-dosing (for pyrazole-5-^{14}C, means of 92.0% and 93.1% in males and females, respectively; for phenyl-U-^{14}C, means of 96.1% and 95.7% in males and females, respectively). Group means of 89.5% and 92.5% of the administered

dose of [pyrazole-5-^{14}C]sedaxane and 93.9% and 87.1% of the administered dose of [phenyl-U-^{14}C]sedaxane were absorbed in males and females, respectively, as calculated from the radioactivity eliminated in urine, daily cage wash and bile over 2 days, together with that present in the residual carcass (Table 1). The total mean per cent recoveries of administered radioactivity including excreta and residues in carcasses following oral gavage dosing at 80 mg/kg bw were 96.6% for males and 95.9% for females with [pyrazole-5-^{14}C]sedaxane and 98.4% for males and 97.7% for females with [phenyl-U-^{14}C]sedaxane (Table 2).

Table 2. Recovery of radioactivity in excreta and bile after administration of a single oral dose of [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane to bile duct–cannulated rats

		Group mean excretion (% of radioactive dose recovered)[a]							
		[Pyrazole-5-^{14}C]sedaxane				[Phenyl-U-^{14}C]sedaxane			
		1 mg/kg bw		80 mg/kg bw		1 mg/kg bw		80 mg/kg bw	
		Males (*n* = 4)	Females (*n* = 4)	Males (*n* = 4)	Females (*n* = 4)	Males (*n* = 4)	Females (*n* = 4)	Males (*n* = 4)	Females (*n* = 4)
Urine	0–24 h	6.5	6.7	5.6	10.0	6.4	7.9	6.4	5.1
	24–48 h	0.2	0.1	0.3	0.2	0.2	0.2	0.4	0.2
	Subtotal	6.7	6.9	5.9	10.2	6.5	8.1	6.7	5.3
Faeces	0–24 h	6.3	4.6	6.5	2.9	5.5	8.3	4.0	10.0
	24–48 h	0.4	0.2	0.6	0.4	0.2	0.3	0.4	0.5
	Subtotal	6.6	4.7	7.1	3.3	5.7	8.6	4.4	10.6
Bile	0–0.5 h	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	0.5–1 h	< 0.2	3.2	0.8	0.4	< 0.3	< 1.8	0.6	< 0.5
	1–2 h	15.6	16.8	5.7	4.1	14.0	15.4	5.1	4.7
	2–4 h	21.0	21.6	11.3	10.6	24.9	18.2	9.4	9.9
	4–8 h	26.7	24.8	23.7	20.8	29.1	24.7	24.1	21.1
	8–24 h	15.2	12.9	38.5	44.3	12.7	17.9	45.2	44.0
	24–48 h	0.4	0.2	1.9	1.0	0.3	0.6	0.9	1.0
	Subtotal	79.0	79.4	81.8	81.2	81.1	78.6	85.3	81.0
Cage wash		1.4	1.5	1.3	0.9	1.2	0.7	1.6	0.7
Gastrointestinal tract + contents		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1
Carcass		0.3	0.1	0.5	0.2	0.3	0.2	0.3	0.1
Total recovery		94.1	92.6	96.6	95.9	94.9	96.1	98.4	97.7

From Shaw (2009c)

[a] Total and subtotal values may not add up exactly due to rounding of values.

In conclusion, irrespective of dose level or sex, a single oral dose of [pyrazole-5-^{14}C]sedaxane or [phenyl-U-^{14}C]sedaxane at 1 or 80 mg/kg bw was extensively absorbed, representing at least 87% of the dose, and was rapidly and extensively eliminated, predominantly via the bile. By 2 days after dosing, carcass residues represented 0.5% of the dose or less for [pyrazole-5-^{14}C]sedaxane and 0.3% of the dose or less for [phenyl-U-^{14}C]sedaxane (Shaw, 2009c,d).

Dermal

Dermal absorption factors for risk assessment were derived from three studies utilizing [pyrazole-5-^{14}C]sedaxane in a flowable concentrate for seed treatment formulation, A16148C. These involved an in vivo rat study (Read & Sweeney, 2009) and in vitro studies with epidermal membranes from rats or humans (Davies, 2009a,b). It has been established in numerous scientific studies that rat skin is more permeable than human skin in the course of typical exposures. Examples where this has been studied in vitro using pesticides can be found in Scott & Corrigan (1990), Scott et al. (1991), Ramsey et al. (1994), Van de Sandt et al. (2000), Cnubben et al. (2002) and Van Ravenzwaay &

Leibold (2004). To account for this difference and to more accurately estimate the dermal absorption in humans, European Union and international guidance utilizes the comparative ratio of rat and human in vitro dermal absorption results to correct the in vivo rat values (EC, 2004; IPCS, 2006).

(b) Pharmacokinetics

The kinetics of [pyrazole-5-^{14}C]sedaxane (lot nos CL-LXII-15 for low dose, CL-LXII-14 for high dose; purity 97.0–99.2%; ratios of isomers 84.6% *trans* to 15.4% *cis* for low dose and 85.9% *trans* to 14.1% *cis* for high dose) was examined using Han Wistar rats given a single oral dose of 1 or 80 mg/kg bw. In addition, excreta samples were collected for total radioactivity analysis (Shaw, 2009b).

Following administration of a single dose of [pyrazole-5-^{14}C]sedaxane at 1 mg/kg bw to male and female rats, the observed maximum concentration (C_{max}) of radioactivity in plasma was 0.11 µg equivalent (Eq) per millilitre in both males and females, with the time taken to reach C_{max} (T_{max}) ranging between 1 and 1.5 hours after dosing. The estimated terminal half-life ($t_{½}$ term.) was 22.65 hours and 24.85 hours in males and females, respectively, and the area under the plasma concentration–time curve ($AUC_{0–t}$) was 1.663 µg Eq·h/ml in males and 1.625 µg Eq·h/ml in females. Only low residues of radioactivity were detectable 72 hours after dosing (Table 3).

Following administration of a single oral dose of [pyrazole-5-^{14}C]sedaxane at 80 mg/kg bw to male and female rats, the observed mean peak concentrations of radioactivity in plasma (C_{max}) were 10.6 and 12.4 µg Eq/ml in males and females, respectively, with T_{max} ranging between 5 and 6 hours after dosing. The estimated $t_{½}$ term. was 28.76 hours and 23.29 hours in males and females, respectively, and the $AUC_{0–t}$ was 233.5 µg Eq·h/ml in males and 192.2 µg Eq·h/ml in females (Table 3). Residues of radioactivity were still detectable 72 hours after dosing.

Systemic exposure to the total radioactivity (AUC) associated with [pyrazole-5-^{14}C]sedaxane in plasma tended to be slightly greater than that in whole blood in both sexes and at both dose levels. This suggests that total radioactivity was relatively evenly distributed between plasma and the cellular component of the blood at both dose levels. Pharmacokinetic parameters of total radioactivity in blood were therefore not appreciably different from those in plasma either between the sexes or between the dose levels.

A comparison between the low and high dose levels showed that increasing the dose of [pyrazole-5-^{14}C]sedaxane from 1 to 80 mg/kg bw produced a 140- and 120-fold increase in plasma AUC in males and females, respectively. However, because of the observed variability in the concentrations for individual rats, this was considered not to be significant.

Table 3. Summary of blood and plasma toxicokinetic parameters following administration of [pyrazole-5-^{14}C]sedaxane to rats

Toxicokinetic parameter	Blood				Plasma			
	1 mg/kg bw		80 mg/kg bw		1 mg/kg bw		80 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
C_{max} (µg Eq/ml)	0.071	0.075	9.035	9.597	0.106	0.110	10.572	12.432
T_{max} (h)	1	1.5	5	4	1	1.5	5	6
$AUC_{0–t}$ (µg Eq·h/ml)	1.464	1.301	196.9	158.2	1.663	1.625	233.5	192.2
$t_{½}$ (h)	39.86	33.92	31.61	20.71	22.65	24.85	28.76	23.29

From Shaw (2009b)

AUC, area under the plasma–concentration time curve; C_{max}, peak plasma concentration; $t_{½}$, half-life; T_{max}, time to reach C_{max}

Following administration of a single oral dose of [pyrazole-5-^{14}C]sedaxane to male and female rats, the mean peak plasma concentrations were reached after approximately 1 hour at the low dose level and 5 hours at the high dose level. Systemic exposure was similar for males and females at both dose levels (Shaw, 2009b).

(c) Distribution

Single dose

A single oral dose of [pyrazole-5-^{14}C]sedaxane (lot nos CL-LXII-15 for low dose, CL-LXII-14 for high dose; purity 97.0–99.2%; ratios of isomers 84.6% *trans* to 15.4% *cis* for low dose and 85.9% *trans* to 14.1% *cis* for high dose) was administered by gavage to 30 male and 30 female rats (Han Wistar) at a nominal dose level of 1 or 80 mg/kg bw. Groups of three rats were killed at various times post-dosing, and selected organs, tissues and body fluids were collected for total radioactivity analysis. The terminal half-life for each tissue depletion was also estimated.

Radioactivity from a single oral dose of 1 mg/kg bw was widely distributed to the tissues in both sexes (Table 4). Peak mean tissue concentrations were attained at the first sampling time. The highest mean concentration of radioactivity was present in the liver of both sexes, with liver and kidney concentrations remaining above plasma concentrations throughout the course of the experiment. High concentrations were also present in the pancreas, adrenals and adipose tissue until approximately 8 hours post-dosing. Thereafter, all but liver and kidney levels declined to concentrations close to or below the limit of reliable measurement.

Radioactivity from a single oral dose of 80 mg/kg bw was widely distributed to the tissues in both sexes (Table 5). Maximum mean tissue concentrations were observed at the first sampling time (5 hours post-dosing in both sexes). The highest mean concentrations of radioactivity were present in the liver and adipose tissue of both sexes. However, the levels in fat declined rapidly and by 48 hours were below plasma concentrations in both sexes. High concentrations were also present in the pancreas, adrenals and thyroid up to approximately 24 hours post-dosing. Thereafter, these concentrations declined to values close to or below mean plasma concentrations. In both sexes, the liver and kidney concentrations remained above mean plasma concentrations throughout the course of the experiment. By 96 hours after dosing, total radioactivity had declined extensively in all other tissues, with mean concentrations close to or below the limit of reliable measurement.

Tissue distribution was generally similar in males and females at both dose levels. The half-lives of tissue depletion of total radioactivity were variable, the shortest and longest estimates occurring in brain (0.1–0.2 day) and thyroid (2.0–3.2 days), respectively (Table 6).

In both male and female rats, the tissue distribution of radioactivity was similar and extensive following a single oral dose of 1 or 80 mg [pyrazole-5-^{14}C]sedaxane per kilogram body weight. Tissue concentrations of radioactivity were highest at the first sampling time (1–1.5 hours for low dose and 5 hours for high dose) and progressively declined thereafter, with elimination half-lives of between 0.1 and 3.2 days. Most mean tissue concentrations were close to or below the limit of reliable measurement by 96 hours post-dosing, when mean total tissue and carcass residues accounted for less than 0.8% of the dose. The high levels of radioactivity in the gastrointestinal tract and its contents throughout the 96-hour time course were consistent with the established biliary excretion and faecal elimination of sedaxane metabolites (Shaw, 2009e).

Repeated dose

Thirty-three male Han Wistar rats were given up to 14 consecutive daily oral doses of [pyrazole-5-^{14}C]sedaxane (lot no. RDR-II-75; purity 98.6%; ratio of isomers 84.2% *trans* to 13.6% *cis*) at 1 mg/kg bw per day. At predetermined intervals during dosing and following the cessation of dosing, groups of rats were killed for the removal of selected tissues or organs to determine the extent of accumulation of radioactivity in tissues and the remaining carcasses and its subsequent elimination. Additionally, the excretion of radioactivity in urine and faeces was monitored in one group of rats for a period of 24 hours following the 1st and 14th doses.

Table 4. Distribution of radioactivity in tissues/organs 1 (males), 1.5 (females), 8, 24, 48 and 96 hours after administration of [pyrazole-5-^{14}C]sedaxane to rats at a dose level of 1 mg/kg bw

	Group mean tissue residues (µg Eq/g or ml)									
	Males					Females				
	1 h	8 h	24 h	48 h	96 h	1.5 h	8 h	24 h	48 h	96 h
Adrenals	0.245	0.084	0.016	< 0.006	< 0.003	0.312	< 0.033	0.008	0.006	< 0.003
Bone mineral	0.024	0.029	0.004	< 0.002	< 0.001	0.021	0.011	0.003	< 0.002	< 0.001
Brain	0.036	0.008	0.001	< 0.001	< 0.001	0.057	0.011	< 0.001	< 0.001	< 0.001
Fat (renal)	0.124	0.097	0.008	< 0.002	< 0.001	0.295	0.133	0.004	< 0.001	< 0.001
Gastrointestinal tract	5.372	4.759	1.037	0.139	0.013	5.923	5.126	1.038	0.118	0.017
Gastrointestinal tract contents	11.131	10.284	2.541	0.244	0.025	10.423	10.609	3.443	0.210	0.027
Heart	0.073	0.029	0.009	0.003	0.001	0.098	0.034	0.005	< 0.001	< 0.001
Kidneys	0.208	0.118	0.035	0.013	0.006	0.227	0.161	0.029	0.008	0.004
Liver	1.103	0.512	0.166	0.055	0.025	1.029	0.700	0.088	0.028	0.014
Lungs	0.086	0.036	0.014	0.007	0.002	0.129	0.043	0.009	0.003	< 0.002
Muscle	0.048	0.043	0.004	0.002	< 0.001	0.057	0.021	0.003	< 0.002	< 0.001
Pancreas	0.162	0.050	0.011	0.004	< 0.001	0.317	0.075	0.010	0.002	< 0.001
Plasma	0.079	0.047	0.028	0.014	0.004	0.115	0.063	0.014	0.004	< 0.002
Residual carcass	0.057	0.047	0.026	0.006	< 0.003	0.087	0.040	0.023	0.008	0.005
Spleen	0.060	0.024	0.008	0.003	0.001	0.323	0.027	0.005	< 0.002	< 0.002
Testes/ovaries	0.032	0.017	0.005	0.002	0.001	0.120	< 0.042	0.005	< 0.002	< 0.002
Uterus	—	—	—	—	—	0.073	0.035	0.004	< 0.002	< 0.001
Thymus	0.042	0.015	0.004	< 0.002	< 0.001	0.063	0.021	0.003	< 0.001	< 0.001
Thyroid	0.077	< 0.029	0.027	< 0.009	< 0.008	0.155	< 0.192	0.018	< 0.007	< 0.010
Whole blood	0.058	0.045	0.028	0.014	< 0.005	0.072	0.047	0.013	0.005	0.002

From Shaw (2009e)

Radioactive residues following administration of [pyrazole-5-^{14}C]sedaxane at a dose of 1 mg/kg bw to male rats for 14 days were well distributed in the extensive range of tissues collected (Table 7). Mean concentrations of total radioactivity in each tissue generally increased with each sampling time during the period of dosing and were detectable in most tissues by 24 hours after the seventh dose. Mean tissue concentrations of total radioactivity were at their highest observed levels 24 hours after the 14th dose, with the exception of blood and plasma, for which the highest mean concentrations were observed 24 hours after the 10th dose. Most mean tissue concentrations appeared either to have attained or to be approaching steady-state kinetics by the end of the 14-day dosing period. Following the cessation of dosing, all tissue concentrations declined, with no evidence of any persistence.

Table 5. Distribution of radioactivity in tissues/organs 5, 12, 24, 48 and 96 hours after administration of [pyrazole-5-^{14}C]sedaxane to rats at a dose level of 80 mg/kg bw

	Group mean tissue residues (µg Eq/g or ml)									
	Males					Females				
	5 h	12 h	24 h	48 h	96 h	5 h	12 h	24 h	48 h	96 h
Adrenals	32.39[a]	11.33	1.28	0.50	< 0.27	65.45	21.36	1.66	0.46	< 0.26
Bone mineral	4.23	1.59	0.27	< 0.11	< 0.06	4.71	2.19	0.36	< 0.10	< 0.06
Brain	12.90	1.79	0.13	< 0.05	< 0.03	20.45	3.45	0.11	< 0.03	< 0.01
Fat (renal)	62.74	49.05	3.17	0.17	< 0.04	107.55	73.66	3.25	0.27	< 0.05
Gastrointestinal tract	605.34	358.62	147.20	22.92	1.60	295.64	370.70	142.70	24.55	1.27
Gastrointestinal tract contents	709.60	554.21	261.53	50.41	2.65	626.13	815.33	433.52	55.69	2.40
Heart	18.93	4.09	0.64	0.27	0.14	20.43	6.21	0.63	0.18	< 0.07
Kidneys	35.18	10.43	2.72	1.05	0.59	31.42	13.51	3.34	1.14	0.45
Liver	71.74	40.14	11.88	5.63	2.85	64.56	34.92	11.38	4.08	1.42
Lungs	18.45[b]	4.37	0.85	0.44	0.19	23.24	6.49	1.05	0.35	0.14
Muscle	9.36	3.02	0.42	0.11	0.06	16.99	4.26	0.34	0.11	< 0.03
Pancreas	47.09	11.68	1.01	< 0.22	< 0.07	65.70	15.57	0.70	0.20	< 0.06
Plasma	10.41	3.67	1.02	0.68	< 0.20	15.48	5.59	1.63	0.53	< 0.20
Residual carcass	19.35	7.07	2.67	0.63	0.21	24.69	13.47	5.72	0.71	0.29
Spleen	14.05	7.09	0.55	< 0.24	< 0.19	20.27	6.98	0.79	0.23	0.13
Testes/ovaries	8.16	3.19	0.43	0.14	< 0.07	46.03	19.75	0.92	0.21	< 0.10
Uterus	—	—	—	—	—	24.89	23.95	0.75	0.19	< 0.10
Thymus	28.45	7.34	0.25	< 0.12	< 0.05	17.96	3.85	0.36	< 0.08	< 0.04
Thyroid	19.35[b]	9.67	2.12	1.35	1.05	79.01	7.80	2.52	1.01	< 0.89
Whole blood	7.60	3.53	1.37	0.72	0.38	11.06	4.26	1.54	0.67	0.29

From Shaw (2009e)

[a] 033M removed from the mean due to inconsistency with other values.

[b] 031M removed from the mean due to inconsistency with other values.

Mean tissue levels of radioactivity were highest in the liver and kidney, and these were the only tissues to consistently exceed blood concentrations throughout the study (Table 8). This is consistent with both biliary and urinary elimination of [pyrazole-5-^{14}C]sedaxane and its metabolites. After the liver and kidney, the thyroid had the next highest mean concentration of radioactivity during the study, followed by the adrenals and spleen. Although thyroid appeared to show some accumulation of radioactivity during dosing, the mean concentration in the thyroid was no longer at a reliably measurable level by 28 days after the 14th dose. Residues in all other tissues were generally below blood and plasma concentrations throughout the course of the study. By the final sampling time (42 days after the 14th dose), mean concentrations of radioactivity were measurable only in the liver, kidney and spleen. The terminal half-life for tissue depletion was variable, with the shortest estimate in the plasma and longest in the spleen, 2.3 days and 33.0 days, respectively (Shaw, 2009f).

Table 6. Elimination of radioactivity from rat tissues/organs after a single administration of [pyrazole-5-^{14}C]sedaxane to rats at a dose level of 1 or 80 mg/kg bw

Tissue	Elimination half-life (h)[a]			
	1 mg/kg bw		80 mg/kg bw	
	Males	Females	Males	Females
Adrenals	11.58	10.62	40.37	7.00
Bone mineral	13.10	16.79	10.92	10.21
Brain	4.59	3.97	2.90	2.51
Fat (renal)	8.34	3.53	4.56	4.68
Heart	27.23	9.11	33.75	27.70
Kidneys	30.62	27.90	34.77	26.02
Liver	27.87	28.52	36.45	24.83
Lungs	25.51	36.11	34.15	26.49
Muscle	42.46	13.87	28.03	21.85
Ovaries	NA	8.42	NA	25.63
Pancreas	21.30	7.39	20.63	25.22
Plasma	25.84	27.53	32.33	27.67
Spleen	25.19	11.05	59.22	30.47
Testes	30.88	NA	29.82	NA
Thymus	14.02	5.01	6.32	7.47
Thyroid	48.38	NC	75.88	47.69
Uterus	NA	10.23	NA	26.70
Whole blood	29.49	31.22	40.10	31.01

From Shaw (2009e)
NA, not applicable; NC, not calculated
[a] Each value is a mean of three rats.

Table 7. Distribution of radioactivity in tissues/organs 24 hours after days 3, 7, 10 and 14 of a repeated administration of [pyrazole-5-^{14}C]sedaxane to male rats at a dose of 1 mg/kg bw

	Group mean tissue residues (µg Eq/g or ml)			
	Day 3	Day 7	Day 10	Day 14
Adrenals	< 0.032	< 0.033	0.047	0.099
Bone mineral	< 0.012	0.010	0.012	0.032
Brain	< 0.003	< 0.005	< 0.003	0.013
Fat (renal)	< 0.013	0.013	0.008	0.023
Gastrointestinal tract	1.609	2.191	1.414	2.639
Gastrointestinal tract contents	4.553	6.120	5.010	5.496
Heart	0.013	0.020	0.024	0.026
Kidney	0.066	0.119	0.094	0.194
Liver	0.287	0.460	0.416	0.507
Lungs	0.023	0.037	0.041	0.044
Pancreas	0.018	0.032	0.017	0.022
Plasma	0.037	0.060	0.088	0.066
Residual carcass	0.034	0.044	0.068	0.083

Table 7 (continued)

	Group mean tissue residues (µg Eq/g or ml)			
	Day 3	Day 7	Day 10	Day 14
Spleen	0.013	0.019	0.022	0.027
Testes	0.008	0.013	0.013	0.013
Thymus	< 0.008	0.010	0.011	0.013
Thyroid	< 0.050	< 0.087	0.140	0.189
Whole blood	0.037	0.062	0.079	0.070

From Shaw (2009f)

Table 8. Distribution of radioactivity in tissues/organs up to 42 days after day 14 of repeated administration of [pyrazole-5-^{14}C]sedaxane to male rats at a dose of 1 mg/kg bw

	Group mean tissue residues (µg Eq/g or ml)						
	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 42
Adrenals	< 0.022	0.028	< 0.034	< 0.010	< 0.026	< 0.005	< 0.009
Bone mineral	< 0.007	< 0.005	< 0.008	< 0.002	< 0.006	< 0.002	< 0.002
Brain	< 0.002	< 0.002	< 0.005	< 0.001	< 0.001	< 0.001	< 0.001
Fat (renal)	< 0.003	< 0.002	< 0.014	< 0.002	< 0.002	< 0.001	< 0.001
Gastrointestinal tract	0.192	0.025	0.007	0.004	0.004	< 0.002	< 0.002
Gastrointestinal tract contents	0.355	0.049	0.011	0.005	0.004	< 0.002	< 0.001
Heart	0.009	0.006	0.026	< 0.003	< 0.003	< 0.001	< 0.001
Kidneys	0.056	0.050	0.039	0.024	0.025	0.014	0.008
Liver	0.177	0.146	0.087	0.048	0.037	0.027	0.009
Lungs	0.017	0.010	0.007	< 0.004	< 0.003	< 0.002	< 0.002
Muscle	0.005	< 0.004	0.004	< 0.001	< 0.002	< 0.001	< 0.001
Pancreas	< 0.007	< 0.004	< 0.006	< 0.001	< 0.002	< 0.001	< 0.001
Plasma	0.034	0.011	< 0.002	< 0.001	< 0.001	< 0.001	< 0.001
Residual carcass	0.028	0.022	0.022	0.010	0.010	0.006	< 0.005
Spleen	0.014	0.012	0.015	0.008	0.008	0.007	0.005
Testes	0.006	0.003	< 0.003	< 0.001	< 0.001	0.006	0.003
Thymus	< 0.005	< 0.003	< 0.006	< 0.001	< 0.002	< 0.001	< 0.001
Thyroid	0.082	< 0.066	0.071	0.048	0.028	< 0.034	< 0.024
Whole blood	0.035	0.022	0.014	0.007	0.006	< 0.002	< 0.002

From Shaw (2009f)

(d) Excretion

A single oral dose of [pyrazole-5-^{14}C]sedaxane (lot nos CL-LXII-15 for low dose, CL-LXII-14 for high dose; purity 99.2% and 97.0% for low and high doses, respectively; ratios of isomers 84.6% *trans* to 15.4% *cis* for low dose and 85.9% *trans* to 14.1% *cis* for high dose) was administered by gavage to eight male and eight female rats (Han Wistar) at a dose of either 1 or 80 mg/kg bw. The excretion of radioactivity was measured over 7 days. After this period, the rats were killed, and residual radioactivity was measured in blood and plasma, selected tissues and the remaining carcasses.

The major route of elimination of a single oral dose of [pyrazole-5-^{14}C]sedaxane at 1 mg/kg bw was via the faeces in both males and females, with respective means of 88.4% and 79.4% of the administered radioactivity recovered by this route over 7 days after dosing. Urinary excretion accounted for means of 11.8% and 19.6% of the administered dose in males and females, respectively, by the end of the sampling period (Table 9).

Table 9. Recovery of radioactivity in excreta and tissues after administration of a single oral dose of [pyrazole-^{14}C]sedaxane to rats

		Group mean excretion (% of radioactive dose recovered)[a]			
		1 mg/kg bw		80 mg/kg bw	
		Males (n = 4)	Females (n = 4)	Males (n = 4)	Females (n = 4)
Urine	0–6 h	3.0	4.0	2.0	1.7
	6–12 h	3.7	6.5	2.0	3.0
	12–24 h	3.0	6.2	3.9	6.8
	24–48 h	1.6	2.4	2.7	4.3
	48–72 h	0.3	0.4	0.9	1.4
	72–96 h	0.1	0.1	0.3	0.2
	96–120 h	< 0.1	0.1	0.1	< 0.1
	120–144 h	< 0.1	< 0.1	< 0.1	< 0.1
	144–168 h	< 0.1	< 0.1	< 0.1	< 0.1
	Subtotal	11.8	19.6	11.9	17.6
Faeces	0–24 h	68.3	57.8	41.0	36.3
	24–48 h	16.2	19.4	28.1	28.7
	48–72 h	2.9	1.8	11.1	8.8
	72–96 h	0.7	0.3	2.3	0.9
	96–120 h	0.3	0.1	0.4	0.2
	120–144 h	0.1	< 0.1	0.1	0.1
	144–168 h	< 0.1	< 0.1	0.1	< 0.1
	Subtotal	88.4	79.4	83.1	74.9
Cage wash		1.7	5.8	2.0	3.7
Gastrointestinal tract + contents		< 0.1	< 0.1	< 0.1	< 0.1
Tissues + carcass		0.2	0.1	0.3	0.1
Total recovery		102.1	104.9	97.2	96.3

From Shaw (2009a)

[a] Total and subtotal values may not add up exactly due to rounding of values.

The major route of elimination of a single oral dose of [phenyl-U-^{14}C]sedaxane at 80 mg/kg bw was similarly via the faeces in both males and females, with respective means of 83.1% and

74.9% of the administered radioactivity recovered by this route over 7 days post-dosing. Urinary excretion accounted for means of 11.9% and 17.6% of the administered dose in males and females, respectively, by the end of the sampling period (Table 9).

Excretion was rapid, with essentially all administered radioactivity being eliminated in the first 72 hours after dosing (means of approximately 100.4% and 103.5% in males and females, respectively, for the low dose and approximately 93.4% and 94.6% in males and females, respectively, for the high dose). The routes and rates of excretion were similar in both sexes, although urinary excretion of absorbed components was slightly higher in females. Seven days after administration of the low and high doses, there was no significant radioactivity remaining in the carcass or gastrointestinal tract, indicating that excretion was essentially complete by 7 days after the single administered dose.

A single oral dose of [pyrazole-^{14}C]sedaxane at 1 or 80 mg/kg bw was rapidly and extensively eliminated, irrespective of dose level or sex. At both dose levels, the major route of elimination was via the faeces, and faecal elimination was slightly higher in males than in females. Accordingly, urinary elimination was slightly higher in females. At both dose levels, residues of radioactivity were very low in blood and tissues by 7 days after dosing and were reliably detected in both sexes only in the liver and kidney. Tissue distribution was generally similar in both sexes at both dose levels, These very low tissue residues were consistent with the extensive excretion of the administered dose (Shaw, 2009a).

1.2 Biotransformation

The biotransformation of sedaxane was investigated using [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane (with a *trans*:*cis* isomer ratio of approximately 6:1) administered in 14 consecutive daily oral doses (low dose level) to male rats and single high and single low oral doses administered to both intact and bile duct–cannulated male and female rats that had been used in previous studies (Shaw, 2009a,b,c,d,f). All metabolites accounting for greater than 5% of the administered dose were identified, and the majority of those accounting for less than 5% were tentatively identified; proposed chemical structures were assigned. Metabolites were identified by radio–high-performance liquid chromatography with mass spectrometry using a combination of comparative chromatography with authentic reference standards, accurate mass measurement and MS^n fragmentation. Minor metabolites were tentatively identified and assigned a proposed chemical structure based on mass spectrometric data. Selected bile and urine samples were subjected to enzyme hydrolysis using a mixture of β-glucuronidase and sulfatase enzymes to assist in the identification of conjugated metabolites.

Sedaxane was extensively metabolized, giving rise to at least 20 types of metabolite (e.g. hydroxy, demethylated hydroxy, glucuronide conjugate, sulfate conjugate), with the potential for multiple isomers within most types. No significant differences in the nature of the metabolites identified were observed in low- and high-dose male and female rats, although some quantitative variation was observed. There was little evidence of significant cleavage of sedaxane into the pyrazole and phenyl moieties, similar metabolic profiles being observed in samples from rats receiving pyrazole- or phenyl-labelled [^{14}C]sedaxane. Small amounts (< 1%) of CSCC210616 (pyrazole amide metabolite) were detected in bile samples. Phenolic and hydroxy metabolites of sedaxane and desmethyl sedaxane were conjugated with glucuronic acid, sulfate and glutathione. Identification of the aglycones following enzymatic hydrolysis using a mixture of β-glucuronidase and sulfatase enzymes confirmed the structural assignments of these conjugated metabolites. Generally, when identification allowed, the metabolites derived from the *trans* isomer of sedaxane were the major components of the metabolic profile. Measurement of the isomers of the remaining sedaxane in samples from rats receiving the low dose of [pyrazole-5-^{14}C]sedaxane showed an approximately similar ratio of *trans* to *cis* isomers as that of the sedaxane administered (ranging from 1.49:1 in plasma to 7.96:1 in faeces from intact rats). In plasma, urine and bile samples from rats receiving the high dose, a much greater proportion of the *trans* isomer (ranging from 12.18:1 in urine from bile duct–cannulated rats to 45.96:1 in plasma from intact rats) was observed.

The biotransformation of sedaxane was postulated to proceed by:

- *N*-demethylation;
- hydroxylation of sedaxane to give the *para*-phenols CSCD658906 and CSCD659090 and the cyclopropyl alcohol CSCD659089; these metabolites were excreted primarily in the bile as glucuronide conjugates;
- hydroxylation of desmethyl sedaxane to give the desmethyl *para*-phenols CSCD659087 and CSCD668404 and the desmethyl cyclopropyl alcohol CSCD659088; these metabolites were excreted primarily in the bile as glucuronide conjugates;
- opening of the terminal cyclopropyl moiety followed by oxidation of the sedaxane and desmethyl sedaxane to give β-hydroxycarbonyl sedaxane (the *trans* isomer CSCD668403 was identified) and β-hydroxycarbonyl desmethyl sedaxane metabolites;
- further hydroxylation and oxidation of hydroxyl and desmethyl hydroxyl metabolites;
- further hydroxylation and oxidation of β-hydroxycarbonyl sedaxane and desmethyl β-hydroxycarbonyl sedaxane metabolites;
- glucuronic acid conjugation together with minor amounts of sulfate conjugation of hydroxylated metabolites;
- glutathione conjugation.

The metabolite profiles in excreta of intact or bile duct–cannulated rats following single or repeated oral doses of [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane at low and high doses are shown in Tables 10–16.

Table 10. Metabolite profile in excreta of rats following a single oral dose of [pyrazole-5-^{14}C]sedaxane at the high dose of 80 mg/kg bw

Compound	% of administered dose (number of isomers)					
	Males			Females		
	Urine (0–96 h)	Faeces (0–96 h)	Total excreta	Urine (0–96 h)	Faeces (0–96 h)	Total excreta
Sedaxane	—	4.25	4.25	—	1.68	1.68
CSCD659087	1.68	15.38	17.06	2.85	22.41	25.26
CSCD668404	0.24	3.04	3.28	0.27	6.99	7.26
CSCD659088	—	2.08	2.08	—	0.96	0.96
CSCD658906	1.70	16.83	18.53	2.02	16.65	18.67
CSCD658089	—	9.77	9.77	—	1.22	1.22
CSCD659090	—	4.27[a]	4.27	0.26	3.37[a]	3.63
CSCD668403	0.25	—	0.25	0.25	—	0.25
β-Hydroxycarbonyl cysteine conjugate	—	0.83	0.83	—	0.75	0.75
Desmethyl hydroxy	—	2.95[a]	2.95	—	2.43[a]	2.43
Desmethyl dihydroxy	—	0.99 (1)	0.99	—	1.84 (3)	1.84
Desmethyl β-hydroxycarbonyl	1.22 (1)	—	1.22	4.78 (1)[b]	—	4.78
Dihydroxy	0.20 (1)	5.03 (3)	5.23	0.23 (1)	2.78 (2)	3.01

Table 10 (continued)

Compound	% of administered dose (number of isomers)					
	Males			Females		
	Urine (0–96 h)	Faeces (0–96 h)	Total excreta	Urine (0–96 h)	Faeces (0–96 h)	Total excreta
Dihydroxy/β-hydroxycarbonyl[c]	0.40 (1)[c]	—	0.40	0.17 (1)[c]	—	0.17
Carboxylic acid	0.73 (1)	—	0.73	—	—	
Hydroxy β-hydroxycarbonyl	2.02 (5)	2.36 (1)	4.38	0.19 (1)	1.54 (1)	1.73
Desmethyl hydroxy sulfate conjugate	1.32 (1)	—	1.32	—	—	—
Desmethyl glucuronide	—	(2)[d]	—	—	(2)[d]	—
Desmethyl hydroxy glucuronide	—	—	—	0.48 (2)[e]	—	0.48
Hydroxy glucuronide	—	2.02 (1)	2.02	3.08 (1)	0.73	3.81
Post-extraction solids	NA	10.00	10.00	NA	8.20	8.20
Total identified	9.76	69.80	79.56	14.58	63.35	77.93
Total unidentified	2.83	1.20	4.03[f]	3.63	2.55	6.18[g]
Total accounted for	12.59	81.00	93.59	18.21	74.10	92.31
Losses/gains[h]	−0.99	0.80	−0.19	−1.11	0.70	−0.41
Total	11.60	81.80	93.40	17.10	74.80	91.90

From Green (2009)
NA, not applicable
[a] Includes an unresolved desmethyl glucuronide metabolite.
[b] Includes an unresolved desmethyl hydroxy glucuronide (phenolic) metabolite.
[c] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass measurement. With the exception of urine obtained from female rats administered (^{14}C-pyrazole)-labelled sedaxane (80 mg/kg bw), in which this component was identified as a β-hydroxycarbonyl, fragmentation data were not available to define the identification.
[d] Two desmethyl glucuronide metabolites were detected and unresolved from CSCD659090 and desmethyl hydroxysedaxane.
[e] Two desmethyl hydroxy glucuronide metabolites were detected, one unresolved from a desmethyl β-hydroxycarbonyl metabolite.
[f] Ten components, none greater than 0.68% of the administered dose.
[g] Eleven components, none greater than 0.89% of the administered dose.
[h] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 11. Metabolite profile in excreta of rats following a single oral dose of [pyrazole-5-^{14}C]sedaxane at the low dose of 1 mg/kg bw

Compound	% of administered dose (number of isomers)					
	Males			Females		
	Urine (0–96 h)	Faeces (0–96 h)	Total excreta	Urine (0–96 h)	Faeces (0–96 h)	Total excreta
Sedaxane	—	1.71	1.71	—	1.56	1.56
CSCD659087	2.58	26.86	29.44	9.33	29.16	38.49
CSCD668404	0.35	5.08	5.43	1.55	9.32	10.87
CSCD659088	—	1.32	1.32	—	1.47	1.47
CSCD658906	1.46	10.05	11.51	2.34	11.74	14.08

Compound	% of administered dose (number of isomers)					
	Males			Females		
	Urine (0–96 h)	Faeces (0–96 h)	Total excreta	Urine (0–96 h)	Faeces (0–96 h)	Total excreta
CSCD659089	—	5.05	5.05	—	—	—
CSCD659090	—	2.29[a]	2.29	0.28	1.98[a]	2.26
Desmethyl hydroxy	—	1.26 (1)[a]	1.26	—	2.99 (1)[a]	2.99
Desmethyl dihydroxy	—	2.17 (1)	2.17	—	1.63 (1)	1.63
Desmethyl β-hydroxycarbonyl	2.11 (1)	—	2.11	2.71 (1)[b]	—	2.71
Dihydroxy	0.22 (1)	5.17 (3)	5.39	—	1.56 (1)	1.56
Dihydroxy/β-hydroxycarbonyl[c]	0.30 (1)[c]	—	0.30	—	—	—
Carboxylic acid	0.56 (1)	—	0.56	—	—	—
Hydroxy β-hydroxycarbonyl	0.70 (3)	0.88 (1)	1.58	—	0.80 (1)	0.80
Desmethyl hydroxy sulfate conjugate	0.42 (1)	—	0.42	—	—	—
Desmethyl glucuronide	—	(2)[d]	—	—	(2)[d]	—
Desmethyl hydroxy glucuronide	—	—	—	0.36 (2)[e]	—	0.36
Hydroxy glucuronide	—	1.21 (1)	1.21	0.96 (1)	2.10 (1)	3.06
Post-extraction solids	NA	13.60	13.60	NA	11.00	11.00
Total identified	8.70	63.05	71.75	17.53	64.31	81.84
Total unidentified	3.01	10.66	13.67[f]	1.87	3.30	5.17[g]
Total accounted for	11.71	87.31	99.02	19.40	78.61	98.01
Losses/gains[h]	−0.01	1.09	1.08	−0.20	0.79	0.59
Total	11.70	88.40	100.1	19.20	79.40	98.60

From Green (2009)
NA, not applicable

[a] Includes an unresolved desmethyl glucuronide metabolite.

[b] Includes an unresolved desmethyl hydroxy glucuronide (phenolic) metabolite.

[c] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass measurement. With the exception of urine obtained from female rats administered (^{14}C-pyrazole)-labelled sedaxane (80 mg/kg bw), in which this component was identified as a β-hydroxycarbonyl, fragmentation data were not available to define the identification.

[d] Two desmethyl glucuronide metabolites were unresolved from CSCD659090 and desmethyl hydroxy sedaxane.

[e] Two desmethyl hydroxy glucuronide metabolites were detected, one unresolved from a desmethyl β-hydroxycarbonyl metabolite.

[f] Fifteen components, none greater than 1.98% of the administered dose.

[g] Nine components, none greater than 1.60% of the administered dose.

[h] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 12. Metabolite profile in excreta of male rats following a repeated oral dosing of [pyrazole-5-^{14}C]sedaxane at the low dose of 1 mg/kg bw

Compound	% of administered dose (number of isomers)					
	0–24 h			312–336 h		
	Urine	Faeces	Total excreta	Urine	Faeces	Total excreta
Sedaxane	—	3.86	3.86	—	—	—
CSCD659087	3.56	21.70	25.26	1.03	35.14	36.17
CSCD668404	0.40	5.26	5.66	0.25	7.46	7.71
CSCD659088	—	—	—	—	—	—
CSCD658906	1.52	7.32	8.84	0.59	11.53	12.12
CSCD659089	—	5.33[a]	5.33	—	2.92	2.92
CSCS659090	—	(1)[b]	—	—	2.03	2.03
Desmethyl β-hydroxycarbonyl	2.96 (1)[c]	—	2.96	5.76 (1)[c]	—	5.76
Dihydroxy/β-hydroxycarbonyl[d]	0.95 (1)[d]	—	0.95	0.95 (1)[d]	—	0.95
Desmethyl hydroxy sulfate conjugate	—	—	—	0.48 (1)	—	0.48
Desmethyl glucuronide	—	(1)[b]	—	—	—	—
Desmethyl hydroxy glucuronide	1.00 (2)[e]	—	1.00	0.64 (2)[e]	—	0.64
Hydroxy glucuronide	1.58 (1)	—	1.58	4.29 (1)	—	4.29
Post-extraction solids	NA	12.10	12.10	NA	8.70	8.70
Total identified	11.97	43.47	55.44	13.99	59.08	72.43
Total unidentified	1.03	4.73	5.76[f]	1.31	3.33	4.64[g]
Total accounted for	13.00	60.30	73.30	15.30	71.11	85.77
Losses/gains[h]	0.00	0.60	0.60	0.60	0.89	2.13
Total	13.00	60.90	73.90	15.90	72.00	87.90

From Green (2009)
NA, not applicable
[a] Includes unresolved components CSCD659090 and a desmethyl glucuronide metabolite.
[b] Component detected within a region of unresolved components, including CSCD659089, CSCD659090 and a desmethyl glucuronide metabolite.
[c] Includes an unresolved desmethyl hydroxy glucuronide metabolite.
[d] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass measurement.
[e] Two desmethyl hydroxy glucuronide metabolites were detected; one was unresolved from a desmethyl β-hydroxycarbonyl metabolite.
[f] Four components, none greater than 4.73% of the administered dose.
[g] Three components, none greater than 3.33% of the administered dose.
[h] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 13. Metabolite profile in excreta of bile duct–cannulated rats following a single oral dose of [pyrazole-5-^{14}C]sedaxane at the high dose of 80 mg/kg bw

Compound	% of administered dose (number of isomers)									
	Males					Females				
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]
			U	H				U	H	
Sedaxane	0.05	6.99[b]	1.40	0.59	8.44	—	2.69[b]	0.98	—	3.67
CSCC210616	—	—	0.96	—	0.96	—	—	0.52	—	0.52
CSCD659087	0.22	—	2.34	14.70	2.56	1.89	—	1.67	20.10	3.56
CSCD668404	—	—	1.01[c]	2.15	1.01	0.34	—	0.85[c]	5.09	1.19
CSCD659088	—	—	0.59	1.60	0.59	—	—	—	1.37	—
CSCD658906	0.23	—	5.30	30.87	5.53	1.84	—	3.15	34.89	4.99
CSCD659089	—	—	—	9.85	—	—	—	—	5.09	—
CSCS659090	0.70	—	2.19[d]	7.53	2.89	—	—	2.18[d]	8.20	2.18
CSCD668403	—	—	—	—	—	0.18	—	—	—	0.18
β-Hydroxycarbonyl cysteine conjugate	—	—	—	0.52 (1)	—	—	—	—	—	—
Desmethyl sedaxane	—	—	—	—	—	—	0.41 (1)	—	—	0.41
Hydroxy	—	—	—	0.96 (1)	—	—	—	—	—	—
Desmethyl β-hydroxycarbonyl	0.73 (1)[e]	—	—	—	0.73	1.29 (1)[e]	—	—	—	1.29
Dihydroxy/β-hydroxycarbonyl[f]	0.16 (1)[f]	—	—	—	0.16	0.33 (1)[g]	—	—	—	0.33
β-Hydroxycarbonyl	—	—	—	—	—	0.57 (1)	—	—	—	0.57
Dihydroxy	—	—	—	0.74 (1)	—	—	—	—	—	—
Carboxylic acid	0.49 (1)	—	—	—	0.49	0.34 (1)	—	—	—	0.34
Hydroxy β-hydroxycarbonyl	0.76 (4)	—	—	—	0.76	0.37 (1)	—	—	—	0.37
Hydroxy sulfate conjugate	0.28 (1)	—	—	—	0.28	0.16 (1)	—	—	—	0.16
Hydroxy cysteine conjugate	—	—	—	0.15 (1)	—	—	—	—	—	—
Desmethyl glucuronide	—	—	1.18 (2)[h]	2.59 (1)	1.18	0.90 (2)	—	1.10 (2)[h]	3.25 (1)	2.00
Desmethyl hydroxy glucuronide	0.09 (2)[i]	—	16.87 (5)[j]	—	16.96	0.20 (2)[i]	—	23.82 (4)[k]	—	24.02
Hydroxy glucuronide	0.32 (1)	—	38.91 (4)[l]	—	40.25	0.71(2)[m]	—	43.40 (4)[l]	—	44.11

Compound	% of administered dose (number of isomers)									
	Males					Females				
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]
			U	H				U	H	
Dihydroxy glucuronide	—	—	1.11 (1)	—	1.11	—	—	—	—	—
Hydroxy glutathione conjugate	—	—	(1)[n]	—	—	—	—	0.32 (1)[n]	—	0.32
Dihydroxy glutathione	—	—	0.61 (1)	—	0.61	—	—	—	—	—
Post-extraction solids	NA	0.20	NA	NA	0.20	NA	0.20	NA	NA	0.20
Total identified	4.03	6.99	72.47	72.25	83.49	9.12	3.10	77.99	77.99	90.21
Total unidentified	1.67	0.00	1.84	2.05	3.51[o]	0.68	0.00	0.00	0.00	0.68[p]
Total accounted for	5.70	7.19	74.31	74.30	87.20	9.80	3.30	77.99	77.99	91.09
Losses/gains[q]	0.20	0.03	7.49	7.50	7.72	0.40	−0.06	3.21	3.21	3.55
Total	5.90	7.22	81.80	81.80	94.92	10.20	3.24	81.20	81.20	94.64

From Green (2009)

H, hydrolysed; NA, not applicable; U, unhydrolysed

[a] Sum of urine, faeces and unhydrolysed bile.

[b] Postulated to include the *trans* and *cis* isomers comprising sedaxane.

[c] Includes an unresolved hydroxy glucuronide (phenolic) metabolite.

[d] Includes an unresolved desmethyl glucuronide metabolite.

[e] Includes an unresolved desmethyl hydroxy glucuronide metabolite.

[f] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass.

[g] Includes an unresolved hydroxy glucuronide metabolite.

[h] Two desmethyl glucuronide metabolites were detected, one unresolved from CSCD659090.

[i] Two desmethyl hydroxy glucuronide metabolites were detected, one unresolved from a desmethyl β-hydroxycarbonyl metabolite.

[j] Includes two hydroxy glutathione metabolites unresolved from a desmethyl hydroxy glucuronide metabolite.

[k] Includes a hydroxy glutathione metabolite unresolved from an isomer of a desmethyl hydroxy glucuronide metabolite.

[l] Four hydroxy glucuronide metabolites were detected, one unresolved from CSCD668404.

[m] Two isomers of hydroxy glucuronide metabolites were detected, one unresolved from a dihydroxy/β-hydroxycarbonyl metabolite.

[n] One hydroxy glutathione metabolite was unresolved from a desmethyl hydroxy glucuronide metabolite.

[o] Eleven components, none greater than 1.04% of the administered dose.

[p] Three components, none greater than 0.36% of the administered dose.

[q] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 14. Metabolite profile in excreta of bile duct–cannulated rats following a single oral dose of [pyrazole-5-^{14}C]sedaxane at the low dose of 1 mg/kg bw

Compound	% of administered dose (number of isomers)									
	Males					Females				
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]
			U	H				U	H	
Sedaxane	—	6.41	0.34	0.72	6.75	—	4.49	0.73	—	5.22
CSCC210616	—	—	0.16	—	0.16	—	—	0.84	—	0.84
CSCD659087	1.04	—	3.46	25.41	4.50	2.36	—	3.05	28.80	5.41
CSCD668404	—	—	0.96[b]	5.13	0.96	0.50	—	1.17[b]	6.59	1.67
CSCD659088	—	—	—	1.32	—	—	—	—	1.52	—
CSCD658906	0.86	—	2.30	22.46	3.16	0.62	—	3.06	25.03	3.68
CSCD659089	—	—	—	4.99	—	—	—	—	2.33	—
CSCS659090	—	—	1.06[c]	5.63	1.06	—	—	0.57[c]	5.99	0.57
Hydroxy	—	—	—	0.57	—	—	—	—	—	—
Desmethyl β-hydroxycarbonyl	0.87[d]	—	—	—	0.87	1.66[d]	—	—	—	1.66
Dihydroxy/β-hydroxycarbonyl[e]	0.39[e]	—	—	—	0.39	—	—	—	—	—
β-Hydroxycarbonyl	—	—	—	—	—	0.22 (1)	—	—	—	0.22
Dihydroxy	—	—	—	0.60	—	—	—	—	—	—
Carboxylic acid	0.36	—	—	—	0.36	0.23	—	—	—	0.23
Hydroxy β-hydroxycarbonyl	0.42	—	—	—	0.42	—	—	—	—	—
Hydroxy cysteine conjugate	—	—	—	2.77	—	—	—	—	—	—
Desmethyl glucuronide	—	—	0.51 (2)[f]	3.07	0.51	—	—	1.20 (2)[f]	3.03	1.20
Desmethyl hydroxy glucuronide	(1)[g]	—	31.68 (4)[h]	—	31.68	(1)[g]	—	33.56 (4)	—	33.56
Hydroxy glucuronide	0.47	—	31.81 (4)[i]	—	32.28	0.47 (1)	—	30.20 (4)[i]	—	30.67
Dihydroxy glucuronide	—	—	0.85 (1)	—	0.85	—	—	1.25 (1)	—	1.25
Hydroxy glutathione	—	—	(1)[j]	—	—	—	—	—	—	—

Compound	% of administered dose (number of isomers)									
	Males					Females				
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]
			U	H				U	H	
conjugate										
Dihydroxy glutathione	—	—	0.32 (1)	—	0.32	—	—	—	—	—
Post-extraction solids	NA	0.30	NA	NA	0.30	NA	0.20	NA	NA	0.20
Total identified	4.41	6.41	73.45	72.67	84.27	6.06	4.49	75.63	73.29	86.18
Total unidentified	2.10	0.00	4.97	5.75	7.07[k]	0.34	0.00	4.19	6.52	4.53[l]
Total accounted for	6.51	6.71	78.42	78.42	91.64	6.40	4.69	79.82	79.81	90.91
Losses/gains[m]	0.19	0.01	0.58	0.58	1.28	0.50	0.04	−0.42	−0.41	0.12
Total	6.70	6.70	79.00	79.00	92.92	6.90	4.73	79.40	79.40	91.03

From Green (2009)

H, hydrolysed; NA, not applicable; U, unhydrolysed

[a] Sum of urine, faeces and unhydrolysed bile.

[b] Includes an unresolved hydroxy glucuronide (phenolic) metabolite.

[c] Includes an unresolved desmethyl glucuronide metabolite.

[d] Includes an unresolved desmethyl hydroxy glucuronide metabolite.

[e] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass.

[f] Two desmethyl glucuronide metabolites were detected, one unresolved from CSCD659090.

[g] A desmethyl hydroxy glucuronide metabolite was unresolved from a desmethyl β-hydroxycarbonyl metabolite.

[h] Includes a hydroxy glutathione metabolite unresolved from a desmethyl hydroxy glucuronide metabolite.

[i] Four hydroxy glucuronide metabolites were detected, one unresolved from CSCD668404.

[j] A hydroxy glutathione metabolite was unresolved from a desmethyl hydroxy glucuronide metabolite.

[k] Eleven components, none greater than 1.96% of the administered dose.

[l] Four components, none greater than 2.59% of the administered dose.

[m] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 15. Metabolite profile in excreta of bile duct–cannulated rats following a single oral dose of [phenyl-U-^{14}C]sedaxane at the high dose of 80 mg/kg bw

Compound	% of administered dose (number of isomers)							
	Males				Females			
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)	Total excreta	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)	Total excreta
Sedaxane	—	3.69	0.87	4.56	—	9.60	—	9.60
CSCD659087	0.31	—	0.75	1.06	1.30	—	0.57	1.87
CSCD668404	—	—	1.35[a]	1.35	0.21	0.20	0.47[a]	0.88
CSCD658906	0.29	0.27	1.55	2.11	0.68	0.31	0.69	1.68
CSCD659090	—	—	1.88[b]	1.88	—	—	2.42[b]	2.42
Desmethyl sedaxane	—	—	—	—	—	0.19 (1)	—	0.19
Desmethyl β-hydroxycarbonyl	0.44 (1)	0.23 (1)	—	0.67	0.96 (1)[c]	—	—	0.96
Dihydroxy/β-hydroxycarbonyl[d]	0.67 (1)[d]	—	—	0.67	0.27 (1)[d]	—	—	0.27
Carboxylic acid	0.56 (1)	—	—	0.56	0.21 (1)	—	—	0.21
Hydroxy β-hydroxycarbonyl	0.50 (4)	—	—	0.50	—	—	—	—
Desmethyl hydroxy sulfate conjugate	0.47 (1)	—	—	0.47	—	—	—	—
Hydroxy sulfate conjugate	0.16 (1)	—	—	0.16	—	—	—	—
Desmethyl glucuronide	—	—	2.11 (2)[e]	2.11	0.38 (2)	—	0.86 (2)[e]	1.24
Desmethyl hydroxy glucuronide	0.58 (2)	—	32.81 (6)[f]	33.39	(1)[g]	—	39.44 (4)[f]	39.44
Hydroxy glucuronide	0.63 (2)	—	48.78 (4)[h]	49.41	0.40 (1)	—	40.71 (4)[h]	41.11
Hydroxy glutathione conjugate	—	—	(1)[i]	—	—	—	(1)[i]	—
Post-extraction solids	NA	0.20	NA	0.20	NA	0.20	NA	0.20
Total identified	4.61	4.19	90.10	98.90	4.41	10.30	85.16	99.87
Total unidentified	1.09	0.00	0.00	1.09[j]	0.50	0.00	0.34	0.84[k]
Total accounted for	5.70	4.39	90.10	100.19	4.91	10.5	85.50	100.91
Losses/gains[l]	1.00	0.01	−4.80	−3.79	0.39	0.1	−4.50	−4.01
Total	6.70	4.40	85.30	96.40	5.30	10.6	81.00	96.90

From Green (2009)
NA, not applicable
[a] Includes an unresolved hydroxy glucuronide (phenolic) metabolite.
[b] Includes an unresolved desmethyl glucuronide metabolite.
[c] Includes an unresolved desmethyl hydroxy glucuronide (phenolic) metabolite.
[d] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass.
[e] Two desmethyl glucuronide metabolites were detected, one unresolved from CSCD659090.
[f] Includes a hydroxy glutathione metabolite unresolved from a desmethyl hydroxy glucuronide metabolite.

Table 15 (continued)

[g] A desmethyl hydroxy glucuronide (phenolic) metabolite was unresolved from a desmethyl β-hydroxycarbonyl metabolite.
[h] Four hydroxy glucuronide metabolites were detected, one unresolved from CSCD668404.
[i] A hydroxy glutathione metabolite was unresolved from an isomer of a desmethyl hydroxy glucuronide metabolite.
[j] Eleven components, none greater than 0.24% of the administered dose.
[k] Five components, none greater than 0.34% of the administered dose.
[l] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 16. Metabolite profile in excreta of bile duct–cannulated rats following a single oral dose of [phenyl-U-^{14}C]sedaxane at the low dose of 1 mg/kg bw

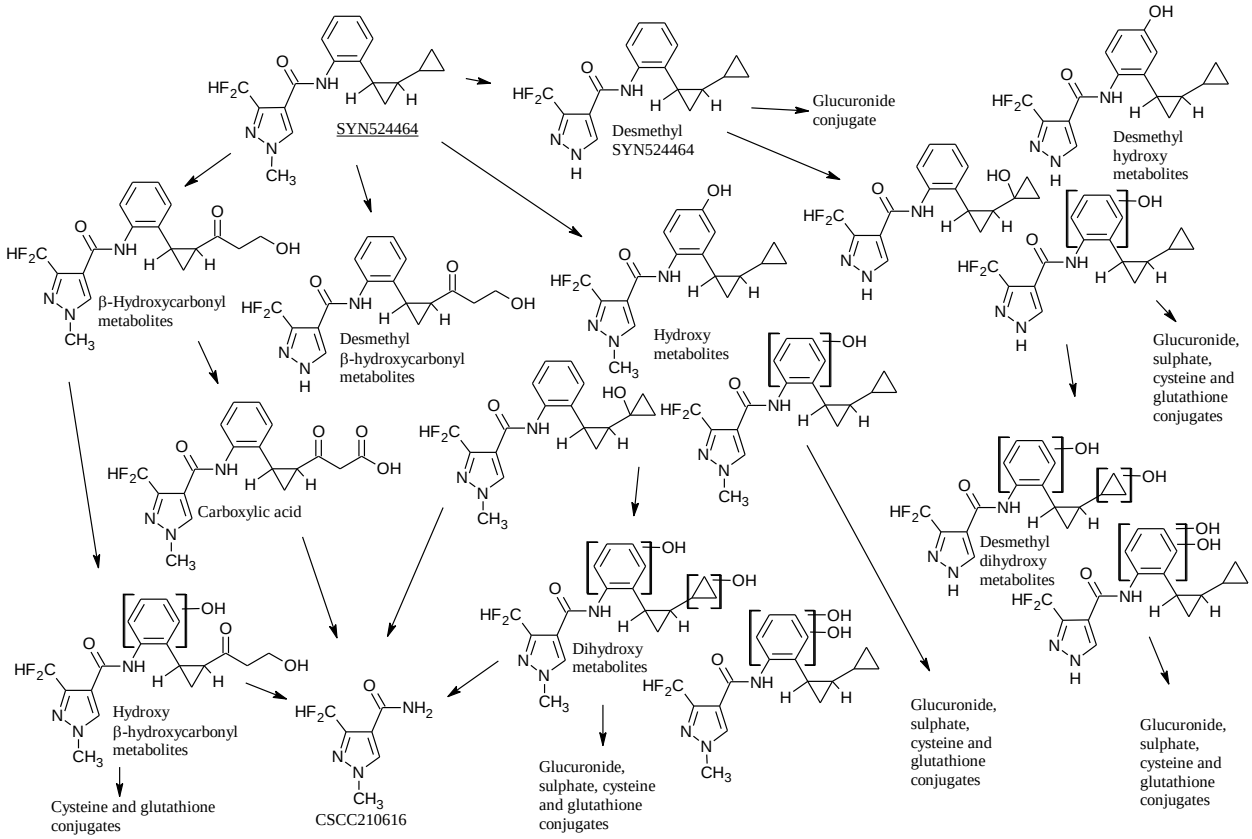
Compound	% of administered dose (number of isomers)							
	Males				Females			
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)	Total excreta	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)	Total excreta
Sedaxane	—	4.31	1.17	5.48	—	7.38	—	7.38
CSCD659087	0.86	—	1.47	2.33	1.62	—	1.27	2.89
CSCD668404	—	—	1.04[a]	1.04	0.54	0.57	—	1.11
CSCD658906	0.46	0.38	1.22	2.06	0.64	0.27	0.79	1.70
CSCD659089	—	0.33	—	0.33	—	0.18	—	0.18
CSCD659090	—	—	1.19[b]	1.19	—	—	0.50[b]	0.50
Desmethyl β-hydroxycarbonyl	—	0.38 (1)	—	0.38	2.51 (1)[c]	—	—	2.51
Dihydroxy/β-hydroxycarbonyl[d]	0.75 (1)[d]	—	—	0.75	0.15 (1)[d]	—	—	0.15
Carboxylic acid	0.43 (1)	—	—	0.43	0.13 (1)	—	—	0.13
Hydroxy β-hydroxycarbonyl	0.29 (1)	—	—	0.29	0.33 (1)	—	—	0.33
Hydroxy cysteine conjugate	—	—	—	—	—	—	2.33 (1)	2.33
Desmethyl glucuronide	—	—	1.02 (2)[e]	1.02	—	—	0.65 (2)[e]	0.65
Desmethyl hydroxy glucuronide	1.12 (2)	—	40.23 (4)[f]	36.37	0.26 (2)[g]	—	40.87 (5)[f]	41.13
Hydroxy glucuronide	0.32 (1)	—	36.93 (4)[h]	37.25	0.85 (1)	—	32.49 (3)	33.34
Hydroxy glutathione conjugate	—	—	(1)[i]	—	—	—	(1)[i]	—
Post-extraction solids	NA	0.20	NA	0.20	NA	0.10	NA	0.10
Total identified	4.23	5.40	84.27	93.90	7.03	8.40	78.90	94.33
Total unidentified	1.57	0.00	1.06	2.63[j]	0.46	0.00	3.21	3.67[k]
Total accounted for	5.80	5.60	85.33	96.73	7.49	8.50	82.11	98.10
Losses/gains[l]	0.70	0.10	−4.23	−3.43	0.61	0.10	−3.51	−2.80
Total	6.50	5.70	81.10	93.30	8.10	8.60	78.60	95.30

From Green (2009)
NA, not applicable
[a] Includes an unresolved hydroxy (phenolic) glucuronide metabolite.
[b] Includes an unresolved desmethyl glucuronide metabolite.
[c] Includes an unresolved desmethyl hydroxy glucuronide (phenolic) metabolite.
[d] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass.
[e] Two desmethyl glucuronide metabolites were detected, one unresolved from CSCD659090.
[f] Includes a hydroxy glutathione metabolite unresolved from a desmethyl hydroxy glucuronide metabolite.
[g] Two desmethyl hydroxy glucuronide metabolites were detected, one unresolved from a desmethyl β-hydroxycarbonyl metabolite.
[h] Four hydroxy glucuronide metabolites were unidentified, one unresolved from CSCD668404.
[i] A hydroxy glutathione metabolite was unresolved from a desmethyl hydroxy glucuronide metabolite.
[j] Eight components, none greater than 1.06% of the administered dose.
[k] Five components, none greater than 2.73% of the administered dose.
[l] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Sedaxane was extensively metabolized by rats via demethylation, hydroxylation, oxidation and conjugation reactions, resulting in an array of hydroxylated metabolites and metabolites formed by cleavage of the terminal cyclopropyl moiety. An equivalent range of metabolites of desmethyl sedaxane was formed. The major metabolites were identified as the *trans-para*-phenol CSCD658906 and the desmethyl *trans-para*-phenol CSCD659087, which, together with the equivalent *cis-para*-phenol isomers CSCD659090 and CSCD668404, accounted for approximately half the administered dose. Glucuronic acid, sulfate and glutathione conjugates were formed (Green, 2009).

On the basis of these studies, the metabolic pathway shown in Figure 2 was proposed.

Figure 2. Overall summary of the biotransformation pathway of sedaxane (SYN 524464) in rat



2. Toxicological studies

2.1 Acute toxicity

Studies on the acute toxicity of sedaxane are summarized in Table 17.

Table 17. Summary of acute toxicity of sedaxane

Species/strain	Route	Sex	Parameter evaluated	Result	Ratio (%) of *trans* to *cis* isomers	Reference
Rat / HanRcc:WIST	Oral	F	LD_{50}	5000 mg/kg bw	83.0:12.3	Arcelin (2008)
Rat / HanRcc:WIST	Dermal	M + F	LD_{50}	> 5000 mg/kg bw	83.0:12.3	Arcelin (2007a)
Rat / HanRcc:WIST	Inhalation	M + F	4 h LC_{50}	> 5.244 mg/l	83.0:12.3	Decker (2008)
Rabbit / NZW	Dermal	M + F	Local irritancy	Non-irritant	83.0:12.3	Arcelin (2007b)
Rabbit / NZW	Ocular	M + F	Local irritancy	Mild irritant	83.0:12.3	Arcelin (2007c)
Mouse / CBA/Ca	Intradermal and topical	M	Local lymph node assay	Non-sensitizer	83.0:12.3	Pooles (2007)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male; NZW, New Zealand White

(a) Oral administration

Rats

In a range-finding acute oral toxicity study, one female HanRcc:WIST (SPF) rat was administered sedaxane (purity 95.3%) orally via gavage at 5000 mg/kg bw. The treated female died shortly after dosing. In a definitive study, 10- to 11-week-old female rats were dosed by gavage with sedaxane in 0.5% carboxymethylcellulose at 175, 550, 1750 or 5000 mg/kg bw (one, one, four and seven females per dose, respectively). All surviving rats were killed and necropsied 15 days after dosing.

No deaths occurred at 175, 550 or 1750 mg/kg bw. At 5000 mg/kg bw, four out of seven rats were killed in extremis on day 1, and one rat died on day 2. Clinical signs recorded included ruffled fur, hunched posture, sedation, poor coordination and ventral recumbency in rats at 5000 or 1750 mg/kg bw. Deep respiration, rales, salivation and bradypnoea were recorded at 5000 mg/kg bw. The single rat given 550 mg/kg bw showed slightly ruffled fur or a hunched posture from 1 hour after dosing up to day 3. The 175 mg/kg bw treated female (one female treated with this dose) had slightly ruffled fur for approximately 0.5–5 hours, a hunched posture for 2–3 hours after the dosing and slight sedation at the 3-hour evaluation. Body weights were normal. At necropsy, a yellowish discoloration of the jejunum was recorded in one 5000 mg/kg bw dose rat killed in extremis, and pale, discoloured lungs were observed in another.

Based on the results of this study, it is estimated that the median lethal dose (LD_{50}) of sedaxane was 5000 mg/kg bw in female rats (Arcelin, 2008).

(b) Dermal application

Rats

A group of five male and five female HanRcc:WIST (SPF) rats was treated with sedaxane (lot no. SMU6LP006; purity 95.3%) at 5000 mg/kg bw by dermal application. In this study, a single animal of each sex was treated first. No deaths and no severe local effects or systemic symptoms were

observed after the 24-hour exposure. Therefore, the test was completed using the remaining four male and four female rats at a dose level of 5000 mg/kg bw with an exposure period of 24 hours. Sedaxane was applied undiluted and moistened with approximately 1 ml purified water for treatment to an area of skin from which hair had been clipped and covered with a semi-occlusive dressing for 24 hours. The rats were observed for 14 days after treatment, after which they were killed and subjected to a macroscopic examination.

All rats survived until the end of the study. No treatment-related effects on mortality, clinical signs or body weight or at gross necropsy were seen.

The acute dermal LD_{50} of sedaxane after a single dermal administration to rats of both sexes was greater than 5000 mg/kg bw (Arcelin, 2007a).

(c) Exposure by inhalation

Rats

A group of five male and five female HanRcc:WIST (SPF) rats 9–10 weeks of age was exposed for 4 hours by nose-only, flow-past inhalation to sedaxane (lot no. SMU6LP006; purity 95.3%) at a gravimetrically determined mean aerosol concentration of 5.244 mg/l air. The particle size distribution of the test atmosphere was analysed twice during the exposure period. The rats were observed for 15 days (including the exposure day as test day 1 of 15). Clinical observations and body weights were recorded throughout the study. The rats were necropsied at the end of the 15-day observation period.

The mass median aerodynamic diameter (MMAD) was 2.97–3.02 μm. There were no deaths and no macroscopic pathological findings. Transient clinical signs including bradypnoea and breath sounds (rales), decreased spontaneous activity, hunched posture and ruffled fur, and transient, slight retardation in body weight gain or marginal to moderate body weight loss in all rats were attributed to sedaxane, although slight physical stress during restraint in the exposure tubes may have contributed to the effects on body weight.

The acute inhalation LC_{50} of sedaxane after a 4-hour exposure in male and female rats was estimated to be greater than 5.244 mg/l air (Decker, 2008).

(d) Dermal irritation

The primary skin irritation potential of sedaxane (lot no. SMU6LP006; purity 95.3%) was investigated. A dose of 0.5 g sedaxane was applied to the shaved intact left flank of each of three young adult New Zealand White rabbits (one male and two females). After 4 hours of semi-occlusive treatment, the dressing was removed, and skin reactions were scored after 1, 24, 48 and 72 hours. The primary irritation index was calculated by totalling the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of data points (Draize, Woodward & Calvery, 1944; Draize, 1959).

No clinical signs were observed, and there were no signs of corrosion, irritation or staining of the skin in any of the rabbits throughout the 72 hours of observation. The primary irritation index was 0.0 (on a scale of 0.0–8.0).

According to Draize classification criteria, sedaxane was not an irritant to rabbit skin (Arcelin, 2007b).

(e) Ocular irritation

The primary eye irritation potential of sedaxane (purity 95.3%) was investigated according to Organisation for Economic Co-operation and Development Test Guideline No. 405. Sedaxane was applied by instillation of 0.1 g into the left eye of each of three young New Zealand White rabbits (one male and two females).

The instillation of sedaxane into the eye resulted in mild, early-onset and transient conjunctival reddening and chemosis. The individual mean scores for the conjunctivae were 0.33, 0.67 and 0.33 for reddening for each of the three rabbits and 0.00 for chemosis. These effects were no

longer observed 72 hours after treatment. No corrosion, staining of the eyes or any other abnormal ocular findings were observed in any rabbit at any examination time. No clinical signs were observed.

Under the conditions of this study, sedaxane was mildly irritating to rabbit eye (Arcelin, 2007c).

(f) Dermal sensitization

A sample of sedaxane (lot no. SUM6LP006; purity 95.3%) was assessed for its skin sensitization potential in CBA/Ca mice using the local lymph node assay. The assay compares the level of T lymphocyte proliferation in the lymph nodes draining the site of chemical application in mice treated with a chemical with T lymphocyte proliferation at a similar site in control group mice, by measuring the amount of radiolabelled thymidine incorporated into the dividing cells. The criterion for a positive response is that one or more of the concentrations tested should elicit a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The assay is able to identify those materials that elicit responses in standard guinea-pig tests for skin sensitization (Kimber et al., 1994). The application of sedaxane at concentrations of 10%, 25% and 50% weight per weight in acetone/olive oil (4:1) resulted in an isotope incorporation ratio that did not exceed 1.12, whereas a positive control treatment with hexylcinnamaldehyde, 15% volume per volume, resulted in an isotope incorporation ratio of 5.67.

Under the conditions of this study, sedaxane was not a skin sensitizer (Pooles, 2007).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Groups of five male and five female CD-1 mice were fed diets containing 0, 1000, 5000 or 7000 ppm sedaxane (lot no. S01F002249U; purity 98.2%; ratio of isomers 83.4% *trans* to 14.8% *cis*) for a period of at least 28 days. The mean compound intakes were 0, 178, 920 and 1268 mg/kg bw per day for males and 0, 248, 1150 and 1800 mg/kg bw per day for females at 0, 1000, 5000 and 7000 ppm, respectively. The mice were monitored regularly for viability and for signs of ill-health or reaction to treatment. Body weights and feed consumption were measured and recorded at predetermined intervals from pretrial until the completion of treatment. Blood samples were collected prior to terminal necropsies during week 5 for laboratory investigations. All animals were subjected to a detailed necropsy examination after the completion of treatment. A limited number of tissues from all mice in the 0 and 7000 ppm dose groups were taken for histological evaluation.

Stability, homogeneity and concentrations in the diet were acceptable. There were no differences in body weight and body weight gain that were attributable to treatment. There were no effects on body weight or feed consumption of males or females at dose levels up to 7000 ppm. A slight increase in haemoglobin value (4%) was observed in males at 7000 ppm that was not considered to be toxicologically significant. Statistically significant decreases in calcium levels compared with controls were observed in males at 5000 and 7000 ppm and in females at 7000 ppm. A 64% higher triglyceride concentration was observed in males at 7000 ppm. The blood chemistry changes were not accompanied by any corroborating histopathology. In addition, these changes were not detected in mice of the 7000 ppm group in the 90-day oral toxicity study described below. Therefore, they were not considered to be toxicologically significant. No treatment-related histopathological changes were observed.

The no-observed-adverse-effect level (NOAEL) was 7000 ppm (equal to 1268 mg/kg bw per day), the highest dose tested (Shearer & Robsertson, 2008).

In a dose-finding study preparatory to a carcinogenicity study, groups of 10 male and 10 female CD-1 mice were fed diets containing sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at a concentration of 0, 500, 3500 or 7000 ppm (equal to 0, 80, 566

and 1167 mg/kg bw per day for males and 0, 112, 810 and 1455 mg/kg bw per day for females, respectively) for a period of at least 90 days. Mice were monitored regularly for viability and for signs of ill-health or reaction to treatment. Body weights and feed consumption were measured and recorded at predetermined intervals from pretrial until the completion of treatment. Blood samples were collected prior to terminal necropsies during week 14 for laboratory investigations. All mice were subjected to a detailed necropsy examination after the completion of treatment. Selected tissues from all mice in the 0 and 7000 ppm dose groups were taken for histological evaluation.

Stability, homogeneity and concentrations in the diet were acceptable. There were no treatment-related clinical signs throughout the study. Males at 7000 ppm had lower body weight and body weight gain throughout the treatment period (Table 18). Lower body weights were also noted in males at 500 or 3500 ppm, but these values were not statistically significant. The differences at 500 and 3500 ppm were not considered to be treatment related because the value in the control group was high compared with the historical control value. Lower feed utilization in males at 7000 ppm was observed along with the lower body weights. Statistically significantly lower white blood cell and lymphocyte counts were noted in all male treated groups, but with no indication of a dose-related response. However, the control values for white blood cells and lymphocytes were considered to be high (10.19×10^9/l and 8.28×10^9/l, respectively) when compared with mean historical control values (7.35×10^9/l and 5.61×10^9/l, respectively, based on eight relevant studies in male mice conducted from 2001 to 2004). There was no decrease in spleen weight, and there was no histopathological finding indicating immunosuppression in the bone marrow, spleen, lymph nodes or thymus. Therefore, these lower levels in haematology were not considered to be treatment related.

Table 18. Intergroup comparison of selected organ weights (absolute and adjusted) and clinical chemistry values of mice treated orally with sedaxane for 90 days

	Males				Females			
	0 ppm	500 ppm	3500 ppm	7000 ppm	0 ppm	500 ppm	3500 ppm	7000 ppm
Terminal body weight ± SD (g)	48 ± 7	45 ± 6	44 ± 4	43 ± 3	32 ± 5	31 ± 3	29 ± 2	34 ± 7
Alkaline phosphatase (IU/l)	62	52	47*	44**	62	60	56	56
Total bilirubin (mmol/l)	3.6	3.6	2.9	2.1**	2.4	2.4	2.8	2.4
Liver (g)	2.24	2.28	2.26	2.49	1.71	1.69	1.66	2.02
Liver, adjusted[a] (g)	2.05	2.29*	2.30*	2.61**	1.69	1.73	1.80	1.85
Kidney (g)	0.720	0.694	0.700	0.730	0.404	0.408	0.384	0.369
Kidney, adjusted[a] (g)	0.695	0.696	0.706	0.748	0.401	0.412	0.400	0.351**
Testis (g)	0.25	0.27	0.29	0.31**	—	—	—	—
Testis, adjusted[a] (g)	0.25	0.27	0.29*	0.31**	—	—	—	—

From Shearer & Foster (2008)
IU, international unit; SD, standard deviation; * $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)
[a] Organ weights were adjusted for terminal body weights.

Blood chemistry analysis indicated statistically significant reductions in alkaline phosphatase activity in male mice of 29% and 24% at 7000 and 3500 ppm, respectively, and in total bilirubin level of 42% at 7000 ppm (Table 18). There were no statistically significant changes in blood chemistry of female mice. Absolute testis weights were significantly higher by 24% in the 7000 ppm group, but there were no other absolute organ weight changes. Relative to body weight, liver weights were increased in male mice of the 7000, 3500 and 500 ppm groups by 27%, 12% and 12%, respectively,

whereas there were no changes in females. The changes in relative liver weights in male mice appear to be attributable to the lower values for terminal body weight, because brain weights were not changed (male mice, 0 ppm, 0.51 g; 7000 ppm, 0.51 g). There was little change in liver weight relative to brain weight at increasing dose. Female mice of the 7000 ppm group had lower relative kidney and heart weights, but only after adjustment for body weight. As these changes were slight or lacked associated histopathological findings, these clinical chemistry and organ weight differences were not considered to be toxicologically significant (Shearer & Foster, 2008).

The NOAEL for this 90-day oral toxicity study in mice was 3500 ppm (equal to 566 mg/kg bw per day), based on a decrease in body weight gain throughout the study in males at 7000 ppm (equal to 1167 mg/kg bw per day) (Shearer & Foster, 2008).

Rats

Two short-term studies were conducted in rats. In the first study (Noakes, 2007), groups of 12 male and 12 female HsdRccHan:WIST rats were fed diets containing sedaxane (lot no. S01F002249U; purity 98.2%; ratio of isomers 83.4% *trans* to *cis* 14.8%) at a concentration of 0, 250, 1000 or 4000 ppm (equal to 0, 18.6, 72.9 and 299.6 mg/kg bw per day for males and 0, 21.4, 85.7 and 315.3 mg/kg bw per day for females, respectively) for 90 consecutive days. Clinical observations, body weights and feed consumption were measured throughout the study; a functional observational battery of tests and locomotor activity monitoring were performed during week 12. An ophthalmoscopic examination was performed on all rats before exposure and on control and high-dose rats in week 13. Urine samples collected during week 13 were analysed. At the end of the scheduled exposure period, the rats were killed and examined post mortem. Cardiac blood samples were taken for blood pathology, selected organs were weighed and specified tissues were taken for subsequent microscopic examination.

Stability, homogeneity and concentrations of sedaxane in the diet were acceptable. No treatment-related clinical signs were detected. No effects on functional observational battery parameters or locomotor activity were observed. At 4000 ppm, terminal body weights were lower in males (10%) and females (15%), and feed consumption by female rats was lower throughout the study. There were no treatment-related changes in ophthalmoscopic examination, functional observational battery assessments and motor activity, or macroscopic findings in any treated groups. Haemoglobin, haematocrit and red blood cell counts were slightly low for females at 4000 ppm. Prothrombin time was slightly, but significantly, longer (4% and 8%, respectively) for males at 1000 and 4000 ppm, and platelet counts were slightly, but significantly, higher (11%) for females at 4000 ppm. In blood chemistry, plasma triglyceride concentrations were higher in male rats at 1000 and 4000 ppm (40% and 63%, respectively), whereas female rats at 4000 ppm had higher triglycerides (64%) and total cholesterol (29%). At 4000 ppm, total protein was slightly increased in males (6%) and females (5%), and albumin was increased in males (5%). In urine analysis, reduced urine volume with high specific gravity was observed for males at 1000 and 4000 ppm. Urinary pH was also marginally, but significantly, lower than the control mean value in males at 4000 ppm (6.86 versus 6.54).

Absolute and adjusted liver weights were higher than control values in males at 4000 ppm (26% and 40%, respectively), in females at 4000 ppm (15% and 27%, respectively) and in females at 1000 ppm (13% and 11%, respectively). Adjusted liver weights only were increased by 8% in males at 1000 ppm. The liver weight changes observed in both sexes at 4000 ppm were accompanied by centrilobular hypertrophy and increased pigmentation. Changes in the weights of other organs occurred only at 4000 ppm. These consisted of increases in males of adjusted kidney weight (6%) and decreases in females of absolute weights of adrenals (14%), brain (4%), heart (15%) and kidney (12%). Neither heart nor kidney weight changes were accompanied by histopathology in these organs at 4000 ppm. Sporadic observation of changes in haematology, blood chemistry, urine analysis or relative organ weights at 1000 ppm were not considered to be toxicologically significant because of their small magnitude and the lack of accompanying histopathology that might point towards hepatotoxicity or renal toxicity at 1000 ppm. The treatment-related changes are shown in Tables 19 and 20.

Table 19. Summary of body weights and feed consumption in rats treated orally with sedaxane for 90 days

	Males				Females			
	0 ppm	250 ppm	1000 ppm	4000 ppm	0 ppm	250 ppm	1000 ppm	4000 ppm
Body weights (g)								
- week 2	196.1	198.3	197.2	185.6**	137.7	137.0	138.4	129.9**
- week 6	293.4	293.4	297.8	268.9**	188.8	184.7	191.2	164.6**
- week 10	343.5	341.0	344.9	310.8**	211.1	207.5	212.6	181.1**
- week 14	371.1	368.0	373.2	334.5**	221.2	218.7	221.4	189.8**
Feed consumption (g/rat per day)								
- week 1	21.2	21.8	21.1	17.3*	14.8	14.3	14.6	11.7**
- week 5	22.1	22.0	21.9	20.3*	17.4	16.2	17.0	13.2**
- week 9	20.5	20.5	20.1	19.7	16.5	15.9	16.2	12.8**
- week 13	19.2	19.8	19.1	19.2	15.2	14.8	15.0	12.9*
Haematological parameters								
Prothrombin time (s)	15.2	15.6	15.8**	16.4**	15.5	15.9	15.8	15.9
Platelets ($\times 10^9$/l)	893	890	897	936	909	955	939	1005*
Haemoglobin (g/dl)	15.2	15.2	15.1	14.9	15.4	14.5**	14.9	14.3**
Haematocrit (l/l)	0.473	0.470	0.473	0.469	0.475	0.445**	0.460	0.440**
Red blood cell count ($\times 10^2$/l)	8.47	8.60	8.61	8.36	8.25	7.86**	8.09	7.75**
Blood biochemical parameters								
Triglyceride (mmol/l)	1.41	1.72	1.98**	2.30**	0.89	0.81	0.97	1.46**
Cholesterol (mmol/l)	1.60	1.59	1.52	1.36	1.69	1.72	1.77	2.18**
Total protein (g/l)	60.1	59.7	61.3	63.7*	62.8	62.5	63.9	65.8*
Albumin (g/l)	33.1	33.3	33.7	34.8*	36.1	35.7	36.0	37.1

From Noakes (2007)
* $P < 0.05$; ** $P < 0.01$ (Student's *t*-test, two-sided)

The NOAEL in the first 90-day dietary study in rats was 1000 ppm (equal to 72.9 mg/kg bw per day), based on reduced body weight gain, liver toxicity (minimal centrilobular hepatocyte hypertrophy and pigmentation, evidence from blood chemistry of liver dysfunction, and increased prothrombin time) at 4000 ppm (equal to 299.6 mg/kg bw per day) (Noakes, 2007).

In the second short-term study, groups of 10 male and 10 female Han Wistar rats were fed sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 300, 2000 or 4000 ppm (equal to 0, 24.8, 168.0 and 325.1 mg/kg bw per day for males and 0, 28.3, 186.0 and 349.8 mg/kg bw per day for females, respectively) for at least 90 days. The rats were monitored regularly for viability and for signs of ill-health or reaction to the diet. Detailed functional observations were performed once during treatment over a 2-week period (weeks 12/13). Grip strength was measured according to the method derived from Meyer et al. (1979). Pain was assessed by measurement of the tail flick response, using a technique based on the method devised by D'Amour & Smith (1941). Body weights and feed consumption were measured and recorded at predetermined intervals from pretrial until the completion of treatment. Ophthalmic assessments were undertaken on all animals pretrial and during week 13. Blood samples were collected at week 14 for haematology and blood chemical analysis. All rats were necropsied. Tissues from all rats in the 0 and

4000 ppm dose groups were subjected to comprehensive histological evaluation. In addition, the liver and thyroid were examined from all rats in the 300 and 2000 ppm dose groups.

Table 20. Summary of selected organ weights and histopathology in rats treated orally with sedaxane for 90 days

	Males				Females			
	0 ppm	250 ppm	1000 ppm	4000 ppm	0 ppm	250 ppm	1000 ppm	4000 ppm
Organ weights								
Liver (g)	12.3	13.1	13.3	15.5**	7.2	7.3	8.1**	8.3**
Liver, adjusted[a] (g)	11.9	11.9	12.8*	16.7**	7.0	7.1	7.8**	8.9**
Heart (g)	1.042	1.023	1.037	0.958	0.777	0.755	0.755	0.657**
Heart, adjusted[a] (g)	1.023	1.008	1.008	1.021	0.762	0.746	0.736	0.700*
Kidney (g)	2.09	2.06	2.12	2.03	1.36	1.36	1.35	1.20**
Kidney, adjusted[a] (g)	2.05	2.02	2.06	2.18*	1.32	1.33	1.30	1.31
Liver histopathology								
Hepatocellular hypertrophy (minimal)	0/12	0/12	0/12	12/12	0/12	0/12	0/12	10/12
Increased pigmentation (minimal)	0/12	0/12	0/12	6/12	0/12	0/12	0/12	10/12

From Noakes (2007)
* $P < 0.05$; ** $P < 0.01$ (Student's *t*-test, two-sided)
[a] Organ weight was adjusted for terminal body weight.

Stability, homogeneity and concentrations of sedaxane in the diet were acceptable. Agitated behaviour was noted occasionally in both sexes at 2000 and 4000 ppm and in two females in the 0 ppm group. This sign was not observed in the more comprehensive functional observational batteries, indicating that the signs in these groups were not treatment related. In male and female animals treated at 4000 ppm, body weight, body weight gain and feed consumption were significantly decreased (Table 21). In females, body weights and body weight gains were reduced at 2000 ppm (Table 21). Sedaxane had no effects on body weight or body weight gain at 300 and 2000 ppm in males or at 300 ppm in females and no effect on feed consumption at 300 or 2000 ppm in either sex. In detailed functional observational battery examinations, males at 4000 ppm showed a significant increase in hunched posture and piloerection in the observation arena. Decreases in forelimb grip strength in females at 2000 and 4000 ppm and hindlimb grip strength in females at 4000 ppm were observed (Table 21). These changes were considered to be indications of general toxicity rather than specific signs of neurotoxicity, because of the lack of any other neurological findings, including changes in motor activity or other functional observational battery parameters or histopathological findings in the nervous system.

In haematology, prothrombin time was significantly increased in both sexes at 4000 ppm, but no differences were detected in activated partial prothrombin time at any dose level; however, a lower number of samples per group were available for this parameter. A slightly lower (5%) statistically significant red blood cell count for male rats at 4000 ppm of 8.67×10^{12}/l was actually in the middle of the reported historical control range ($8.28–9.10 \times 10^{12}$/l), whereas the concurrent 0 ppm value of 9.15×10^{12}/l was at the upper end of this range. Consequently, the finding was interpreted as having no toxicological significance. At 4000 ppm, plasma gamma-glutamyl transferase was increased in both sexes, triglyceride and total protein concentrations were increased in males and plasma

cholesterol concentrations were increased in females. Haematological and blood biochemical changes are summarized in Table 22.

Table 21. Body weights and grip strength in functional observational battery in rats treated orally with sedaxane for 90 days (second study)

Parameter	Males				Females			
	0 ppm	300 ppm	2000 ppm	4000 ppm	0 ppm	300 ppm	2000 ppm	4000 ppm
Forelimb grip strength (g)	868	838	668*	697	810	708	632*	562**
Hindlimb grip strength (g)	604	595	575	551	572	595	507	459*
Body weight (g)								
- day 0	167	167	166	165	121	121	121	123
- day 7	217	217	212	197**	145	146	141	139
- day 28	289	284	274	263*	188	189	180	178*
- day 49	356	356	338	316*	217	217	200	198*
- day 70	392	397	382	351	237	235	215*	211*
- day 91	420	421	406	375	247	244	222*	218*

From Shearer & Foster (2009)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

Table 22. Summary of haematology and blood biochemistry in rats treated orally with sedaxane for 90 days (second study)

Parameter	Males				Females			
	0 ppm	300 ppm	2000 ppm	4000 ppm	0 ppm	300 ppm	2000 ppm	4000 ppm
Haematology								
Prothombin time (s)	14.8	14.7	16.1	16.8*	15.2	15.2	15.4	16.5*
Red blood cell count ($\times 10^{12}$/l)	9.15	8.92	8.75	8.67*	8.32	8.03	8.01	8.20
Blood biochemistry								
Aspartate aminotransferase (IU/l)	83	92	67	67	77	71	69	63*
Gamma-glutamyl transferase (IU/l)	3	3	3	4*	3	3	3	5**
Cholesterol (mmol/l)	2.2	2.0	1.8	2.0	1.6	1.7	1.8	2.4**
Triglycerides (mmollL)	2.00	2.04	2.27	3.18**	1.58	1.26	1.98	2.12
Total protein (g/l)	70	69	71	73**	74	70	73	75

From Shearer & Foster (2009)
IU, international units; * $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

No treatment-related effects were observed at necropsy. In both male and female rats, increases were observed in absolute liver weights and liver weights adjusted for body weight at 4000 ppm and in adjusted liver weights at 2000 ppm. The liver weight changes in both male and female rats at 4000 ppm were accompanied by mild to moderate centrilobular hepatocyte hypertrophy and minimal to mild hepatocyte pigmentation. In addition, minimal to moderate diffuse thyroid follicular cell hypertrophy was observed in five males and one female at 4000 ppm (Table 23).

Table 23. Liver weights and selected histopathology in rats treated orally with sedaxane for 90 days (second study)

	Males				Females			
	0 ppm	300 ppm	2000 ppm	4000 ppm	0 ppm	300 ppm	2000 ppm	4000 ppm
Liver weight (g)	15.98	16.50	17.72	19.64**	9.18	9.03	10.17	11.85**
Liver weight, adjusted (g)[a]	15.38	15.71	17.73**	20.94**	8.46	8.51	10.60**	12.65**
Histopathology								
Liver								
Centrilobular hypertrophy (mild to moderate)	0/10	0/10	0/10	10/10***	0/10	0/10	0/10	10/10***
Hepatocyte pigment (minimal to mild)	0/10	1/10	0/10	8/10***	0/10	0/10	1/10	7/10**
Thyroid								
Follicular cell hypertrophy (minimal to moderate)	0/10	0/10	0/10	5/10**	0/10	0/10	0/10	1/10

From Shearer & Foster (2009)
* $P < 0.05$ (Fisher's exact probability test); ** $P < 0.01$ (organ weights, Dunnett's test, two-sided; histopathological findings, Fisher's exact probability test); *** $P < 0.001$ (Fisher's exact probability test)
[a] Organ weight was adjusted for terminal body weight.

The NOAEL in the second 90-day dietary study in rats was 300 ppm (equal to 28.3 mg/kg bw per day), based on decreases in forelimb grip strength in both sexes and body weight gains in females at 2000 ppm (equal to 168.0 mg/kg bw per day), supported by decreases in hindlimb grip strength, reduced body weight gain, liver toxicity and thyroid follicular cell hypertrophy at 4000 ppm (equal to 325.1 mg/kg bw per day) (Shearer & Foster, 2009).

Dogs

A small study was conducted as a guide to doses to be used in larger, 3- and 12-month studies with Beagle dogs. Groups of one male and one female Beagle dog were dosed orally (by capsule) with sedaxane (lot no. S01F002249U; purity 98.2%; ratio of isomers 83.4% *trans* to 14.8% *cis*) at 0, 50, 100 or 300 mg/kg bw per day for 4 weeks.

All dogs survived the scheduled treatment period. There were no treatment-related clinical signs at any dose level. There were no effects on ophthalmic examinations or other changes at veterinary examination and no effects on urine analysis parameters. Body weight and feed consumption reductions were recorded for the female dosed at 300 mg/kg bw per day during the first 2 weeks. Subsequently, the feed consumption improved, but body weight gain remained lower than the control value. An increase in liver weight associated with slight hepatocellular hypertrophy and vacuolation was observed in the male at 300 mg/kg bw per day. The female dog at 300 mg/kg bw per day had slight hepatocyte vacuolation. The female at 100 mg/kg bw per day also had minimal hepatocyte vacuolation. Although the number of dogs used was very small, there was a suggestion that hepatocellular vacuolation might be a treatment-related effect, as it was observed in both sexes at 300 mg/kg bw per day. Hepatocellular hypertrophy observed in one dog was not considered to be adverse, because there was no indication of hepatotoxicity (Jackson, 2008a).

Groups of four male and four female 5- to 6-month-old Beagle dogs were dosed orally by capsule with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*)

at 0, 50, 150 or 400 mg/kg bw per day for a period of 13 weeks. Clinical signs, body weight and feed consumption were recorded throughout the study. Ophthalmoscopic examination of the eye and detailed physical examination of all dogs were performed and blood and urine samples were collected for laboratory investigations at intervals during the study. Following completion of the scheduled treatment period, a detailed necropsy was performed on all dogs, and a number of selected organs were weighed. A comprehensive list of tissues and organs was prepared and examined microscopically.

All dogs survived the scheduled treatment period. No adverse clinical signs were observed that were considered to be related to treatment with the test compound. Slightly higher incidences of vomiting of feed (males and females) and vomiting of mucous (females only) at 400 mg/kg bw per day were considered not to be of toxicological significance. Cumulative body weight gains were lower in both sexes at 400 mg/kg bw per day throughout the study (Table 24). Cumulative body weight gain in females at 150 mg/kg bw per day was lower than the control values from day 71 to the end of the study. The body weights were higher than control values at the start of the study in males at 50 and 150 mg/kg bw per day, but were not different at termination. After week 1, feed consumption was consistently lower than the control group value in females at 400 mg/kg bw per day (Table 24).

Table 24. Cumulative body weight gain and feed consumption in dogs treated orally with encapsulated sedaxane for 90 days

	Males				Females			
	0 mg/kg bw per day	50 mg/kg bw per day	150 mg/kg bw per day	400 mg/kg bw per day	0 mg/kg bw per day	50 mg/kg bw per day	150 mg/kg bw per day	400 mg/kg bw per day
Cumulative body weight gain (kg)								
- day 8	0.03	0.11	−0.21	−0.62**	0.09	0.06	−0.30**	−0.57**
- day 15	0.37	0.28	0.33	−0.17	0.54	0.74	0.43	−0.17**
- day 22	0.87	0.52	0.62	0.15**	0.93	0.93	0.64	0.22*
- day 64	2.14	1.38	1.51	0.81**	2.07	1.85	1.45	1.01*
- day 71	2.40	1.51	1.66	1.02**	2.20	1.90	1.38*	1.07**
- day 78	2.60	1.70*	1.66*	0.99**	2.38	2.05	1.43*	1.33**
- day 85	2.58	1.66	1.65	1.14**	2.51	1.93	1.35**	1.15**
- day 92/93[a]	2.57	2.02	1.73	1.07**	2.56	1.77	1.22**	1.03**
Feed consumption (g)								
- pretest (day −6 to 1)	269	343*	331*	324	272	210**	247	246
- day 1	7.03	8.07	8.26	7.94	6.00	5.60	5.82	6.05
- day 8	7.06	8.18	8.05	7.32	6.10	5.66	5.53	5.48
- day 15	7.40	8.35	8.59	7.77	6.54	6.34	6.25	5.88
- day 71	9.43	9.58	9.92	8.96	8.20	7.50	7.20	7.12*
- day 92/93[a]	9.60	10.09	9.99	9.01	8.56	7.37*	7.04**	7.08*

From Jackson (2008b)

* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

[a] Day 92 (males), day 93 (females).

In haematological examination at termination, lower total leukocyte, lymphocyte and monocyte counts in females at 150 and 400 mg/kg bw per day and lower total leukocyte count in males at 400 mg/kg bw per day were observed, whereas no differences were observed at other time points. The lower leukocyte counts were not considered to be treatment related because they were relatively small and there was an absence of corresponding histopathological changes in bone marrow or lymph nodes.

The treatment-related difference in blood biochemistry was lower cholesterol in males at 400 mg/kg bw per day from week 4 to week 13 (Table 25). Lower cholesterol in males at 150 mg/kg bw per day was not considered to be treatment related, because only one male showed a slightly lower cholesterol level at this dose level at week 13.

Table 25. Cholesterol levels in male dogs treated orally with encapsulated sedaxane for 90 days

Week	Plasma cholesterol level (mmol/l)			
	0 mg/kg bw per day	50 mg/kg bw per day	150 mg/kg bw per day	400 mg/kg bw per day
Pretest	3.64	2.74**	3.26	3.27
1	2.73	2.11	2.57	2.83
4	3.05	2.31*	2.62	2.41*
8	3.49	2.53**	3.03	2.72*
13	3.43	2.34**	2.93*	2.53**

From Jackson (2008b)

* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

There were no differences in ophthalmoscopy, veterinary examinations, organ weights or macroscopic findings between the control and treated groups. Microscopically, thyroid follicular cell hypertrophy (0, 1, 2, 1 in males; 0, 0, 0, 2 in females; at 0, 50, 150 and 400 mg/kg bw per day, respectively) was observed in some male dogs in all treated groups and in females at 400 mg/kg bw per day. In the absence of a dose–response relationship and considering the minimal severity, thyroid follicular cell hypertrophy is considered not to be treatment related.

The NOAEL in the 90-day oral (capsule) study in dogs was 50 mg/kg bw per day, based on lower cumulative body weight gain in females at 150 mg/kg bw per day (Jackson, 2008b).

Sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) was administered to four Beagle dogs of each sex per group orally, by capsule, at a dose level of 0, 15, 50 or 200 mg/kg bw per day for 52 weeks. Clinical signs, body weight and feed consumption were recorded throughout the study. Ophthalmoscopy and veterinary examinations were performed and blood and urine samples were collected for clinical laboratory investigations at intervals during the study. After the completion of treatment, all dogs were examined macroscopically and microscopically.

All dogs survived the treatment period. No treatment-related clinical signs were observed. Decreased feed consumption with a corresponding loss of body weight or low body weight gain was observed throughout the treatment period in the animals dosed at 200 mg/kg bw per day (Table 26). Feed intake was reduced from the 1st week of the treatment period. Following extension of the daily feeding period, feed intake improved transiently in the males but remained low in the females over the remainder of the treatment period.

Table 26. Body weights, cumulative body weight gains and feed consumption in dogs treated orally with encapsulated sedaxane for 1 year

	Males				Females			
	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day
Body weight (kg)								
- day 1	9.25	8.84	9.18	8.96	7.37	7.14	7.75	7.29
- day 8	9.59	9.26	9.47	8.98	7.56	7.28	7.91	7.00
- day 92	10.83	11.02	11.25	9.59	9.01	8.70	9.63	7.40*
- day 365/366	11.50	12.54	12.71	10.54	10.07	10.16	10.98	8.21
Feed consumption								
- day 8	0.34	0.42	0.28	0.02*	0.19	0.15	0.16	−0.29**
- day 15	0.34	0.42	0.48	0.17	0.38	0.29	0.32	−0.29**
- day 50	1.26	1.62	1.48	0.51**	1.27	1.10	1.33	0.14**
- day 92	1.58	2.18	2.06	0.63*	1.64	1.56	1.88	0.11**
- day 169	1.67	2.55	2.52	1.26	1.82	2.20	2.64	0.60*
- day 239	1.76	3.06	2.96	1.68	2.02	2.26	3.14	0.69

From Braun (2009)

* $P < 0.05$; ** $P < 0.01$ (Dunnett's test)

In haematology, treatment-related changes were not observed. In blood chemistry, lower glucose levels and higher alkaline phosphatase activity were observed in males and females dosed at 200 mg/kg bw per day. Plasma cholesterol and phosphorus concentrations in males of the 200 mg/kg bw per day group were lower than those of controls (Table 27).

Liver weights, absolute and adjusted for terminal body weight, were increased in both sexes at 200 mg/kg bw per day (Table 28). Testes weights were lower than those of controls at 200 mg/kg bw per day. The testes weights in all groups except for one dog were within the range of historical control data of the laboratory (10.9–26.6 kg in 2000–2007, 11 studies), although they were in the lower range. A dog with the lowest weight of testes at 200 mg/kg bw per day showed inflammation with tubular atrophy and spermatogenic giant cells in the testes, and these changes were considered to be incidental. Treatment-related histopathological changes were not observed in other animals.

The NOAEL in the 1-year oral (capsule) study in dogs was 50 mg/kg bw per day, based on decreased feed consumption with corresponding initial body weight loss and lower body weight gain, minor changes in clinical biochemistry and higher liver weights in both sexes seen at 200 mg/kg bw per day (Braun, 2009).

(b) Dermal application

Rats

Groups of 10 male and 10 female HanRcc:WIST (SPF) rats were administered dermal doses of sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0 (control), 100, 300 or 1000 mg/kg bw per day in bi-distilled water under semi-occlusive conditions for 6 hours/day, 5 days/week, over a period of 28 days. An area of skin of approximately 25 cm^2 was exposed. General clinical observations, detailed behavioural observations, body weight measurements and feed consumption measurements were made once weekly during the acclimatization and treatment periods. A functional observational battery, including grip strength and locomotor activity measurements, was conducted during the last week of treatment before the dermal administration for

that day. Eye examinations were performed in all rats during acclimatization and during week 4 in animals of the control and high-dose groups. Haematology and blood chemistry investigations were conducted. At necropsy, selected organs were weighed, and a range of tissues and organs was examined macroscopically and microscopically.

Table 27. Selected blood chemistry parameters in dogs treated orally with encapsulated sedaxane for 1 year

Parameter	Males				Females			
	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day
Glucose (mmol/l)								
- pretest	5.75	6.07	6.11	5.77	6.22	5.95	5.96	5.52*
- week 13	5.49	5.78	5.76	5.01*	5.82	5.82	5.64	4.98
- week 26	5.25	5.70	5.57	4.93	5.58	5.55	5.80	5.03
- week 52	5.71	5.55	5.61	4.90*	5.89	5.89	6.01	5.10*
Alkaline phosphatase (U/l)								
- pretest	189	163	214	161	142	175	134	147
- week 13	112	99	145	155	96	108	86	118
- week 26	78	77	114	145*	72	79	79	114*
- week 52	66	81	112	180*	61	86	63	132**
Cholesterol (mmol/l)								
- pretest	2.82	2.39	2.75	2.43	2.37	2.56	2.30	2.42
- week 13	3.25	2.62	2.99	2.30	3.00	3.12	2.94	2.67
- week 26	3.31	3.02	3.46	2.46*	3.28	3.27	3.13	2.77
- week 52	3.52	3.27	3.63	2.35	3.90	3.93	3.15	3.32
Phosphorus (mmol/l)								
- pretest	2.28	2.27	2.28	2.28	2.03	2.09	2.15	2.24
- week 13	1.86	1.72	1.67	1.58*	1.71	1.61	1.57	1.58
- week 26	1.43	1.32	1.24	1.22*	1.15	1.03	1.13	1.25
- week 52	1.28	1.12	1.13	1.10	1.06	0.94	0.97	1.04

From Braun (2009).
U, unit; * $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

All rats survived the treatment. No sedaxane-related effects were recorded at the treated skin sites. General clinical observations, detailed behavioural observations, eye examination and functional observational battery investigations, including grip strength and locomotor activity measurements, revealed no effects related to sedaxane treatment. No treatment-related changes were observed in feed consumption, body weight, haematology, clinical biochemistry, urine analysis, organ weights or macroscopic and microscopic findings.

The NOAEL in the 28-day dermal toxicity study in rats was 1000 mg/kg bw per day, based on an absence of treatment-related changes at 1000 mg/kg bw per day, the highest dose tested (Sommer, 2009a).

Table 28. Organ weights in dogs treated orally with encapsulated sedaxane for 1 year

	Organ weight (g)							
	Males				Females			
	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day
Liver weight	356	366	384	416*	325	365	343	338
Liver weight adjusted for body weight	357	362	379	423*	318	356	311	384
Testes weight	16.9	14.4	15.3	13.2*	—	—	—	—
Testes weight adjusted for body weight	16.9	14.4	15.2	13.3*	—	—	—	—

From Braun (2009)
* $P < 0.05$ (Dunnett's test)

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female CD-1 mice were fed diets containing sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 200, 1250 or 7000 ppm (equal to 0, 25, 157 and 900 mg/kg bw per day for males and 0, 29, 185 and 1001 mg/kg bw per day for females, respectively) for 80 weeks. The mice were monitored regularly for viability and for signs of ill-health or reaction to treatment. Body weights and feed consumption were measured and recorded at predetermined intervals from pretrial until the completion of treatment. Blood samples for haematology were collected from all surviving mice prior to terminal kill at week 80. Blood films were made from all surviving mice during week 52/53 and at week 80. All surviving mice were necropsied after the completion of treatment. Tissues from all mice were subjected to a comprehensive histological evaluation.

Homogeneity, stability and achieved concentration of sedaxane diets were acceptable. Body weights and body weight gains in both sexes at 7000 ppm were slightly lower than those of controls (Table 29). The maximum difference from control for body weight was 7% in males and 9% in females. Whereas feed consumptions in treated groups were comparable to the control group value, lower values of food utilization during two of the three measurement intervals at 7000 ppm were considered to be treatment related.

There were no treatment-related effects on haematological parameters in male or female mice. Liver weight was increased in males at 7000 ppm (+16%), but this change was considered to be an adaptive response due to the absence of any indication of hepatotoxicity on microscopic examination (Table 30). There were no treatment-related macroscopic or microscopic findings in any treated groups. A lower incidence of lymphomas in females was not considered to be toxicologically significant.

In male mice at 7000 ppm, the incidences of hepatocellular adenoma and adenomas and carcinomas combined were slightly, but statistically significantly, higher than those of the control group (Table 31). All other neoplastic alterations in the treated groups were comparable to controls in the laboratory.

The NOAEL in the 18-month dietary study in mice was 1250 ppm (equal to 157 mg/kg bw per day), based on reduced body weight and body weight gain in both sexes seen at 7000 ppm (equal to 900 mg/kg bw per day). A slightly increased incidence of hepatocellular adenomas and carcinomas combined was observed in male mice at the high dose in comparison with the control group incidence.

The NOAEL for equivocal carcinogenicity in mice was 1250 ppm (equal to 157 mg/kg bw per day) (Perry, 2010b).

Table 29. Summary of body weight changes in mice orally treated with sedaxane for 80 weeks

	Body weight / body weight change (g)							
	Males				Females			
	0 ppm	200 ppm	1250 ppm	7000 ppm	0 ppm	200 ppm	1250 ppm	7000 ppm
Body weight								
- week 0	34.0	34.2	33.0	33.3	24.0	23.8	24.2	23.9
- week 1	36.3	36.0	35.0*	34.9*	25.2	24.8	25.3	25.1
- week 3	38.5	38.6	37.7	37.5	27.2	26.7	27.1	26.3*
- week 13	48.0	47.3	46.8	45.6	32.8	31.8	32.6	31.1
- week 26	55.1	53.9	52.8	52.4	40.6	38.3	39.4	36.9*
- week 52	59.8	57.6	56.7	57.9	46.1	44.3	45.0	42.7
- week 80	61.3	59.6	58.7	57.6	49.4	48.2	49.2	45.6
Cumulative body weight change								
- weeks 0–1	2.2	1.8**	2.0	1.6**	1.2	1.1	1.2	1.2
- weeks 0–3	4.4	4.3	4.7	4.2	3.3	2.9	2.9	2.4**
- weeks 0–13	14.0	13.0	13.7	12.3	8.9	8.1	8.4	7.2
- weeks 0–80	27.5	25.7	25.8	24.7	25.5	24.3	25.1	21.7

From Perry (2010b)

* $P < 0.05$; ** $P < 0.01$ (Dunnett's test)

Table 30. Absolute and adjusted liver and adrenal weights in mice orally treated with sedaxane for 80 weeks

	Organ weight (g)							
	Males				Females			
	0 ppm	200 ppm	1250 ppm	7000 ppm	0 ppm	200 ppm	1250 ppm	7000 ppm
Liver, absolute	3.20	3.13	3.09	3.44	2.15	1.98	2.02	2.11
Liver, adjusted	3.04	3.13	3.15	3.53*	2.13	1.97	1.99	2.17
Adrenal, absolute	0.0057	0.0058	0.0051	0.0061	0.0084	0.0095	0.0101*	0.0100
Adrenal, adjusted	0.0056	0.0057	0.0052	0.0061	0.0084	0.0095	0.0101*	0.0100

From Perry (2010b)

* $P \leq 0.05$ (Dunnett's test)

Rats

Four groups of 52 male and 52 female Han Wistar rats were assigned to the carcinogenicity study and administered diets containing sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 200, 1200 or 3600 ppm (equal to 0, 11, 67 and 218 mg/kg bw per day for males and 0, 14, 86 and 261 mg/kg bw per day for females, respectively) for 104 weeks. A 52-week toxicity study comprising an additional four groups of 12 male and 12 female rats was combined with the 104-week component to examine clinical observations, body weight, feed consumption, haematology, coagulation potential, clinical chemistry, organ weights, and gross and microscopic lesions. Additionally, carcinogenicity study rats underwent eye examinations and had urine samples taken from them for analysis. The 52-week toxicity study rats underwent a detailed

Table 31. Incidence of hepatocellular adenoma and carcinoma in male mice orally treated with sedaxane for 80 weeks

	Incidence of finding[a]				Historical control incidence	
	0 ppm	200 ppm	1250 ppm	7000 ppm	Laboratory (range)[b]	RITA (range)[c]
Adenoma						
Intercurrent	1/48	2/45	1/45	3/48	Four studies including concurrent study. 14/50, 5/50, 11/50, 30/150 (range, 10–28%)	0.0–13.6%
Terminal kill	6/48	7/45	9/45	12/48		
Total (%)	7/48 (15%)	9/45 (20%)	10/45 (22%)	15/48* (31%)		
	0 ppm	*Low*	*Mid*	*High*		
From concurrent study (%)	14/50 (28%)	17/50 (34%)	17/50 (34%)	13/50 (26%)		
Carcinoma						
Intercurrent	1/48	0/45	0/45	4/48	Four studies including concurrent study. 3/50, 5/50, 3/50, 11/150 (range, 6–10%)	4.0–22.0%
Terminal kill	4/48	5/45	3/45	6/48		
Total (%)	5/48 (10%)	5/45 (11%)	3/45 (7%)	10/48 (21%)		
	0 ppm	*Low*	*Mid*	*High*		
From concurrent study (%)	3/50 (6%)	10/50 (20%)	3/50 (6%)	4/50 (8%)		
Adenoma and carcinoma combined[d]						
Combined adenoma and carcinoma (%)	9/48 (19%)	13/45 (29%)	12/45 (27%)	19*/48 (40%)		

From Perry (2010b)

RITA, Registry of Industrial Toxicology Animal-data; * $P < 0.05$ (Fisher's exact test)

[a] Number of tumour-bearing animals/number of animals examined, excluding those that died before week 49.

[b] Laboratory historical control data = four studies, all started in 2007.

[c] RITA historical control data are shown for studies of 18–19 months' duration.

[d] Number of animals bearing both an adenoma and a carcinoma were 3, 1, 1 and 6 at 0, 200, 1250 and 7000 ppm, respectively.

functional observational battery assessment at week 51/52. Grip strength was measured according to the method derived from Meyer et al. (1979). Pain was assessed by measurement of the tail flick response, using a technique based on the method devised by D'Amour & Smith (1941). All surviving carcinogenicity and toxicity study rats were killed at 104 or 52 weeks of treatment, respectively.

Dietary concentrations were measured according to the methodology developed by Currie (2007). Homogeneity, stability and achieved concentrations of sedaxane in the rat diet were acceptable. There were no treatment-related differences in mortality or clinical signs between the control and treated groups in both sexes. At 52 weeks, no treatment-related effects were observed in detailed clinical observations, motor activity or any other functional observational battery parameters. There were no ophthalmoscopic findings associated with treatment. There was clearly a treatment-related effect on body weight in both sexes at 3600 ppm throughout the study (Table 32). At termination, the body weight gains at 3600 ppm were depressed by 24% and 50% in males and females, respectively.

Table 32. Body weights and cumulative body weight changes in rats treated orally with sedaxane for 2 years

Parameter	Body weight / body weight change (g)							
	Males				Females			
	0 ppm	200 ppm	1200 ppm	3600 ppm	0 ppm	200 ppm	1200 ppm	3600 ppm
Body weight								
- week 0	148.5	150.6	145.6	149.3	129.4	129.9	129.9	132.5
- week 1	197.6	201.3	191.0	182.7**	152.7	152.5	150.4	144.9**
- week 3	260.6	267.5	254.8	232.4**	183.4	185.0	180.5	167.9**
- week 13	381.0	398.6*	373.7	336.6**	235.5	238.0	232.2	205.4**
- week 26	438.5	453.7	425.4	379.2**	259.0	259.0	249.0**	219.4**
- week 30	450.7	467.7	438.9	394.1**	261.1	262.8	254.8	224.2**
- week 32	460.3	473.8	440.4	383.7**	264.8	266.5	257.4	209.3**
- week 34	467.0	478.1	448.2	404.0**	268.3	269.4	258.6*	228.1**
- week 52	518.8	537.9	506.5	450.0**	292.9	295.5	281.1	240.2**
- week 104	613.5	657.2	590.5	503.6**	392.5	389.9	362.2*	264.1**
Body weight change								
- weeks 0–1	49.1	50.7	45.4**	33.3**	23.2	22.6	20.5**	12.4**
- weeks 0–3	112.1	116.8	109.2	83.0**	54.0	55.1	50.6	35.4**
- weeks 0–13	232.5	247.9*	228.0	187.3**	106.1	108.1	102.2	72.9**
- weeks 0–52	370.3	387.3	360.9	300.7**	163.5	165.6	151.1**	108.1**
- weeks 0–104	464.9	509.7*	447.9	355.7**	262.6	259.2	232.7*	132.4**

From Perry (2010a)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

Female rats treated at 3600 ppm showed statistically significantly lower feed consumption compared with their respective controls (about 11–17%) throughout the treatment period, whereas males of the same treatment group had statistically significantly lower feed consumption only during weeks 1–7 (< 10%). Both males and females of the 3600 ppm sedaxane group had statistically significantly reduced feed utilization during weeks 1–4 and 9–13. The feed consumption and feed utilization profiles of males and females receiving 200 or 1200 ppm closely resembled those of their respective controls.

Prothrombin time was higher in males of the 3600 ppm group at multiple measurement times, but no other haematological differences between control and treatment groups were consistently observed. Blood chemistry analysis (Table 33) showed that plasma protein, albumin and globulin levels were increased in males and females at 3600 ppm. Slightly higher plasma gamma-glutamyl transferase values were observed in males at 3600 ppm at 104 weeks. Glucose and phosphate levels were high in males at 3600 ppm at several time points, and increases in plasma glucose were observed at 1200 ppm at two time points. Cholesterol was higher in females at 3600 ppm at weeks 14 and 27, but not later. Considering the small magnitude of these changes and lack of consistency, these changes are not considered to be treatment related except the increases in cholesterol in females at 3600 ppm. There were no treatment-related macroscopic findings at either 52 or 104 weeks.

Liver weights (adjusted for terminal body weight) were higher in both sexes in the 1200 ppm and 3600 ppm dose groups at both 52 and 104 weeks (Table 34).

Table 33. Summary of blood chemistry in rats orally treated with sedaxane for 2 years

Parameter	Males				Females			
	0 ppm	200 ppm	1200 ppm	3600 ppm	0 ppm	200 ppm	1200 ppm	3600 ppm
GGT (IU/l)								
- week 14	3	3	3	3**	3	3	3	4**
- week 27	3	3	3	4*	3	3	3	3
- week 52	3	3	3	3	3	3	3	3
- week 53	3	3	3	3*	3	3	3	3
- week 79	3	3	3	4**	3	5	3	3
- week 104	3	3	3	6**	3	3	3	3
Total protein (g/l)								
- week 14	67	69	69	71**	72	74	72	74
- week 27	68	70	71*	71**	73	73	72	73
- week 52	73	73	75	78**	78	77	80	79
- week 53	72	74	76*	77**	77	78	79	81*
- week 79	73	72	76	76	74	75	78	79*
- week 104	73	74	75*	76**	75	75	77	81**
Globulin (g/l)								
- week 14	24	26	25	27**	22	23	23	26**
- week 27	25	26	27	27	22	22	23	25**
- week 52	30	30	32*	32*	26	25	27	28
- week 53	29	30	31	32*	25	26	27	28**
- week 79	30	31	33	33	26	28	27	28
- week 104	31	34**	33**	33*	27	27	27	29
Cholesterol (mmol/l)								
- week 14	1.7	1.8	1.6	1.5	1.6	1.5	1.5	2.2**
- week 27	1.8	1.9	1.7	1.5*	1.8	1.5	1.6	2.1*
- week 52	2.3	2.3	2.2	1.9	2.1	2.1	2.1	2.6
- week 53	2.1	2.2	2.0	1.9	2.2	1.9	1.9	2.4
- week 79	2.8	2.6	2.7	2.2	2.2	2.3	2.2	2.4
- week 104	3.5	3.7	3.4	3.1	2.8	2.9	2.4*	2.6
Phosphate (mmol/l)								
- week 14	1.80	1.71	2.00*	2.00*	1.50	1.48	1.68	1.92**
- week 27	1.48	1.51	1.61	1.69	1.36	1.30	1.48	1.56
- week 52	1.36	1.32	1.42	1.43	1.21	1.17	1.28	1.21
- week 53	1.31	1.34	1.42	1.45	1.10	1.11	1.26	1.23
- week 79	1.24	1.46*	1.43*	1.49**	1.17	1.37	1.45*	1.40
- week 104	1.06	1.12	1.20**	1.30**	1.06	1.11	1.16	1.16
Glucose (mmol/l)								
- week 14	6.87	7.14	7.18	7.26	7.09	7.30	7.55	7.28
- week 27	6.98	7.43	7.75*	7.71	7.10	7.57	7.96*	7.13
- week 52	7.47	8.16	8.09	7.35	7.98	7.25*	7.12*	7.04**
- week 53	7.43	7.66	8.27	8.79**	7.64	7.67	8.15	7.27
- week 79	7.58	7.93	8.34*	8.32*	7.62	7.43	7.47	7.22
- week 104	6.60	6.99	7.25**	7.63**	5.92	6.62**	7.26**	6.87**

From Perry (2010a)

* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

Table 34. Liver weights in rats orally treated with sedaxane for 2 years

	Liver weight (g)							
	Males				Females			
	0 ppm	200 ppm	1200 ppm	3600 ppm	0 ppm	200 ppm	1200 ppm	3600 ppm
52 weeks								
- absolute	16.30	17.09	19.29*	21.01**	9.21	9.33	9.89	10.39
- adjusted[a]	16.10	16.03	18.94**	22.63**	8.86	9.18	9.50	11.39**
104 weeks								
- absolute	18.57	19.40	20.21*	22.52**	12.72	13.24	13.07	12.17
- adjusted[a]	18.10	18.06	20.21**	24.20**	11.56	12.05	12.65**	14.64**

From Perry (2010a)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)
[a] Adjusted to terminal body weight.

The liver weight changes were accompanied at 52 weeks by centrilobular hepatocellular hypertrophy and pigmentation in males and females of the 3600 ppm dose group (Table 35). Treatment-related microscopic findings at 104 weeks included centrilobular hepatocyte hypertrophy in males at 1200 ppm and in both sexes at 3600 ppm. The incidence of hepatocellular pigmentation was increased in females at 3600 ppm. Eosinophilic cell foci were increased in males at 3600 ppm and in females in all treated groups. The increases in females in all treated groups were within the range of historical control data (7–32%) and considered to be attributed to low incidences in the control group, which were below the range of historical control data.

At 52 weeks, low incidences of thyroid follicular cell hypertrophy were observed in males at 1200 and 3600 ppm, without dose dependency (Table 35). At 104 weeks, an increase in focal follicular cell hyperplasia in the thyroid was noted in males at 3600 ppm. At 104 weeks, the incidences of colloid basophilia and desquamation of the follicular epithelium in the thyroid were increased in females at 1200 and 3600 ppm. Colloid basophilia was increased in males of the 3600 ppm dose group (Table 35).

Uterine adenocarcinoma was slightly, but significantly, increased at 3600 ppm (Table 36). The incidence of endometrial hyperplasia, a precancerous lesion, was not increased, and any changes indicating a higher ratio of estrogen to progesterone were not found in the ovary, uterus or vagina in short-term, long-term and reproductive toxicity studies of sedaxane. The incidence of endometrial adenocarcinoma in the control group was the lowest value of historical control data in the laboratory, and the incidence at 3600 ppm was close to the upper limit. The increase in uterine adenocarcinoma, however, was considered to be treatment related, because information about exclusion of the increase from the treatment was inadequate.

Decreased incidences of several neoplastic or non-neoplastic lesions, including mammary fibroadenoma, mammary lobular hyperplasia and chronic progressive nephropathy, in females at 3600 ppm were considered to be secondary effects corresponding to body weight depression at this dose level.

The NOAEL in the 104-week dietary study in rats was 200 ppm (equal to 11 mg/kg bw per day), based on increases in liver weight and histopathological changes in the liver (centrilobular hypertrophy) in males and in the thyroid in males and females and reduced body weight gain in females at 1200 ppm (equal to 67 mg/kg bw per day). The NOAEL for carcinogenicity in rats was 1200 ppm (equal to 86 mg/kg bw per day), based on uterine tumours in female rats (Perry, 2010a).

Table 35. Summary of histopathological changes in rats orally treated with sedaxane for 2 years

Non-tumour findings	Incidence of finding							
	Males				Females			
	0 ppm	200 ppm	1200 ppm	3600 ppm	0 ppm	200 ppm	1200 ppm	3600 ppm
52 weeks								
Liver								
Number examined	12	12	12	12	12	12	12	12
Basophilic cell focus, tigroid	1	1	0	0	5	1	0*	0*
Clear cell focus	11	6	9	4**	1	0	1	1
Hepatocyte hypertrophy, centrilobular	0	0	0	11***	0	0	0	12***
Hepatocyte pigment	0	0	0	7**	1	1	1	7*
Thyroid gland								
Number examined	12	12	12	12	12	12	12	12
Follicular cell hypertrophy	0	0	5*, +	4+	0	0	2	3
104 weeks								
Liver								
Number examined	52	52	52	52	52	52	52	52
Eosinophilic cell focus	8 (15.4%)	7 (13.5%)	15 (28.8%	25*** (48.1%)	2 (3.8%)	10* (19.2%)	12** (23.1%)	14** (26.9%)
Historical range at laboratory	16–86% (2002–2005, 5 studies)				7–32% (2002–2005. 5 studies)			
Basophilic cell focus, homogenous	2	0	1	0	1	0	2	1
Basophilic cell focus, tigroid	4	7	6	5	36	35	42	31
Clear cell focus	33	39	37	19*	12	16	14	12
Hepatocyte hypertrophy, centrilobular	0	0	8**	16***	0	0	1	38***
Hepatocyte pigment	0	1	0	1	2	3	1	15***
Thyroid gland								
Number examined	52	52	52	52	52	52	52	52
Desquamation, epithelial follicular	7	8	11	16	2	5	9*	14**
Basophilia, colloid	7	9	12	16+	3	6	11*	17***
Diffuse C-cell hyperplasia	27	27	24	10***	29	31	27	5***
Focal follicular cell hyperplasia	7	8	8	16+	0	4+	0	4+
Thymus								
Number examined	52	50	48	49	50	52	50	51
Hyperplasia, epithelial tubular	3	1	5	5	18	17	18	31*
Vagina								
Number examined	—	—	—	—	51	52	52	52
Mucification	—	—	—	—	15	22	16	3**
Mammary gland								
Number examined	43	43	45	41	52	50	51	52
Lobular hyperplasia	7	1	1*	4	34	34	32	21*

From Perry (2010a)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (pairwise Fisher's exact test); + $P < 0.05$ (Mann-Whitney U-test). Other Mann-Whitney results for the indicated statistical results were similar to Fisher's test results.

Table 36. Incidence of and historical control data on uterine adenocarcinoma and adenoma in rats orally treated with sedaxane for 2 years

	Incidence of finding				Historical control data[a]	
	0 ppm	200 ppm	1200 ppm	3600 ppm	Laboratory	RITA
No. of uteri examined	52	52	52	52	2002–2005 (5 studies)	
Adenocarcinoma	0^	3 (4.7%)	2 (3.1%)	9** (17.3%)	0/50, 9/100, 10/99, 7/50, 21/110 (0–19%)	0–28%
Adenoma	0	0	1	0		

From Perry (2010a)
RITA, Registry of Industrial Toxicology Animal-data; ** $P < 0.01$ (pairwise Fisher's exact test); ^ $P < 0.05$ (positive trend by Peto trend test, groups 1–4; *P*-value for linear trend including groups 1–4 = 0.002; *P*-value for linear trend including groups 1–3 = 0.22)
[a] Laboratory historical control data = five studies, all started between 2002 and 2007; RITA historical control data are shown for studies of 22–25 months' duration.

2.4 Genotoxicity

Sedaxane was tested for genotoxicity in a range of assays, both in vitro and in vivo (Table 37). There was no evidence of mutagenic activity in a bacterial reverse mutation assay performed over a dose range up to 5000 µg/plate. In cultured mammalian cell assays, there was no evidence for clastogenicity or aneuploidy in primary cultures of human peripheral blood lymphocytes or of mutagenicity in mouse lymphoma L5178Y cells ($tk^{+/-}$ locus) over dose ranges that included moderately toxic concentrations. An in vivo study for the induction of micronuclei in polychromatic erythrocytes in the bone marrow of male NMRI mice showed no evidence of an effect of sedaxane at either 24 or 48 hours following a single oral dose of up to 2000 mg/kg bw. In a second study in vivo, there was no induction of unscheduled deoxyribonucleic acid (DNA) synthesis in hepatocytes isolated from male Sprague-Dawley rats treated orally with sedaxane at dose levels up to 2000 mg/kg bw and killed 2 hours later. Thus, sedaxane showed no evidence of genotoxicity or mutagenicity in any of these guideline studies, whereas significant responses to positive control treatment were observed in all studies.

Table 37. Summary of genotoxicity studies on sedaxane[a]

	Test system	Dose levels	Result	References
In vitro				
Bacterial reverse mutation (Ames test)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2(pKM101), WP2*uvrA*(pKM101)	3–5000 µg/plate	Negative (±S9)	Sokolowski (2009)
Test for clastogenicity in mammalian cells	Human peripheral blood lymphocytes; 4 or 22 h exposure	23.1–216.8 µg/ml	Negative (±S9)	Bohnenberger (2009)
Mammalian cell gene mutation	Mouse lymphoma L5178Y cells, $tk^{+/-}$ locus forward mutation	6.9–110 µg/ml	Negative (±S9)	Wollny (2009)
In vivo				
Mouse bone marrow micronucleus test	NMRI mouse bone marrow from 6 male mice per dose, obtained 24 or 48 h after exposure	500–2000 mg/kg bw	Negative	Reichenbach (2010)
Unscheduled DNA synthesis	Rat hepatocytes; 2 or 16 h exposure	667–2000 mg/kg bw	Negative	Durward (2009)

S9, 9000 × *g* supernatant fraction of rat liver homogenate
[a] Lot no. SMU6LP006 95.3% (ratio of isomers 83.0% *trans* to 12.3% *cis*) used in all assays.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

A dose range–finding reproductive toxicity study in rats was performed. Sedaxane (lot no. SMU6LP0006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) was administered to four groups of F_0 generation male and female rats (Crl:WI (Han)) (10 of each sex per group) in the diet at a nominal dose level of 0, 500, 1500 or 3600 ppm for 10 weeks before pairing through to weaning of the F_1 generation. Toxicity of sedaxane was also investigated in the F_1 generation when continuously available in the diet from birth up to day 35 of age. After the F_1 generation pups were weaned (day 28 of age), up to three males and three females were selected from each litter from the 0, 500 and 1500 ppm diet groups and retained until day 35 of age. High-dose (3600 ppm) pups were not retained after day 28 of lactation. The F_1 generation rats received sedaxane in the diet at the same nominal dose level as the F_0 generation from which they were selected. All rats were examined for effects on general condition, body weight and feed consumption. Organ weights were recorded at necropsy for all F_0 generation rats, for one male and one female pup per litter not selected for retention after day 28, and for one male and one female pup per litter selected for rearing on day 35 of age.

Mean body weight gain was statistically significantly lower than the control value for males given 3600 ppm throughout the majority of the study. Body weight gains in F_0 females at 1500 ppm during pre-pairing, gestation and lactation were generally lower than control values, on occasion achieving statistical significance. Body weight gains of males given 500 or 1500 ppm and females given 500 ppm were similar to control values. Mean F_1 body weight, body weight gain and feed consumption values were slightly lower than those of controls in the group given 1500 ppm. Body weights, body weight gains and feed consumption of animals given 500 ppm were generally similar to control values for both males and females.

The NOAEL in this range-finding study in parents and offspring was 500 ppm (equivalent to 50 mg/kg bw per day), based on lower feed consumption throughout treatment, lower body weight gain that attained statistical significance on occasion and markedly higher liver weights in the F_0 and F_1 generations at 1500 ppm (equivalent to 150 mg/kg bw per day). Occasionally lower feed consumption values at 500 ppm were not considered adverse (Richmond, 2009).

A two-generation study of reproductive toxicity in rats was conducted. Four groups of HanRcc:WIST (SPF) rats (P generation) (25 of each sex per group) received sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) in the diet at 0, 200, 500 or 1500 ppm for 10 weeks and were then paired (one male with one female) for mating. The mean compound intakes during the different parts of this study are given in Table 38. The F_1 generation rats were selected from the weaned F_1 litters. The F_1 parents were maintained on test diets for at least 91 days and were then paired for mating. The F_2 offspring were killed at weaning. Dietary sedaxane was administered continuously throughout the study. All dams and remaining pups were killed on day 21 postpartum, and males were killed when they were no longer needed for reproduction.

Homogeneity, stability and achieved concentrations of sedaxane diets were acceptable. All parental animals survived until the scheduled necropsy. Treatment-related findings in clinical observations were not noted at any dose level during the study. In the 1500 ppm group, a reduction in mean feed consumption was noted in the P generation males and in the females in both generations. In F_1 males of the 1500 ppm diet group, body weight and body weight gain were lower throughout the study (Table 39). In females of the 1500 ppm diet group, body weight gain was reduced in the pre-pairing period in both generations and during the lactation period in the P generation, and mean body weight was reduced throughout the study, except on lactation day 14, in both generations.

Table 38. Sedaxane intake of all generations in a multigeneration reproductive toxicity study in rats

	Mean consumption (mg/kg bw per day)		
	200 ppm	500 ppm	1500 ppm
Males P pre-pairing period	16	41	120
Males P after pairing period	11	29	87
Females P pre-pairing period	18	46	143
Females P gestation period	15	40	120
Females P lactation period	36	87	252
Males F_1 pre-pairing period	17	43	134
Males F_1 after pairing period	12	29	89
Females F_1 pre-pairing period	19	47	141
Females F_1 gestation period	16	40	117
Females F_1 lactation period	38	93	282

From Whitlow (2010)

Table 39. Body weights in male rats of P and F_1 generations orally treated with sedaxane

	Body weight (g)							
	P				F_1			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
Males								
No. of animals/group	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Pre-pairing day 1	231	234	231	232	106	105	105	95**
Pre-pairing day 8	267	270	264	267	159	163	162	152
Pre-pairing day 70/71	411	409	405	404	417	414	420	395
Females								
No. of animals/group	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Pre-pairing day 1	161	161	160	158	96	98	95	89
Pre-pairing day 15	198	201	197	190**	157	158	157	152
Pre-pairing day 70/71	261	263	255	243**	243	241	244	229*
Gestation day 0	259	260	254	240**	254	249	251	237**
Gestation day 21	385	381	380	359**	363	360	365	344
Lactation day 1	279	282	277	263*	273	268	275	253**
Lactation day 7	304	304	301	285**	295	295	298	276**
Lactation day 14	287	299	296	291	289	293	289	284
Lactation day 21	313	314	310	292**	301	300	303	284*

From Whitlow (2010)
* $P < 0.05$; ** $P < 0.01$ (Dunnett test based on pooled variance)

There were no differences in oestrous cyclicity, sperm measurement or reproductive performance between the control and treated animals (Table 40).

Table 40. Reproductive performance in two-generation reproductive toxicity study in rats orally treated with sedaxane

	P generation				F_1 generation			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
Number of animals paired	25	25	25	25	25	25	25	25
Mean precoital interval (days)	3.6	3.1	3.2	3.5	4.3	4.0	3.0	5.0
Number of litters	23	25	24	25	23	23	23	25
Mean gestation (days)	21.7	21.6	21.4	21.6	21.7	21.6	21.7	21.6
Percentage mating (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Fertility index (%)	92.0	100.0	96.0	100.0	96.0	92.0	92.0	100.0
Conception rate (%)	92.0	100.0	96.0	100.0	96.0	92.0	92.0	100.0
Gestation index (%)	100.0	100.0	100.0	100.0	95.8	100.0	100.0	100.0

From Whitlow (2010)

Increases in liver weights were observed in males and females of both generations at 1500 ppm (Table 41). In F_1 females, ovary and uterus weights were significantly decreased. Slight but significant changes in organ weights were noted in the adrenal, kidney and thyroid in both sexes in P generation and F_1 females. They were not considered to be treatment related because they were unilateral changes and did not correspond to histopathological changes.

Microscopically, the incidences of centrilobular hepatocellular hypertrophy were increased in males and females of both generations at 1500 ppm (Table 42). The liver hypertrophy corresponding to the increases in liver weights at all treated doses was not considered to be adverse because of the lack of histopathological changes indicating hepatotoxicity. Diffuse follicular cell hypertrophy in the thyroid was slightly increased in F_1 males at 1500 ppm. The increases in vaginal lactation diestrus in F_1 females at 1500 ppm were not considered to be direct effects or hormonal effects of sedaxane on the female reproductive tracts, but rather were considered to reflect a delay in the return to normal cycling due to the lower body weights of the pups that were still suckling at weaning.

In F_1 females at 1500 ppm, ovary and uterus weights were slightly decreased. In morphometric analysis of the ovary at 0 and 1500 ppm, decreases in the numbers of corpora lutea at 1500 ppm in both generations, primordial follicles in the P generation, and growing and antral follicles in the F_1 generation were observed (Table 43). The decrease in primordial follicles in the P generation was not observed in the F_1 generation, which was exposed for a longer time, including the developmental period, the period most sensitive to ovarian toxicants (Hoyer, 2004; Sanbuissho et al., 2009). The decreases in numbers of corpora lutea in the P and F_1 generations might correspond to their litter sizes, which were smaller than those of the controls, but not statistically significantly. Therefore, these decreases in numbers of corpora lutea in the ovary were not considered to be toxicologically significant.

In offspring, there were no effects of sedaxane on litter size, viability or clinical signs. At 1500 ppm, there were no effects on body weights, but there were growth delays 14 days after birth in both sexes of the F_1 and F_2 generations (Table 44).

Although F_1 females at 1500 ppm showed delay in time to vaginal opening, the body weights at sexual maturation were similar to control values, suggesting that the delayed maturation was caused by lower body weights (Table 45). In males in this group, no effect on time until preputial separation was observed. In F_2 pups, anogenital distances in females were slightly, but statistically significantly, increased at 1500 ppm. Although statistically significant, the increase was only slight and was considered to be equivocal. There were no data supporting endocrinological effects on the female reproductive system. Considering the inherent variability in the measurement of anogenital distance

(Tyl, 1999) and the small magnitude of the difference, this was considered not to be toxicologically significant.

Table 41. Selected organ weights in two-generation reproductive toxicity study in rats orally treated with sedaxane

	Mean weight (g)							
	P generation				F_1 generation			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
Males								
No. of animals	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Liver								
- absolute	12.18	12.69	12.93*	14.51**	13.45	13.59	14.23	15.42**
- adjusted	11.995	12.623**	13.086**	14.597**	13.183	13.554	13.962*	15.997**
Females (at the end of lactation)								
No. of animals	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Liver								
- absolute	13.29	13.35	13.62	15.97**	12.56	13.03	12.73	16.29**
- adjusted[a]	12.937	13.003	13.472	16.786**	12.094	12.713	12.616	17.115**
Ovary (right)								
- absolute	0.056	0.055	0.055	0.049	0.057	0.056	0.054	0.043**
- adjusted[a]	0.055	0.054	0.055	0.052	0.055	0.055	0.054	0.045**
Ovary (left)								
- absolute	0.059	0.056	0.056	0.050*	0.055	0.058	0.054	0.047**
- adjusted[a]	0.058	0.056	0.056	0.051	0.055	0.058	0.054	0.048
Uterus								
- absolute	0.83	0.87	0.96	0.74	0.79	0.80	0.78	0.62**
- adjusted[a]	0.838	0.877	0.964	0.735	0.780	0.791	0.781	0.634*

From Whitlow (2010)
* $P < 0.05$; ** $P < 0.01$ (Dunnett test based on pooled variance)
[a] Organ weight is adjusted for terminal body weight.

Liver weights adjusted for body weight were increased in both sexes of the F_1 and F_1 generations at 1500 ppm.

In this multigeneration reproduction study, the NOAEL for parental toxicity was 500 ppm (equal to 41 mg/kg bw per day), based on lower body weights in both sexes at 1500 ppm (equal to 120 mg/kg bw per day) in P generation males. The NOAEL for reproductive toxicity was 1500 ppm (equal to 120 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 500 ppm (equal to 43 mg/kg bw per day), based on lower body weights of F_1 generation males during the pre-pairing period at 1500 ppm (equal to 134 mg/kg bw per day) (Whitlow, 2010).

Table 42. Selected histopathological changes in two-generation reproductive toxicity study in rats orally treated with sedaxane

	Males				Females			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
P generation								
Number of animals	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Hepatocellular hypertrophy	13	6	6	20	0	1	0	10
Thyroid diffuse follicular cell hypertrophy	11	14	11	14	2	0	0	3
Vaginal lactation diestrus	—	—	—	—	7	NE	NE	12
F_1 generation								
Number of animals	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Hepatocellular hypertrophy	10	7	3	22	0	0	0	17
Thyroid diffuse follicular cell hypertrophy	5	7	3	9	1	0	0	1
Vaginal lactation diestrus	—	—	—	—	8	10	10	20

From Whitlow (2010)
NE, not examined

Table 43. Classification of follicles and the number of corpora lutea in the ovary at the end of lactation in P and F_1 female rats orally treated with sedaxane

Generation	Dose (ppm)	Number of follicles			Number of corpora lutea
		Primordial	Growing	Antral	
P	0	212.10	18.70	8.60	16.10
	1500	139.70*	18.80	7.80	12.20*
F_1	0	218.00	24.50	10.50	15.50
	1500	201.50	13.00*	5.00*	11.00*

From Whitlow (2010)
* $P < 0.05$ (Wilcoxon test)

Table 44. Body weights of F_1 and F_2 pups in rats orally treated with sedaxane

	Males				Females			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
F_1 generation								
Number of litters/group	23	25	24	25	23	25	24	25
Mean pup body weight (g)								
- day 1	6.3	6.2	6.1	6.3	6.0	6.0	5.7	6.0
- day 4	8.9	9.1	8.6	9.3	8.6	8.8	8.3	8.9
- day 7	15.0	15.1	14.4	14.3	14.3	14.6	13.9	13.9

Table 44 (continued)

	Males				Females			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
- day 14	30.7	31.3	29.8	28.4**	29.6	30.5	29.4	27.7*
- day 21	50.0	49.9	48.2	45.1**	47.8	48.5	46.9	43.3**
F_2 generation								
Number of litters/group	23	23	23	25	23	23	23	25
Mean pup body weight (g)								
- day 1	6.5	6.5	6.5	6.5	6.2	6.1	6.1	6.1
- day 4	9.7	9.7	9.4	9.5	9.4	9.3	9.0	9.1
- day 7	22.7	22.6	21.8	21.5	22.0	21.9	21.3	21.1
- day 14	31.8	32.0	31.0	29.9*	31.1	31.1	30.2	29.3*
- day 21	51.4	51.6	50.1	46.9**	49.6	49.5	48.0	45.7**

From Whitlow (2010)
* $P < 0.05$; ** $P < 0.01$ (Dunnett test based on pooled variance)

Table 45. Selected sexual developmental landmarks (preputial separation, vaginal opening and anogenital distance) in F_1 and F_2 offspring in rats orally treated with sedaxane

	Males				Females			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
F_1 generation								
Number of animals	25	25	25	25	25	25	25	25
Day of preputial separation/vaginal opening	27.6	27.9	28.0	28.1	32.5	33.3	32.7	34.2**
Weight at preputial separation/vaginal opening (g)	82.98	83.64	81.96	75.58**	99.59	104.52	100.70	102.58
F_2 generation								
Number of litters	23	23	23	25	22	23	23	25
Anogenital distance (mm)	3.63	3.66	3.80	3.73	1.79	1.85	1.88	1.93*

From Whitlow (2010)
* $P < 0.05$; ** $P < 0.01$ (Dunnett test based on pooled variance)

(b) Developmental toxicity

Rats

A dose range–finding developmental toxicity study was performed in which groups of 10 mated female Han Wistar rats were dosed orally by gavage with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 200, 500 or 750 mg/kg bw per day on days 6–20 post-coitum using 0.5% carboxymethylcellulose as the vehicle. Dams were examined for mortality or morbidity twice daily. Clinical signs were assessed and recorded daily. Individual weights were recorded daily. Feed consumption was recorded on days 0–4, 4–6, 6–9, 9–12, 12–15, 15–18 and 18–21 post-coitum. All surviving dams were killed on day 21 post-coitum, and the fetuses were

removed by caesarean section. Examination of dams and external and visceral examinations of all fetuses were performed.

The dams in the groups receiving 500 and 750 mg/kg bw per day were killed before the scheduled end of the study because of their poor condition, consisting of decreased activity, generally poor clinical condition and reduced feed consumption and body weight gains. Feed consumption, body weight gain and corrected body weight gain were statistically significantly reduced at 200 mg/kg bw per day. No sedaxane-related effects were noted in the reproduction or fetal data at this dose level. Based on the results of this study, sedaxane dose levels of up to 200 mg/kg bw per day delivered orally by gavage were considered suitable for a main developmental toxicity study in rats (Whitlow, 2009).

In a developmental toxicity study, groups of 24 mated female Han Wistar rats were dosed orally by gavage with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 25, 100 or 200 mg/kg bw per day on days 6–20 post-coitum using 0.5% carboxymethylcellulose as the vehicle. Dams were examined for mortality or morbidity twice daily. Clinical signs were assessed and recorded daily. Individual body weights were recorded daily. Feed consumption was recorded on days 0–4, 4–6, 6–9, 9–12, 12–15, 15–18 and 18–21 post-coitum. All surviving dams were killed on day 21 post-coitum, and the fetuses were removed by caesarean section. External examinations of all dams and fetuses were conducted. In addition, external examination of all fetuses and visceral or skeletal examination of approximately equal numbers of fetuses from each treatment group were conducted.

At 200 mg/kg bw per day, mean feed consumption and mean body weights were reduced during the treatment period in dams. At 100 mg/kg bw per day, feed consumption and cumulative body weight gain of dams were slightly, but significantly, reduced during the 1st and 2nd weeks of gestation. A slight reduction in fetal weight was noted only in females at 200 mg/kg bw per day (4.5 g) compared with controls (4.7 g). This reduction in fetal body weights in female pups was not considered to be adverse because it represented a difference of only 4.3% compared with controls, this effect was not observed in male pups and there were no differences in the combined (both sexes) fetal body weights. In addition, no effects were observed on fetal development, and there was no delay in the stage of ossification observed. No sedaxane-related effects on fetal survival or the numbers and types of fetal abnormalities and variations were noted in any group.

In this developmental toxicity study in rats, the NOAEL for maternal toxicity was 25 mg/kg bw per day, based on reduction of feed consumption and body weight gain at 100 mg/kg bw per day. The NOAEL for developmental toxicity was 200 mg/kg bw per day, the highest dose tested. Sedaxane was not teratogenic (Cappon et al., 2005; Senn, 2009).

Rabbits

A dose range–finding developmental toxicity study was performed in which groups of 10 time-mated female New Zealand White (Hra:(NZW)SPF) rabbits 6 months of age were dosed orally by gavage with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 100, 300 or 500 mg/kg bw per day on days 7–28 post-coitum using 0.5% carboxymethylcellulose as the vehicle. Mortality, clinical observations, body weights and feed consumption were recorded. On gestation day 29, the uteri, placentae and ovaries of all surviving dams were examined, and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights and liver weights from all females were recorded. The fetuses were weighed, sexed and examined for external and visceral malformations and developmental variations.

Severe toxicity observed in two dams of the 500 mg/kg bw per day group led to their euthanasia on gestation day 16. A dose-related increase in the incidence of decreased defecation was noted among surviving rabbits in all treated groups. Body weight losses were noted at 300 and 500 mg/kg bw per day immediately following administration of sedaxane on gestation days 7–10, and

lower body weight gains continued at 500 mg/kg bw per day during gestation days 10–13. Reductions in feed consumption at 300 and 500 mg/kg bw per day were continued during gestation days 13–21, but without corresponding effects on body weight gains and when the overall treatment period was evaluated. Body weights were depressed throughout the treatment period at 300 and 500 mg/kg bw per day by up to 8.9%. No treatment-related changes were observed at necropsy. Dose-related higher absolute and body weight–relative liver weights were noted at all dose levels, the increases being statistically significant at 300 and 500 mg/kg bw per day. There was no evidence of developmental toxicity at any dose level. No external or visceral fetal malformations or developmental variations were attributable to sedaxane administration to the dams.

On the basis of these results, dose levels of 25, 100 and 200 mg/kg bw per day were selected for a definitive prenatal developmental toxicity study of sedaxane in New Zealand White rabbits (Sawhney Coder, 2010a).

In the definitive developmental toxicity study, groups of 25 time-mated female New Zealand White (Hra:(NZW)SPF) rabbits approximately 6 months of age were dosed orally by gavage with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at a dose of 0, 25, 100 or 200 mg/kg bw per day on days 7–28 post-coitum, using 0.5% carboxymethylcellulose as the vehicle. All rabbits were observed twice daily for mortality and morbidity. Clinical observations, body weights and feed consumption were recorded. On gestation day 29, the uteri, placentae and ovaries from all surviving dams were examined, and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights and net body weight (the gestation day 29 body weights exclusive of the weight of the uterus and contents), net body weight change (the gestation days 0–29 body weight change exclusive of the weight of the uterus and contents) and cumulative body weight were recorded. In addition, the livers from all females examined at the scheduled necropsy were weighed. The fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations.

At 200 mg/kg bw per day, decreased maternal body weight gain, cumulative body weight gain and feed consumption were observed during treatment (Table 46). Slight increases in liver weights, both absolute weight (13%) and adjusted to net body weight as a covariate (14%), were observed at 200 mg/kg bw per day when compared with controls. Liver weight increases of 11% (absolute) and 9% (adjusted) observed at 100 mg/kg bw per day were considered adaptive.

Fetal examination revealed slightly lower mean fetal weights at 200 mg/kg bw per day, although there were no treatment-related effects on fetal development in this group.

In the developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on the reduction of body weight gain and feed consumption at 200 mg/kg bw per day. The NOAEL for developmental toxicity was 100 mg/kg bw per day, based on slight reduction of fetal body weights at 200 mg/kg bw per day (Sawhney Coder, 2010b).

2.6 Special studies

(a) Acute neurotoxicity

In a preliminary acute oral neurotoxicity study in rats, groups of three male and three female HanRcc:Wistar (SPF) rats were administered a single oral dose of sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 80, 1000 or 2000 mg/kg bw and observed for 8 days.

All animals survived. At 1 hour after dosing, one female exhibited slight inactivity at 2000 mg/kg bw. Treatment-related effects at 1000 and 2000 mg/kg bw were a transient occurrence of reduced activity, recumbency, hunched posture, spasms, ruffled fur, piloelection and uncoordinated movements, with a peak effect observed on day 1 in males and females at 3 hours after the dosing. Sedaxane did not affect body weight or macroscopic findings.

Table 46. Body weight and cumulative body weight gain in pregnant rabbits orally treated with sedaxane

	Body weight / body weight gain (g)			
	0 mg/kg bw per day (control)	25 mg/kg bw per day	100 mg/kg bw per day	200 mg/kg bw per day
Body weight				
- GD 0	3348	3346	3355	3365
- GD 7	3451	3511	3479	3462
- GD 8	3440	3508	3460	3425
- GD 14	3507	3606	3550	3455
- GD 19	3539	3687	3590	3484
- GD 24	3588	3747	3637	3557
- GD 29	3602	3711	3643	3549
Body weight gain				
- GD 7	0	0	0	0
- GD 8	−11	−3	−19	−37**
- GD 9	−10	−3	−7	−35*
- GD 11	4	20	3	−30
- GD 14	55	95	71	−7*
- GD 18	89	161	99	10
- GD 20	97	192*	122	36
- GD 23	126	235*	163	73
- GD 29	150	196	159	71

From Sawhney Coder (2010b)

GD, gestation day; * $P < 0.05$; ** $P < 0.01$

Based on the results, it was determined that a single oral sedaxane dose of 2000 mg/kg bw would be an appropriate high dose for an acute neurotoxicity study in rats (Sommer, 2008).

To detect acute oral neurotoxicity in rats, four groups of 10 male and 10 female HanRcc:Wistar (SPF) rats were administered a single oral dose of sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0 (0.5% carboxymethylcellulose, the vehicle control), 30, 250 or 2000 mg/kg bw per day, respectively, and observed for 15 days. General cage-side observations, detailed clinical observations comprising open-field evaluation of clinical signs, functional observational battery evaluations and locomotor activity were assessed. Feed consumption and body weights were measured during the test period. On day 16, five rats of each sex per group were perfusion fixed in situ, and selected nervous system tissues were collected, processed and examined microscopically.

Four males and three females in the 2000 mg/kg bw group were killed due to the severity of the clinical signs in response to sedaxane on day 1. Clinical signs observed in these rats included markedly laboured respiration (bradypnoea), abnormal gait, moderately reduced activity, weakened condition, recumbency, piloerection and/or a decrease in rectal temperature. No remarkable macroscopic findings were noted in these rats at necropsy. The remaining rats survived until the end of the scheduled post-dosing observation period.

At approximately 1 hour after dosing, treatment-related cage-side observations for the 250 and 2000 mg/kg bw females included weakened condition, swaying gait and decreased activity. At approximately 2 hours after dosing, treatment-related clinical observations noted during the functional

observational battery included increased incidences of reduced activity, decreased rearing, initial inactivity, piloerection (250 and 2000 mg/kg bw males and females), reduced muscle tone (250 mg/kg bw females and 2000 mg/kg bw males and females), hunched posture (2000 mg/kg bw males), recumbency (250 and 2000 mg/kg bw males), abnormal gait (2000 mg/kg bw females) and bradypnoea (2000 mg/kg bw males and females). Similar signs and incidences were noted when detailed observations were made approximately 5 hours after dosing. Other treatment-related findings noted in the functional observational battery evaluations at approximately 2 hours after dosing included lower mean body temperatures and decreases in both mean forelimb and hindlimb grip strength for the 2000 mg/kg bw males and females. At approximately 3–4 hours after dosing, the locomotor parameters for total moved distance and rearing activity were reduced at 250 and 2000 mg/kg bw in males and females. The decreases in these locomotor activity parameters are attributed to treatment and correlate with the increased incidences of reduced activity and decreased rearing activity observed in the detailed observations approximately 2–3 hours after dosing. During days 2–7, several treatment-related daily clinical signs were noted for the 250 and 2000 mg/kg bw males and females and included ruffled fur (250 mg/kg bw males and 2000 mg/kg bw males and females), rough coat (2000 mg/kg bw males), weakened condition (250 mg/kg bw females and 2000 mg/kg bw males and females), swaying gait (250 and 2000 mg/kg bw females), decreased activity and prostrate and hunched posture (2000 mg/kg bw females). Treatment with doses of 250 and 2000 mg/kg bw resulted in a dose-dependent decrease in mean feed consumption for males and females on days 1–2. Lower mean body weights for the 250 mg/kg bw males and 2000 mg/kg bw males and females were observed on day 8, and these reflected the lower mean body weight gains noted in these groups during the 1st week post-dosing. No treatment-related cage-side and/or detailed clinical observations were noted subsequent to the 1st week of the study (i.e. days 8–16). There were no effects on brain weight or macroscopic or microscopic findings. At study termination, no neurohistopathology was observed in any treated groups.

The NOAEL for systemic toxicity following a single oral dose was 30 mg/kg bw, based on severe loss of general condition, decreased body weight and decreased feed consumption at 250 mg/kg bw in both males and females (Sommer, 2009b).

(b) Subchronic neurotoxicity

A 28-day dietary toxicity study was conducted as a preliminary range-finder to a 90-day neurotoxicity study using groups of eight male and eight female Wistar (HanRcc:WIST) rats orally treated with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0 (control), 500, 2000 or 5000 ppm (equal to 0, 37.8, 153.5 and 360.1 mg/kg bw per day for males and 0, 40.1, 156.4 and 338.8 mg/kg bw per day for females, respectively). All animals were checked for clinical condition and detailed behavioural observations. Body weights and feed consumption were recorded. At the end of the scheduled period, animals were necropsied.

All animals survived their scheduled study period. No treatment-related clinical signs were observed in any rats of any dose groups throughout the study period. Reduced feed intake was observed in males and females at 5000 ppm and females at 2000 ppm. Feed utilization was significantly reduced in males and females at 5000 ppm. Body weights and cumulative body weight gains were reduced in males and females at 5000 ppm and in females at 2000 ppm.

The lower body weights (−10.0% in males, −16.5% in females) and cumulative body weight gain (−27.1% in males, −52.8% in females) at 5000 ppm over 4 weeks indicated that 5000 ppm was too high to be the top dose in the subchronic neurotoxicity study. Consistently lower feed consumption and feed utilization also confirmed the observation concerning the 5000 ppm dose level. No macroscopic findings were recorded at necropsy in any animals of any dose groups (Sommer, 2009c).

Groups of 12 male and 12 female HanRcc:WIST (SPF) rats were fed sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) in the diet at a concentration of 0, 300, 1000 or 4000 ppm (equal to 0, 19.7, 66.0 and 260.0 mg/kg bw per day for males and 0,

24.3, 79.7 and 302.9 mg/kg bw per day for females, respectively) for up to 92 days. All animals were observed prior to the study start and daily throughout the study for any changes in clinical condition, detailed clinical observations, a functional observational battery and an assessment of locomotor activity. Body weight and feed consumption were measured. Ophthalmoscopic examination was performed in all animals. At the end of the scheduled period, five rats of each sex per group were perfused in situ, brain weight was recorded, and organs and selected nervous tissues were assessed for macroscopic changes. The remaining animals were killed and discarded. Tissues of the peripheral and central nervous systems of the control and 4000 ppm dose groups were examined neurohistopathologically.

Homogeneity, stability and achieved concentration of sedaxane diets were acceptable. All animals survived the scheduled study period. Detailed clinical observations and functional observational battery evaluations of clinical symptoms revealed no treatment-related effects. Body temperature, landing foot splay and grip strength in the forelimbs and hindlimbs were not affected. Feed consumption was significantly reduced in both sexes at 4000 ppm. At 4000 ppm, body weights were generally lower in both sexes throughout the study (Table 47). Cumulative body weight gains were statistically significantly lower in both sexes at 4000 ppm from day 8 to the end of study. Mean locomotor activity total distance values in both sexes at 4000 ppm were generally lower than in controls. These lower total distance values are not considered to represent a direct neurotoxic insult, but rather are reflective of the treatment-related decreases in feed consumption and body weight. No gross findings were present at necropsy. Brain weights and adjusted brain weights were not affected by treatment with sedaxane. There was no treatment-related neuropathology.

Table 47. Body weight changes and feed consumption in rats orally treated with sedaxane in a 90-day neurotoxicity study

	Males				Females			
	0 ppm	300 ppm	1000 ppm	4000 ppm	0 ppm	300 ppm	1000 ppm	4000 ppm
Body weight (g)								
- day 1	188.4	194.0	195.3	191.5	150.9	147.7	146.6	151.2
- day 8	233.6	241.1	243.1	225.8	173.1	171.7	168.2	164.7
- day 15	268.1	277.4	279.2	258.6	188.2	185.7	182.6	174.8**
- day 29	320.7	331.3	332.3	299.6	218.6	213.7	213.7	202.2*
- day 57	383.1	398.0	397.7	350.9*	243.2	248.0	238.8	225.3
- day 85	426.9	438.8	441.4	386.8*	254.7	258.1	248.9	229.3**
Feed consumption (g/day)								
- week 1	19.925	19.670	20.071	14.645**	15.770	15.749	15.560	10.280**
- week 7	23.325	24.008	23.093	21.055*	17.135	18.586	17.519	15.650
- week 8	22.761	23.289	23.403	20.712*	19.457	18.985	18.210	15.016**
- week 9	23.135	23.221	23.403	20.917*	15.931	17.978	16.866	15.143
- week 10	22.758	22.903	23.029	20.815	17.359	17.278	16.641	14.805**
- week 11	22.877	22.870	23.687	21.465	17.258	19.200	17.802	14.855*

From Sommer (2009d)

* $P < 0.05$; ** $P < 0.01$ (Dunnett's *t*-test, two-sided)

The NOAEL for systemic toxicity in a 13-week neurotoxicity study in rats was 1000 ppm (equal to 66 mg/kg bw per day), based on decreased body weight, body weight gain, feed consumption and feed efficiency, as well as reduced locomotor activity, at 4000 ppm (equal to 260 mg/kg bw per day). Sedaxane has no neurotoxicity (Sommer, 2009d).

(c) Immunotoxicity

To provide information on suppression of the immune system that might occur as a result of repeated exposure to a test chemical, the effects of oral (dietary, ad libitum) treatment with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) on the humoral component of the immune system were investigated in male CD-1 mice. The following immunological parameters were evaluated: spleen weights, thymus weights, spleen cell number and the spleen immunoglobulin M (IgM) antibody response to the T cell–dependent antigen, sheep erythrocytes (sRBC). Four groups of male Crl:CD1 (ICR) mice were fed sedaxane ad libitum in the diet for a minimum of 28 consecutive days at 0, 500, 2000 or 5500 ppm (equal to 0, 93, 637 and 1084 mg/kg bw per day, respectively). Five mice were administered the positive control substance, cyclophosphamide monohydrate, via intraperitoneal injection (50 mg/kg bw per day) for 4 consecutive days (study days 24 through 27). Additionally, all mice were immunized with an intravenous injection of sRBC on study day 24, approximately 96 hours prior to the scheduled necropsy. Each group consisted of 10 males. All animals were euthanized on study day 28. Spleen samples were processed into single-cell suspensions, and an antibody-forming cell (AFC) assay was performed to determine the number of specific IgM antibody-forming cells directed towards sRBC. The AFC assay was a modification of the Jerne plaque assay (Jerne, Nordin & Henry, 1963).

Homogeneity, stability and achieved concentration of sedaxane diets were acceptable. All animals survived to the scheduled necropsy. There were no test substance–related clinical observations, body weights, feed consumption, macroscopic findings, terminal body weights or absolute, adjusted or relative spleen or thymus weights. There were no effects on the mean number of IgM antibody-forming splenocytes that were attributed to sedaxane.

Adjusted liver weights were increased at 5500 ppm, but this finding was considered non-adverse due to the lack of histopathology indicating hepatotoxicity. In the cyclophosphamide group, there were findings consistent with the known immunosuppressant effects (Table 48). In the sedaxane-treated groups, there was no suppression of the humoral immune component of the immune system, including a lack of changes in spleen and thymus weights, spleen cell numbers and the T cell–dependent antibody response of splenocytes, also referred to as the IgM AFC assay.

Table 48. Selected organ weights in an immunotoxicity study in mice orally treated with sedaxane for 28 days

Parameter	Mean values				
	0 ppm	500 ppm	2000 ppm	5500 ppm	Positive control CP: 50 mg/kg bw
Terminal body weight (g)	35.1	33.9	35.2	34.4	34.3
Absolute liver weight (g)	2.1248	2.0143	2.1504	2.2802	2.0587
Adjusted liver weight (g)	2.0858	2.0669	2.1034	2.2930**	2.0794
Absolute thymus weight (g)	0.0377	0.0379	0.0387	0.0428	0.0122**
Adjusted thymus weight (g)	0.0377	0.0378	0.0387	0.0428	0.0122**
Relative thymus weight (% of body weight)	0.11	0.11	0.11	0.12	0.04**
Absolute spleen weight (g)	0.1358	0.1271	0.1355	0.1469	0.0629**
Adjusted spleen weight (g)	0.1338	0.1297	0.1331	0.1476	0.0640**
Relative spleen weight (% of body weight)	0.38	0.38	0.39	0.43	0.19**

From Crittenden (2010)
CP, cyclophosphamide monohydrate; ** $P < 0.01$ (Dunnett's test, two-sided)

In this immumotoxicity study in mice, sedaxane was not immunotoxic at doses up to 5500 ppm (equal to 1084 mg/kg bw per day) (Crittenden, 2010).

(d) Comparative toxicities of trans *and* cis *isomers and their mixture*

To investigate the relative toxicity profiles of the *trans* isomer, the *cis* isomer and a 1:1 mix of these isomers (lot no. KI 7193/5 for *trans* isomer and lot no. KI-7245/5 for *cis* isomer; purity not confirmed), an exploratory investigation of 28-day dietary toxicity in rats, with supplemental examination of liver biochemistry, toxicokinetic and serum thyroid hormone parameters, was conducted.

Groups of five male and five female HsdBrlHan:Wistar rats were fed diets containing 0, 500, 2000 or 5000 ppm of the *trans* or *cis* isomers or a 1:1 mixture of the two isomers for 28 consecutive days (see Table 49 for mean intakes). Clinical observations, body weights and feed consumption were measured throughout the study. At the end of the exposure period, the rats were killed and examined post mortem. Cardiac blood samples were taken for clinical chemistry and analysis of thyroid hormones, selected organs were weighed and specified tissues were taken for subsequent microscopic examination. Samples of liver were analysed for total cytochrome P450 (CYP) and CYP-dependent isoenzyme activities. Satellite groups of three male and three female rats per sampling day (exposure days 1 and 14) were treated with the same test diets and utilized for toxicokinetic analysis. Blood samples for toxicokinetic analysis were taken 8 hours after the administration of test diets and at 4-hour intervals thereafter (approximately at 17:00, 21:00, 01:00, 05:00, 09:00 and 13:00) on day 1/2 and on day 14/15 of the study.

Table 49. Mean chemical intakes

	Mean intake (mg/kg bw per day)								
	1:1 mixed isomers			*Trans* isomer			*Cis* isomer		
	500 ppm	2000 ppm	5000 ppm	500 ppm	2000 ppm	5000 ppm	500 ppm	2000 ppm	5000 ppm
Males	47.5	181.2	444.6	47.0	187.4	438.2	45.9	182.7	438.2
Females	46.7	181.1	428.1	48.4	177.1	384.3	47.6	179.6	435.8

From Peffer & Noakes (2010)

There were no deaths or treatment-related clinical signs. Body weights adjusted for initial weights were lower, compared with the controls, for males and females that received the *trans* or *cis* isomer or mixed isomers at 5000 ppm (Table 50). The magnitude of the difference from the controls was consistent across all three test compounds. There was a slightly lower body weight, compared with the controls, for males and females at 2000 ppm, although this was generally not of statistical significance.

Feed consumption was lower than that of the controls for both males and females that received the *trans* isomer, *cis* isomer or mixed isomers at 5000 ppm and to a lesser extent for females at 2000 ppm. In the toxicokinetics analysis, there were no clear differences in mean T_{max} between the *trans* isomer, *cis* isomer and mixed isomers. The data were limited (no available data to determine the overall absorption and rate of conversion to metabolites for the *trans* and *cis* isomers); however, mean C_{max} and AUC were generally considerably lower (approximately 5- to 20-fold, depending on sex, sampling day, dietary concentration and parameter) for the *cis* isomer compared with the *trans* isomer when administered separately or when administered as mixed isomers with *trans* and *cis* isomers analysed separately in the plasma (Table 51).

Table 50. Body weights of rats orally treated with* trans, cis *and mixed isomers of sedaxane

Day	Body weight[a] (g)							
	Males				Females			
	0 ppm	500 ppm	2000 ppm	5000 ppm	0 ppm	500 ppm	2000 ppm	5000 ppm
trans isomer								
- day 1	158.0	156.6	158.4	157.4	124.8	123.0	119.8	123.4
- day 7	193.4	193.8	193.0	174.1**	137.9	141.1	138.4	124.9**
- day 14	227.9	230.9	225.5	203.1**	154.4	158.1	151.5	133.9**
- day 21	253.9	254.0	254.3	221.0**	166.5	170.6	162.8	143.8**
- day 29	280.7	284.5	284.5	238.4**	180.5	183.9	171.2	149.3**
cis isomer								
- day 1	158.0	158.0	157.8	158.6	124.8	123.2	121.2	127.8
- day 7	193.4	194.8	188.5	172.8**	137.9	139.1	138.7	123.8**
- day 14	227.9	228.9	221.9	194.2	154.4	156.7	153.0	135.4**
- day 21	253.9	256.1	240.1	212.3**	166.5	168.6	164.6	148.0**
- day 29	280.7	281.5	260.3*	231.1**	180.5	182.5	170.9	155.4**
Mixture								
- day 1	158.0	159.6	156.2	159.8	124.8	122.0	120.0	117.2
- day 7	193.4	194.5	190.6	169.1**	137.9	144.2	133.2	133.9
- day 14	227.9	234.5	223.7	196.4**	154.4	160.8	153.7	142.7**
- day 21	253.9	264.0	245.1	217.2**	166.5	168.2	164.8	152.5**
- day 29	280.7	294.7	268.0	236.1**	180.5	183.0	176.6	160.5**

From Peffer & Noakes (2010)

* $P < 0.05$; ** $P < 0.01$ (Student's *t*-test, two-sided)

[a] Values are the mean body weights adjusted for initial body weights by analysis of covariance.

Haematology parameters were unaffected, with the exception of a slightly higher, but not dose-related, prothrombin time in males treated with the *trans* isomer at 2000 and 5000 ppm.

In blood chemistry investigations, increases in plasma total protein, cholesterol and triglyceride concentrations were common changes in both sexes receiving the *trans* isomer, *cis* isomer or mixed isomers at higher doses (Table 52); triglyceride concentrations were increased in females in all groups treated with the *trans* isomer and mixed isomers, and cholesterol levels were increased in females in all groups treated with the *cis* isomer. Slight increases in alkaline phosphatase, gamma-glutamyl transferase or alanine aminotransferase were also noted in males or females dosed with the three compounds at 5000 ppm.

None of the three test compounds caused an effect on serum thyroid hormone levels (triiodothyronine, thyroxine or thyroid stimulating hormone).

Analyses of hepatic CYP-related enzymes showed that pentoxyresorufin *O*-dealkylase (PROD) activity was increased in males only when receiving the *trans* isomer at 500 ppm, in both males and females receiving the *cis* isomer or the mixed isomers at 500 ppm and in both males and females receiving all three test substances at 2000 or 5000 ppm. Increases were greater in males (up to about 40-fold) than in females (up to about 10-fold). The marked increase in hepatic PROD activity with all three test compounds indicates that they are potent inducers of CYP2B isoforms. Analysis of ethoxyresorufin *O*-dealkylase (EROD) activity showed that, in male rats, the *cis* isomer apparently caused increases in activity in the 500 and 2000 ppm diet groups of 2-fold and 1.6-fold, respectively,

but there was no increase in activity in the 5000 ppm diet group, suggesting either that the higher activities observed in the lower dose groups were not an effect of treatment or that it was the lower activity in the highest dose level group that was the statistical anomaly. In female rats, EROD activity (per milligram protein) was slightly increased by the *cis* isomer in all dose groups by about 1.5-fold. The *trans* isomer had no clear effect on the activity of EROD in male rats, and in females, there was only a slight increase in activity in the 5000 ppm *trans* isomer diet group. The diet containing the mixture of isomers was associated with higher EROD activity in male rats of the 2000 ppm diet group and in females of the 2000 and 5000 ppm diet groups. In both sexes, the increases were modest (1.5- to 1.7-fold in specific activity). The weakly increased EROD activity indicates that the three different test compounds possess low potential for inducing CYP1A isoforms. There were no consistent effects of treatment on total CYP in the liver.

Table 51. Toxicokinetics analysis of* trans, cis *and mixed isomers in rats orally treated with sedaxane for 28 days

Dietary concentration (ppm)	Sex	Day	*Trans*			*Cis*		
			Mean T_{max} (h)	Mean C_{max} (µg/ml)	Mean AUC (µg·h/ml)	Mean T_{max} (h)	Mean C_{max} (µg/ml)	Mean AUC (µg·h/ml)
500	Male	1	12	0.391	4.89	—	—	—
		14	13	0.202	1.85	—	—	—
	Female	1	13	0.198	2.73	—	—	—
		14	17	0.222	2.74	—	—	—
2000	Male	1	16	4.61	59.0	20	0.676	5.53
		14	16	0.568	6.18	20	0.315	3.15
	Female	1	13	2.50	28.6	16	0.266	2.63
		14	17	2.43	25.0	15 (18)[a]	0.681 (0.265)	6.01 (2.46)
5000	Male	1	19	3.40	44.8	16	0.526	6.45
		14	19	4.75	83.8	18	0.196	2.17
	Female	1	20	3.37	44.6	16	0.635	6.38
		14	17	5.11	81.7	17	1.50	10.0
Mixture (*trans*:*cis* 1:1)								
500	Male	1	17	0.112	1.42	20	0.0275	0.320
		14	12	0.0603	0.633	8	0.0319	0.231
	Female	1	12	0.0915	1.23	—	—	—
		14	16	0.145	1.51	—	—	—
2000	Male	1	19	1.22	14.3	19	0.101	1.42
		14	19	0.153	2.16	—	—	—
	Female	1	16	0.555	8.48	17	0.134	1.50
		14	19	0.471	5.34	20	0.0412	0.348
5000	Male	1	23	1.61	21.2	19	0.120	2.03
		14	17	0.613	6.15	18	0.0318	0.328
	Female	1	19	0.843	11.4	19	0.147	1.78
		14	20	1.290	14.1	20	0.0644	0.926

From Peffer & Noakes (2010)

—, data not available; AUC, area under the plasma–concentration time curve; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}

[a] Standard deviation given in parentheses.

Table 52. Summary of comparison of clinical chemistry in a 4-week oral study in rats treated with* trans *or* cis *isomers or their mixture

	Males				Females			
	0 ppm[a]	500 ppm	2000 ppm	5000 ppm	0 ppm[a]	500 ppm	2000 ppm	5000 ppm
***Trans* isomer**								
Total protein (g/l)	58.3	57.9	58.8	64.5**	59.3	62.5	64.4*	66.7**
Total bilirubin (µmol/l)	4.79	4.06	3.97	10.36**	4.00	5.42	4.38	7.92*
Cholesterol (mmol/l)	1.63	1.67	1.39	2.66**	1.50	1.70	2.34**	3.61**
Triglycerides (mmol/l)	1.26	1.42	1.59	5.29**	0.95	1.75*	2.46**	4.91**
Alkaline phosphatase (IU/l)	398	395	322*	495*	225	224	220	264
Gamma-glutamyl transferase (IU/l)	6.8	7.0	7.3	6.8	5.5	5.5	6.8	10.2**
Alanine aminotransferase (IU/l)	67.4	65.6	61.4	74.6	44.0	42.0	53.0	62.6*
***Cis* isomer**								
Total protein (g/l)	58.3	59.5	62.7*	63.1*	59.3	63.3*	62.9	65.2*
Total bilirubin (µmol/l)	4.79	3.19	3.85	5.76	4.00	4.10	4.31	5.48
Cholesterol (mmol/l)	1.63	1.92	1.94	2.32**	1.50	1.83*	2.07**	2.90**
Triglycerides (mmol/l)	1.26	1.34	1.77	2.41*	0.95	1.32	2.38**	2.76**
Alkaline phosphatase (IU/l)	398	363	348	421	225	221	259	230
Gamma-glutamyl transferase (IU/l)	6.8	7.1	8.2	13.4**	5.5	5.9	7.0	11.7**
Alanine aminotransferase (IU/l)	67.4	64.8	49.6	54.8	44.0	58.2	46.2	51.8
Mixture (*trans:cis*, 1:1)								
Total protein (g/l)	58.3	59.2	59.8	66.7**	59.3	62.9	63.5	64.7**
Total bilirubin (µmol/l)	.4.79	4.09	4.59	6.71	4.00	5.35	3.35	10.28**
Cholesterol (mmol/l)	1.63	1.64	1.67	2.83**	1.50	1.65	2.15**	2.94**
Triglycerides (mmol/l)	1.26	1.66	1.69	3.36**	0.95	2.04*	1.77*	3.14**
Alkaline phosphatase (IU/l)	398	458	375	477*	225	228	198	246
Gamma-glutamyl transferase (IU/l)	6.8	6.7	6.8	12.1**	5.5	6.0	7.6	8.2*
Alanine aminotransferase (IU/l)	67.4	67.2	63.8	61.2	44.0	44.2	45.3	64.2*

From Peffer & Noakes (2010)
IU, international unit; * $P < 0.05$; ** $P < 0.01$
[a] Compared with satellite control group.

All three compounds significantly increased the 16β-hydroxylation of testosterone in both males (36-fold) and females (20-fold), consistent with being potent inducers of CYP2B isoforms. In addition, the three test compounds caused decreased 16α- and 2α-testosterone hydroxylase activities in male rats but increased their activities in female rats, and there was a greater increase in the 2β- and 6β-hydroxytestosterone hormonal levels in female rats than in males. Immunoblotting showed increased levels of CYP2B and CYP3A relative to the controls for all three compounds, providing further qualitative support for the increased enzyme activity, noted in liver biochemistry, of these two CYP isoenzymes.

There were statistically significantly higher liver weights and liver weights adjusted for body weights for males and females at 2000 and 5000 ppm for all three compounds. There was a consistent response across males and females and with each test substance.

There were no treatment-related gross findings. Microscopically, minimal centrilobular hypertrophy was observed in both sexes after treatment with all three compounds at 5000 ppm, except in males treated with the *trans* isomer or mixed isomers at 2000 and 5000 ppm. The liver from a small number of rats in the control and 5000 ppm groups of the three compounds was examined using electron microscopy on both semi-thin toluidine blue–stained sections and ultra-thin sections. Some changes, such as a proliferation of smooth endoplasmic reticulum, increased fat and a more frequent occurrence of condensed cells, were found in the liver from sedaxane-treated rats. The changes that were observed were similar with all three test compounds. No treatment-related changes were detected in other tissues except the liver.

In conclusion, the toxicological target organ of *trans* and *cis* isomers and their mixture was the liver. The toxicological profiles of the three compounds were qualitatively similar, and there were very few differences in the incidence or severity of findings observed in response to the three isomer preparations. They all induced drug metabolizing enzyme activity (mainly PROD), although there were some treatment-related differences in testosterone hydroxylation activities. Toxicokinetic analysis indicated higher C_{max} and AUC values for the *trans* isomer than for the *cis* isomer and for the mixture (Peffer & Noakes, 2010).

(e) Toxicity of metabolite CSCD465008

CSCD465008, or 3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid, has been assessed for acute oral toxicity, in vitro genotoxicity (bacterial reverse mutation, in vitro cytogenetics and mammalian gene cell mutation) and repeated-dose oral toxicity for up to 28 days, as summarized in Table 53. CSCD465008 was not acutely toxic by the oral route, was not genotoxic in vitro and did not result in any toxicologically significant effects at dose levels exceeding 1000 mg/kg bw per day, the limit dose for a 28-day toxicity study in rats. These data indicate that CSCD465008 is of lower toxicity than the parent sedaxane.

Table 53. Summary of toxicity studies with CSCD465008

Study	Result	Reference
Acute oral toxicity	LD_{50} > 2000 mg/kg bw	Simon (2008)
Bacterial reverse mutation	Negative	Sokolowski (2008)
In vitro cytogenetics	Negative	Bohnenberger (2008)
Mammalian cell gene mutation (mouse lymphoma)	Negative	Wollny (2008)
28-day dietary toxicity in the rat	NOAEL = 1018 mg/kg bw per day (highest dose tested)	Walraven (2008)

LD_{50}, median lethal dose; NOAEL, no-observed-adverse-effect level

In a 28-day oral toxicity study, CSCD465008 (lot no. MES-103/1; purity 94%), a metabolite of sedaxane, was administered ad libitum via the basal diet for 28 consecutive days to three groups of Crl:WI(Han) rats. Dietary concentrations were 0, 2000, 6000 and 12 000 ppm (equal to 0, 175, 497 and 1018 mg/kg bw per day for males and 0, 176, 525 and 1107 mg/kg bw per day for females, respectively). Each group consisted of five animals of each sex. Following 4 weeks of test diet administration, all animals were euthanized and necropsied. All animals were checked for mortality, clinical examinations and detailed physical examinations. Body weights and feed consumption were recorded. Functional observational battery and locomotor activity data were recorded for all animals. Ophthalmic examinations, clinical pathology evaluations (haematology, coagulation, serum chemistry and urine analysis) were performed on all animals. After necropsies, selected organs were weighed and examined microscopically from all control and the highest dose groups. All gross lesions were examined in all animals. Sections of the liver were collected from all animals for CYP enzyme analysis.

There were no test substance–related effects on clinical observations, body weights, feed consumption, functional observational battery, motor activity, haematology, coagulation, serum chemistry, urine analysis parameters, P450 content, P450 activity (for those enzymes evaluated), ophthalmic examinations and macroscopic or microscopic findings.

The NOAEL was 12 000 ppm (equal to 1018 mg/kg bw per day), the highest dose tested (Walraven, 2008).

In conclusion, CSCD465008 is of low acute toxicity and has no genotoxicity and no toxicity in a 4-week oral toxicity study in rats. The toxicities were weak compared with those of sedaxane.

3. Observation in humans

Analysis of the company's internal database produced no reports of adverse health effects that relate to the synthesis or formulation of sedaxane. As sedaxane has not yet been introduced into the market, there is no information on record of clinical cases and poisoning incidents or observations on exposure of the general population and epidemiological studies. Sedaxane is of low acute toxicity, and no cases of intoxication with sedaxane have yet been observed. No specific monitoring programmes have been performed in humans.

Comments

Biochemical aspects

In rats given [^{14}C]sedaxane labelled in either the phenyl or pyrazole ring as a single oral dose of 1 or 80 mg/kg bw, the radiolabelled material was rapidly and extensively absorbed, based on recoveries in excreta from bile duct–cannulated rats. The times to reach C_{max} were approximately 1 hour and 5–6 hours following the low and high doses, respectively. The mean C_{max} and AUC values for the *trans* isomers were higher than those for the *cis* isomers and their mixture in rats. Approximately 90% of the administered dose was absorbed at both the low and high doses. Radiolabelled material was widely distributed throughout the body within 5 hours. The half-lives of elimination of total radioactivity from different tissues varied from 0.1–0.2 day in brain to 2.0–3.2 days in thyroid. Elimination half-lives from blood ranged from 30 to 40 hours and were generally similar in males and females at both dose levels. Less than 0.8% of the administered dose remained in the body at 96 hours after dosing.

The sedaxane administered to rats was rapidly excreted, predominantly in the faeces (75–88%) and in urine (12–20%). Sedaxane was extensively metabolized in rats by demethylation, hydroxylation, oxidation and conjugation, resulting in many hydroxylated metabolites and metabolites formed by cleavage of the terminal cyclopropyl moiety. The major metabolites have been identified as the *trans*-*para*-phenol sedaxane and the desmethyl *trans*-*para*-phenol sedaxane, which, together with the equivalent *cis*-*para*-phenol isomers of sedaxane, account for approximately half of the administered dose. There appear to be no major sex- or dose-related differences in the qualitative metabolite profile of sedaxane. There is little evidence of any cleavage between the phenyl and pyrazole moieties of the sedaxane molecule. A small amount (< 1%) of a pyrazole amide metabolite of sedaxane also found in plants can be found in bile samples. The phenolic and hydroxy metabolites of sedaxane and desmethyl sedaxane are subject to glucuronic acid, sulfate and glutathione conjugation.

Toxicological data

The oral LD_{50} was 5000 mg/kg bw in rats. Significant clinical signs of toxicity (ruffled fur, hunched posture, sedation, poor coordination, ventral recumbency, deep respiration, rales, salivation and bradypnoea) were observed at lower doses (1750 and 550 mg/kg bw) for a few hours following treatment. The dermal LD_{50} in rats was greater than 5000 mg/kg bw. The 4-hour acute inhalation LC_{50} in rats was greater than 5.2 mg/l. Sedaxane was not irritating to rabbit skin and minimally irritating to rabbit eyes. Sedaxane was not a skin sensitizer in the mouse local lymph node assay.

The short-term oral toxicity of sedaxane was evaluated in mice, rats and dogs, in which the main effects were on body weight gain and liver. In a 28-day study of toxicity in mice, no toxicity was observed at doses up to 7000 ppm (equal to 1268 mg/kg bw per day). In a 90-day dietary toxicity study in mice, the NOAEL was 3500 ppm (equal to 566 mg/kg bw per day), based on a decrease in body weight gain throughout the study in males at 7000 ppm (equal to 1167 mg/kg bw per day).

Two 90-day toxicity studies were conducted in rats, each demonstrating the liver as the target for sedaxane. In the first study, the NOAEL was 1000 ppm (equal to 72.9 mg/kg bw per day), based on lower body weights, centrilobular hepatocyte hypertrophy and pigmentation, and blood chemistry indicating liver dysfunction in males and females at 4000 ppm (equal to 299.6 mg/kg bw per day). In the second study, the NOAEL was 300 ppm (equal to 28.3 mg/kg bw per day), based on reduced body weight and body weight gain and significant decreases in forelimb grip strength at 2000 ppm (equal to 168.0 mg/kg bw per day); liver toxicity was observed at 4000 ppm (equal to 325.1 mg/kg bw per day). The overall NOAEL from these studies was 1000 ppm (equal to 72.9 mg/kg bw per day).

The toxicity of sedaxane administered in capsules was tested in dogs in 90-day and 1-year toxicity studies. The overall NOAEL was 50 mg/kg bw per day, based on reduced body weight gain in females at 150 mg/kg bw per day.

The NOAEL in an 18-month dietary study in mice was 1250 ppm (equal to 157 mg/kg bw per day), based on a decrease in body weight and body weight gain in both sexes at 7000 ppm (equal to 900 mg/kg bw per day). A slightly increased incidence of hepatocellular adenomas and carcinomas combined was observed in male mice at the high dose in comparison with the control group incidence. The NOAEL for equivocal carcinogenicity in mice was 1250 ppm (equal to 157 mg/kg bw per day).

The NOAEL in a 104-week dietary study in rats was 200 ppm (equal to 11 mg/kg bw per day), based on increases in liver weight and histopathological changes (centrilobular hypertrophy) in the liver in males, histopathological changes in the thyroid in males and females, and reduced body weight gain in females at 1200 ppm (equal to 67 mg/kg bw per day). Hepatocellular eosinophilic foci were also increased at 200 ppm in females at 52 weeks, but did not persist at 2 years. Uterine adenocarcinomas were increased at 3600 ppm (equal to 261 mg/kg bw per day). The NOAEL for carcinogenicity was 1200 ppm (equal to 86 mg/kg bw per day), based on uterine tumours in female rats.

Sedaxane was tested for genotoxicity in vitro and in vivo in an adequate range of assays. In none of these assays was there any evidence of genotoxic potential.

The Meeting concluded that sedaxane is unlikely to be genotoxic.

On the basis of the absence of genotoxicity and the fact that equivocal increased incidences of hepatocellular adenomas and carcinomas combined in male mice and uterine endometrial adenocarcinomas in rats occurred only at the highest doses tested, the Meeting concluded that sedaxane is unlikely to pose a carcinogenic risk to humans at dietary exposure levels.

In a multigeneration reproductive toxicity study in rats, the NOAEL for parental toxicity was 500 ppm (equal to 41 mg/kg bw per day), based on significantly reduced body weight gain at 1500 ppm (equal to 120 mg/kg bw per day) in parental generation males. Decreased ovarian follicle counts were observed at 1500 ppm (low and middle doses not examined). Slightly decreased ovary weights were observed at 1500 ppm. The NOAEL for reproductive toxicity was 1500 ppm (equal to 120 mg/kg bw per day). The NOAEL for offspring toxicity was 500 ppm (equal to 43 mg/kg bw per day), based on significantly lower body weights of F_1 generation males during premating at 1500 ppm (equal to 134 mg/kg bw per day).

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 25 mg/kg bw per day, based on reductions in body weight gain and feed consumption at 100 mg/kg bw per day. The NOAEL for developmental toxicity was 200 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on reductions in body weight gain and feed consumption at 200 mg/kg bw per day.

The NOAEL for developmental toxicity was 100 mg/kg bw per day, based on slight reductions in fetal body weights at 200 mg/kg bw per day.

The Meeting concluded that sedaxane is not teratogenic in rats or rabbits.

The NOAEL in a single-dose neurotoxicity study in rats was 30 mg/kg bw, based on severe loss of general condition, decreased body weight and decreased feed consumption at 250 mg/kg bw.

The NOAEL for systemic toxicity in a 13-week neurotoxicity study was 1000 ppm (equal to 66 mg/kg bw per day), based on decreased body weight, body weight gain, feed consumption and feed efficiency, as well as reduced locomotor activity, at 4000 ppm (equal to 260 mg/kg bw per day).

The Meeting concluded that sedaxane is not neurotoxic.

In an immunotoxicity study in mice, sedaxane was not immunotoxic at doses up to 5500 ppm (equal to 1084 mg/kg bw per day).

A 28-day comparative study of the toxicities of *trans* and *cis* isomers and their mixture in rats demonstrated that their toxicological profiles were qualitatively similar.

The toxicity of a sedaxane plant metabolite (3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid) has been investigated. The LD_{50} value in rats was greater than 2000 mg/kg bw, and the NOAEL in a 28-day oral (gavage) toxicity study in rats was 12 000 ppm (equal to 1018 mg/kg bw per day), the highest dose tested. There was no evidence for genotoxicity in in vitro assays.

No information on medical surveillance or poisoning incidents was available.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.1 mg/kg bw on the basis of a NOAEL of 200 ppm (equal to 11 mg/kg bw per day) in a 2-year study of toxicity and carcinogenicity in rats, based on reduced body weight gain in females and histopathological changes in the liver in males and in the thyroid in males and females at 1200 ppm (equal to 67 mg/kg bw per day). A safety factor of 100 was applied. The ADI provides a margin of exposure of at least 860 relative to the NOAEL for uterine tumours in rats and at least 1570 for equivocal liver tumour response in mice. Thus, the Meeting considered that sedaxane is not likely to pose a carcinogenic risk to humans at dietary levels of exposure.

An acute reference dose (ARfD) of 0.3 mg/kg bw was established on the basis of a NOAEL of 30 mg/kg bw in a single-dose neurotoxicity study in rats, based on severe loss of general condition, decreased body weight and decreased feed consumption. A safety factor of 100 was applied.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	1250 ppm, equal to 157 mg/kg bw per day	7000 ppm, equal to 900 mg/kg bw per day
		Carcinogenicity (equivocal)	1250 ppm, equal to 157 mg/kg bw per day	7000 ppm, equal to 900 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
Rat	Ninety-day studies of toxicity[a,b]	Toxicity	1000 ppm, equal to 72.9 mg/kg bw per day	2000 ppm, equal to 168 mg/kg bw per day
	Twenty-four-month study of toxicity and carcinogenicity[a]	Toxicity	200 ppm, equal to 11 mg/kg bw per day	1200 ppm, equal to 67 mg/kg bw per day
		Carcinogenicity	1200 ppm, equal to 86 mg/kg bw per day	3600 ppm, equal to 261 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	1500 ppm, equal to 120 mg/kg bw per day[d]	—
		Parental toxicity	500 ppm, equal to 41 mg/kg bw per day	1500 ppm, equal to 120 mg/kg bw per day
		Offspring toxicity	500 ppm, equal to 43 mg/kg bw per day	1500 ppm, equal to 134 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	25 mg/kg bw per day	100 mg/kg bw per day
		Developmental toxicity	200 mg/kg bw per day[d]	—
	Single-dose test of neurotoxicity[c]	Toxicity	30 mg/kg bw	250 mg/kg bw
Rabbit	Developmental toxicity study[c]	Maternal toxicity	100 mg/kg bw per day	200 mg/kg bw per day
		Developmental toxicity	100 mg/kg bw per day	200 mg/kg bw per day
Dog	Ninety-day and 12-month studies of toxicity[b,c]	Toxicity	50 mg/kg bw per day	150 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Gavage administration.
[d] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.1 mg/kg bw

Estimate of acute reference dose

0.3 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 sedaxane

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid , > 87%
Dermal absorption	No data

Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid, > 99.5% within 2 days
Metabolism in animals	Main four metabolites by demethylation, hydroxylation, oxidation and conjugation
Toxicologically significant compounds in animals, plants and the environment	Parent compound and all of the individual isomers
Acute toxicity	
Rat, LD_{50}, oral	5000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw per day
Rat, LC_{50}, inhalation	> 5.244 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Mildly irritating
Dermal sensitization	Not sensitizing (local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Liver and reduced body weight gain
Lowest relevant oral NOAEL	50 mg/kg bw per day
Lowest relevant dermal NOAEL	1000 mg/kg bw per day
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver, thyroid and reduced body weight gain
Lowest relevant NOAEL	11 mg/kg bw per day
Carcinogenicity	Equivocal hepatic tumours in mice and uterine tumours in rats; unlikely to pose a carcinogenic risk at dietary exposure levels
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Target/critical effect	No reproductive toxicity
Lowest relevant reproductive NOAEL	120 mg/kg bw per day (highest dose tested)
Lowest relevant parental NOAEL	41 mg/kg bw per day
Lowest relevant offspring NOAEL	43 mg/kg bw per day
Developmental toxicity	
Target/critical effect	No developmental toxicity
Lowest relevant maternal NOAEL	25 mg/kg bw per day
Lowest relevant developmental NOAEL	100 mg/kg bw per day
Neurotoxicity	
Acute neurotoxicity	Not neurotoxic; 250 mg/kg bw (highest dose tested) NOAEL for toxicity: 30 mg/kg bw
Subchronic neurotoxicity	Not neurotoxic; 260 mg/kg bw per day (highest dose tested)
Other toxicological studies	
Comparative toxicity	Toxicological profile similar for *trans* and *cis* isomers and their mixture in rats
Immunotoxicity	Not immunotoxic; 1084 mg/kg bw per day (highest dose tested)

Medical data	
	No reports of toxicity in workers exposed during manufacture or use

Summary

	Value	Study	Safety factor
ADI	0–0.1 mg/kg bw	Two-year study (rats)	100
ARfD	0.3 mg/kg bw	Single-dose study (rats)	100

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