SPIROTETRAMAT

First draft prepared by
D. Kanungo[1] & A. Moretto[2]

[1] Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, New Delhi, India; and
[2] Department of Environmental and Occupational Health, University of Milan, International Centre for Pesticide and Health Risk Prevention, Luigi Sacco Hospital, Milan, Italy

Explanation

Spirotetramat is the ISO approved name for *cis*-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro[4,5]dec-3-en-2-one (IUPAC). The CAS No. for spirotetramat is 203313-25-1. Spirotetramat belongs to the chemical class of ketoenols, subclass tetramic acid derivatives, and is intended for use as an insecticide on a range of agricultural crops. The pesticidal mechanism of action is disruption of lipogenesis as a result of inhibition of acetyl CoA carboxylase.

The JMPR has not previously evaluated spirotetramat. Spirotetramat was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR) at its 40th Session.

The impurity profiles of batches of spirotetramat used in studies of toxicity were variable. Some impurities were absent in the material used in long-term studies of toxicity and studies of genotoxicity, or were present at a low concentration. However, the results of studies with impurities indicated that this was not a critical issue in the toxicological evaluation. All critical studies complied with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

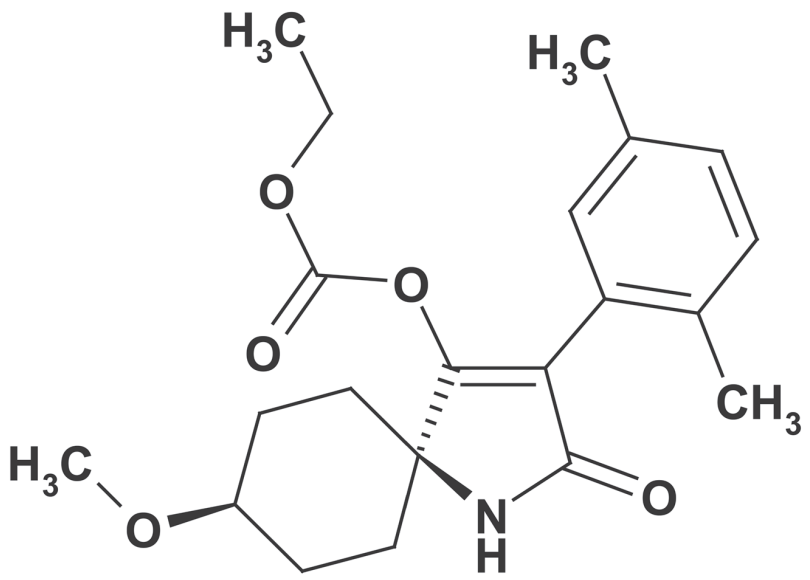

Figure 1. Chemical structure of spirotetramat

Rats

In a study of absorption, distribution, metabolism and excretion, groups of four male or four female rats (*Rattus norvegicus domesticus*, strain Wistar Hsd/Cpb:WU) were given a single dose of [azaspirodecenyl-3-^{14}C]spirotetramat (purity, > 99%; radiochemical specific activ-

ity, 100.2–99.1 µCi/mg, equal to 3.67–3.71 MBq/mg) at a target dose of 2 or 100 mg/kg bw by gavage in aqueous Tragacanth®. Two additional groups of four male or four female rats were pretreated for 14 days with non-radiolabelled spirotetramat at a dose of 2 mg/kg bw per day followed by a single dose of radiolabelled spirotetramat at 2 mg/kg bw. The active substance was radiolabelled with ^{14}C in the 3-position of the spirodecenyl ring of the molecule. The rats were aged 9 weeks (male) and 12–13 weeks (female) at study initiation. All rats were sacrificed 2 days after dosing.

Total radioactivity (including test substance and its metabolites) was determined in samples of plasma, excreta (urine and faeces) and in organs and tissues. Metabolism was investigated by high-performance liquid chromatography (HPLC), normal-phase thin-layer chromatography (TLC) and spectroscopic methods in selected samples of urine and faecal extracts.

Between 91.4% and 99.8% of the administered dose was recovered in the urine, faeces and organs and tissues at sacrifice. The recovery of radiolabel in the excreta, organs and tissues is summarized in Table 1.

[Azaspirodecenyl-3-^{14}C]Spirotetramat was very rapidly absorbed from the gastrointestinal tract of male and female rats in all test groups. The absorption commenced immediately after dosing. Values calculated for the absorption half-lives were in the range of 0.6–10 min. In this study, the minimal absorption rate in all tests was between 90% and 98% of the total radioactivity recovered, calculated from the values of the urine and the body without the gastrointestinal tract. No significant differences were seen between the lower, higher and repeated doses.

The maximum plasma concentration was reached for all dose groups within 0.09 to 2.03 h after administration (values calculated by pharmacokinetic modelling). From the maximum, the radioactivity concentrations in plasma declined steadily by several orders of magnitude within 48 h for all dose groups. The pharmacokinetic parameters (three-compartment modelling using the TOPFIT software program) are given in Table 2.

The results obtained from the experiments with the lower dose were nearly identical for males and females. Absorption of radiolabel was slightly slower in males than in females when comparing t_{max} values. Absorption was followed by a fast initial elimination phase and a moderate terminal elimination phase. The area under the curve [AUC (0–∞)] indicated a slightly higher systemic exposure for males than for females. The elimination rate constant was very similar for males and females. The mean residence time was low in males and slightly higher in females. The results obtained with the lower dose were in the same range after repeated and single doses.

At the higher dose, the C_{max} was significantly higher for males than for females. Compared with the lower dose, the plasma concentrations in males were proportional to the dose ratio with a slightly higher value for AUC (0–∞) in the test with the higher dose. The C_{max} was slightly lower for females at the higher dose than for females at the lower dose, while the value for AUC (0–∞) was proportional to the dose ratio. This indicated that the absorption process had not been (over) saturated at the respective highest dose. As with the lower dose, absorption was followed by a fast initial elimination phase and a moderate terminal elimination phase. The elimination rate constant was lower than for the lower dose and lower for males than for females. The mean residence time was in the same range as for the lower dose and was quite similar for males and females. Comparison of the absorption phases of the kinetic curves between the groups at the lower and higher dose showed a broader maximum for males at the higher dose. The maximum concentration (C_{max}) was reached slightly later at this dose and the following initial elimination phase was slightly longer than observed for the lower dose. The curves at the end of the terminal elimination phase were comparable. Plasma concentrations of radiolabel are summarized in Table 3.

The distribution of radioactive residues in the body was analysed at the time of sacrifice, 48 h after dosing, by measurement of the concentrations of radiolabel in the major organs and tissues. Less than 0.2% of the administered dose was detected in the body. The residues in all organs and

tissues at the time of sacrifice were low in all tests and sometimes below the limit of detection (LOD). The highest equivalent concentrations were detected in the liver (0.002–0.18 mg/kg) and kidney (0.001–0.11 mg/kg), the organs responsible for degradation and excretion. The recovery of radiolabel in organs and tissues of rats 48 h after oral administration of [azaspirodecenyl-3-^{14}C]spirotetramat is shown in Table 4.

Excretion was fast and almost complete within 24 h after dosing. There were no significant differences between males and females or between doses. Excretion was mainly renal and quite similar for males and females given either dose, either as a single dose or as repeated doses. About

Table 1. Recovery of radiolabel from rats killed 48 h after dosing with [azaspirodecenyl-3-^{14}C] spirotetramat

Sample	Recovery of radiolabel (% of administered dose)					
	Dose (mg/kg bw)					
	No pretreatment				Pretreatment[a]	
	2		100		2	2
	Male	Female	Male	Female	Male	Female
Urine	93.34	87.92	89.14	93.78	91.48	94.78
Faeces	5.11	3.34	10.51	2.98	6.59	1.78
Total excreted	98.45	91.26	99.65	96.76	98.07	96.56
Skin	0.004	0.070	0.015	0.014	0.011	0.024
Organs, total	0.019	0.058	0.112	0.021	0.067	0.036
Body, excluding gastrointestinal tract	0.023	0.129	0.126	0.035	0.078	0.060
Gastrointestinal tract	0.014	0.043	0.012	0.012	0.035	0.024
Total, body	0.038	0.171	0.138	0.047	0.113	0.083
Balance[b]	98.49	91.44	99.78	96.81	98.19	96.65
Recovery of radiolabel (% of total recovered radioactivity)						
Urine	94.79	96.06	89.34	96.86	93.23	98.06
Faeces	5.18	3.75	10.52	3.10	6.66	1.85
Total excreted	99.97	99.81	99.86	99.96	99.89	99.91
Skin	0.004	0.078	0.015	0.014	0.012	0.025
Organs, total	0.019	0.064	0.113	0.022	0.069	0.037
Body, excluding gastrointestinal tract	0.024	0.142	0.127	0.036	0.080	0.062
Gastrointestinal tract	0.014	0.047	0.012	0.013	0.036	0.025
Total, body	0.038	0.189	0.139	0.049	0.117	0.086
Normalization factor	1.015	1.101	1.002	1.034	1.022	1.036
Absorption rate	94.81	96.20	89.47	96.90	93.31	98.12

From Klempner (2006a)

[a] Fourteen doses of non-radiolabelled spirotetramat plus a single dose of radiolabelled spiriotetramat.

[b] Balance = total excreted + total retained in the body.

88–95% of the administered dose was eliminated in the urine and about 2–11% in the faeces. The concentration of radioactive residues in the organs and tissues were determined at sacrifice, 48 h after dosing. Negligible amounts of radiolabel were found in the carcass (including organs), showing that the elimination of compound-related radioactivity was nearly complete. No sex differences were observed in the amounts of residues in the organs and tissues. The time-course of excretion of radioactivity after oral administration of [azaspirodecenyl-3-^{14}C]spirotetramat is shown in Table 5.

Parent compound was not detected in the urine and faeces of rats in any of the tests. Two metabolites were prominent in all samples: the spirotetramat-enol, which was the major metabolite in urine and faeces of males and females in all tests, and the spirotetramat-desmethyl-enol. A sex-related difference in the ratio of the two main metabolites was observed in males and females in all

Table 2. Distribution and plasma kinetics of radiolabel in rats given [azaspirodecenyl-3-^{14}C] spirotetramat

Parameter	Units	Dose (mg/kg bw)					
		No pretreatment				Pretreatmenta	
		2	2	100	100	2	2
		Male	Female	Male	Female	Male	Female
C_{max} (model)	µg/g	4.41	4.15	210	117	5.21	2.98
t_{max} (model)	h	0.89	0.09	2.03	0.77	0.45	0.35
C_{max} (experiment)	µg/g	4.40	3.84	204	116	4.96	2.69
t_{max} (experiment)	h	1.00	0.17	1.50	0.66	0.66	0.66
$t_{1/2}$ a	h	< 0.01	< 0.01	0.17	0.06	0.10	0.07
$t_{1/2}$ e (1)	h	0.31	4.79	1.70	0.19	3.62	0.47
$t_{1/2}$ e (2)	h	20.1	29.7	17.5	27.2	92.7	13.2
t_{lag} a	h	< 0.01	0.08	0.06	0.05	0.03	0.03
AUC (0–∞)	µg/g × h	16.4	10.2	1380	451	14.6	7.64
K_1 e	1/h	246	258	0.98	3.53	5.61	8.53
CL/f	ml/min per kg	2.03	3.27	1.21	3.70	2.28	4.36
CLR	ml/min per kg	1.89	2.89	1.08	3.47	2.09	4.13
MRT	h	3.39	9.68	4.90	4.26	5.29	4.38
MRTabs	h	2.55	1.16	2.53	2.99	1.91	1.94
MRTdisp	h	0.84	8.52	2.37	1.27	3.38	2.44
Vss	l	0.10	1.67	0.17	0.28	0.46	0.64
Weighting function[b]	Number	g = 1	g = 1/y	g = 1	g = 1/y	g = 1	g = 1
Compartment[c]		3	3	3	3	3	3

From Klempner (2006a)

AUC, area under the curve of conentration–time; CL, clearance; CL/f, Total clearance of radioactivity from plasma; CLR, renal clearance radioactivity; C_{max}, maximum plasma concentration; K_{1e}, Elimination rate constant; MRT, mean residence time; $t_{1/2}$a, half-life of absorption; $t_{1/2}$ e (1), half-life of the elimination phase 1, equals initial elimination phase; $t_{1/2}$ e (2), half-life of the elimination phase 2, equals intermediate elimination phase; t_{laga}, lag between administration and the onset of absorption; t_{max}, time at which the maximum radioactivity concentration occurs in plasma after administration of extravascular dose; Vss, volume of distribution (apparent) of the radioactivity under steady-state conditions based on compound-related radio activity in plasma.

[a] Fourteen doses of non-radiolabelled spirotetramat plus a single dose of radiolabelled spirotetramat.

[b] For some individual data points, weighting factors were used for better curve-fitting.

[c] Three-compartment modelling using the TOPFIT software program.

tests, but the difference was greater in tests with the higher dose. The quantity of the major metabolite, spirotetramat-enol, was lower in males than in females. Conversely, the quantity of the second main metabolite, spirotetramat-desmethyl-enol, was higher in males than in females. The recovery of spirotetramat-enol ranged from 53% to 66% of the administered dose in males and from 81% to 87% in females. The recovery of spirotetramat-desmethyl-enol ranged from 25% to 37% of the dose in males and from 5% to 10% in females. The results for the single lower dose and repeated lower dose with pretreated rats were in the same range. Four more metabolites identified in the excreta were of minor importance. They ranged from 0.1% to 1.6% of the administered dose. They all were degradation products of the spirotetramat-enol. The first important metabolic reaction in males and females was the cleavage of the ester bond of the side-chain, yielding spirotetramat-enol, followed by demethylation of the methoxy group at the cyclohexyl ring. Cleavage of the molecule was not observed. All other metabolic reactions, such as conjugation of the spirotetramat-enol with glucuronic acid, hydroxylation in the pyrrolidine ring of spirotetramat-enol resulting in spirotetramat-ketohydroxy and oxidation of one of the methyl groups of the phenyl ring, were of minor importance. The proposed metabolic pathway of spirotetramat in male and female rats is shown in Figure 2. The study complied with GLP and a statement of quality assurance (QA) was provided (Klempner, 2006a).

A study of metabolism and pharmacokinetics was undertaken to investigate the depletion of residues of spirotetramat from the plasma, testes, liver and kidney, excretion in the urine and metabolism in groups of four male Wistar Hsd/Cpb:WU rats (aged 9 weeks) given a single dose of [azaspirodecenyl-3-^{14}C]spirotetramat (radiochemical purity, >98%) at 2 or 1000 mg/kg bw (three groups per

Table 3. Time-course of plasma concentrations of radiolabel in rats given oral doses of [azaspirodecenyl-3-^{14}C]spirotetramat

Timepoint (after dosing)	Equivalent concentration [µg/g] (measured values) (n = 4)					
	Dose (mg/kg bw)					
	No pretreatment				Pretreatmenta	
	2		100		2	2
	Male	Female	Male	Female	Male	Female
0.08	1.435	2.225	14.436	16.071	1.784	1.389
10.2 min	2.568	3.836	45.424	52.592	3.935	2.301
20 min	3.396	3.239	101.600	89.249	4.891	2.673
40 min	4.139	2.925	157.374	116.044	4.962	2.687
1 h	4.402	2.394	189.735	115.816	4.383	2.179
1.5 h	4.004	1.598	204.390	100.464	3.401	1.531
2 h	3.390	1.067	201.130	87.011	2.581	0.929
3 h	2.358	0.659	194.017	58.094	1.661	0.395
4 h	1.558	0.515	168.045	40.126	1.114	0.229
6 h	0.676	0.356	122.464	21.094	0.495	0.142
8 h	0.327	0.280	78.375	16.608	0.246	0.132
24 h	0.005	0.035	0.415	0.204	0.009	0.014
32 h	0.003	0.023	0.189	0.150	0.003	0.010
48 h	0.002	0.011	0.154	0.098	0.002	0.009

From Klempner (2006a)

[a] Fourteen doses of non-radiolabelled spirotetramat plus a single dose of radiolabelled spirotetramat.

dose) by oral gavage in aqueous Tragacanth®. The lower dose was selected to match the lower dose of spirotetramat administered in the previously described study of absorption, distribution, metabolism and excretion (Klempner, 2006a). Taking into account the findings from a mechanistic study, in which degenerative effects on round/elongating spermatids in the testes were found in male rats given more than 10 doses of spirotetramat at 1000 mg/kg bw per day; the higher dose selected was 1000 mg/kg bw (**Kennel, 2005**).

The rats were sacrificed 1 h, 8 h and 24 h after dosing. The total quantity of radiolabel, including parent compound and metabolites, was determined in samples of urine and faeces, as well as in the plasma, testes, liver and kidney at sacrifice. Investigations on metabolites were performed by determination of radiolabel in eluates from high-performance liquid chromatography (radio-HPLC) with selected samples of urine and plasma, and with extracts from the testes, liver and kidney.

Between 100.3% and 121.8% (lower dose) and 94.8% and 98.9% (higher dose) of the administered doses were recovered in the urine and faeces, and in organs and tissues at sacrifice. The slightly

Table 4. Recovery of radiolabel from tissues and organs of rats killed 48 h after oral administration of [azaspirodecenyl-3-^{14}C]spirotetramat

Tissue/organ	Equivalent concentration [µg/g][a] (n = 4)					
	Dose (mg/kg bw)					
	No pretreatment			Pretreatmentb		
	2	2	100	100	2	2
	Male	Female	Male	Female	Male	Female
Erythrocytes	0.0010	0.0013	0.0385	0.0250	0.0007	0.0007
Plasma	0.0011	0.0015	0.0703	0.0267	0.0009	0.0010
Spleen	0.0006	0.0009	0.0626	< LOD	0.0006	0.0006
Gastrointestinal tract	0.0024	0.0094	0.0809	0.0999	0.0035	0.0046
Liver	0.0076	0.0035	0.1792	0.0502	0.0094	0.0019
Kidney	0.0009	0.0040	0.1065	0.0609	0.0024	0.0027
Perirenal fat	< LOD	< LOD	< LOD	< LOD	0.0047	< LOD
Adrenal gland	< LOD	< LOD	< LOD	< LOD	0.0062	< LOD
Testes	0.0008	—	0.0622	—	0.0003	—
Ovaries	—	< LOD	—	< LOD	—	< LOD
Uterus	—	< LOD	—	< LOD	—	0.0015
Skeletal muscle	< LOD	0.0013	0.0377	< LOD	0.0006	< LOD
Bone (femur)	< LOD	0.0030	0.0855	0.0534	0.0009	< LOD
Heart	0.0006	0.0010	0.0332	0.0189	0.0006	0.0005
Lung	0.0005	0.0011	0.0327	0.0220	0.0006	0.0007
Brain	< LOD	0.0005	< LOD	< LOD	< LOD	< LOD
Thyroid gland	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Skin	0.0008	0.0060	0.0567	0.0529	0.0008	0.0022
Carcass	< LOD	0.0015	0.1649	0.0257	0.0009	0.0010

From Klempner (2006a)

< LOD, below the limit of detection.

[a] The concentrations are mean values for the four rats in each group.

[b] Fourteen doses of non-radiolabelled spirotetramat plus a single dose of radiolabelled spirotetramat.

higher recoveries obtained from two groups of rats at the lower dose were caused by the foamy consistence of the suspension of spirotetramat, which made it difficult to accurately measure the volume to be administered by gavage. The entire balances for the total amount of radiolabel detected in the urine, faeces, gastrointestinal tract plus faeces, skin and organs and tissues at sacrifice are shown in Table 7.

A summary of the experimental protocol is shown in Table 6.

Table 5. Time-course of excretion of radiolabel in the urine and faeces of rats given oral doses of [azaspirodecenyl-3-^{14}C]spirotetramat

Timepoint (hours after dosing)	Cumulative excretion of radiolabel (% of administered dose)					
	Dose (mg/kg bw)					
	No pretreatment				Pretreatmenta	
	2	2	100	100	2	2
	Male	Female	Male	Female	Male	Female
Urine						
4	34.48	36.50	17.95	55.19	40.65	56.35
8	77.48	45.10	50.87	78.95	77.77	57.55
12	89.95	[b]	[b]	[b]	[b]	[b]
24	92.96	85.68	88.32	93.03	90.86	93.16
48	93.34	87.92	89.14	93.78	91.48	94.78
Faeces						
24	4.89	2.30	9.95	2.79	5.94	1.44
48	5.11	3.34	10.51	2.98	6.59	1.78
Total excreted	98.45	91.27	99.64	96.76	98.08	96.56

From Klempner (2006a)

[a] Fourteen doses of non-radiolabelled spirotetramat plus a single dose of radiolabelled spirotetramat.

[b] No samples collected.

Table 6. Experimental protocol in a study of the metabolism and disposition of [azaspirodecenyl-3-^{14}C]spirotetramat in rats

Dose (mg/kg bw)	Time-point (h)	Comments
Single (2 mg/kg bw)	1	Urine collected at 0–1 h; blood, testes, liver, kidney, gastrointestinal. tract (+ contents), skin and carcass assessed at sacrifice.
	8	Urine collected at 0–1 and 4–8 h; blood, testes, liver, kidney, skin and carcass assessed at sacrifice.
	24	Urine collected at 0–1, 4–8 h, 8–24 h; blood, testes, liver, kidney, gastrointestinal tract (+ contents) skin and carcass assessed at sacrifice.
Single (1000 mg/kg bw)	1	Urine collected at 0–1 h; blood, testes, liver, kidney, skin and carcass assessed at sacrifice.
	8	Urine collected at 0–1 and 4–8 h; blood, testes, liver, kidney, skin, carcass assessed at sacrifice.
	24	Urine collected at 0–4, 4–8 h, 8–24 h; blood, testes, liver, kidney, gastrointestinal tract (+ contents) skin and carcass assessed at sacrifice.

From Klempner (2006b)

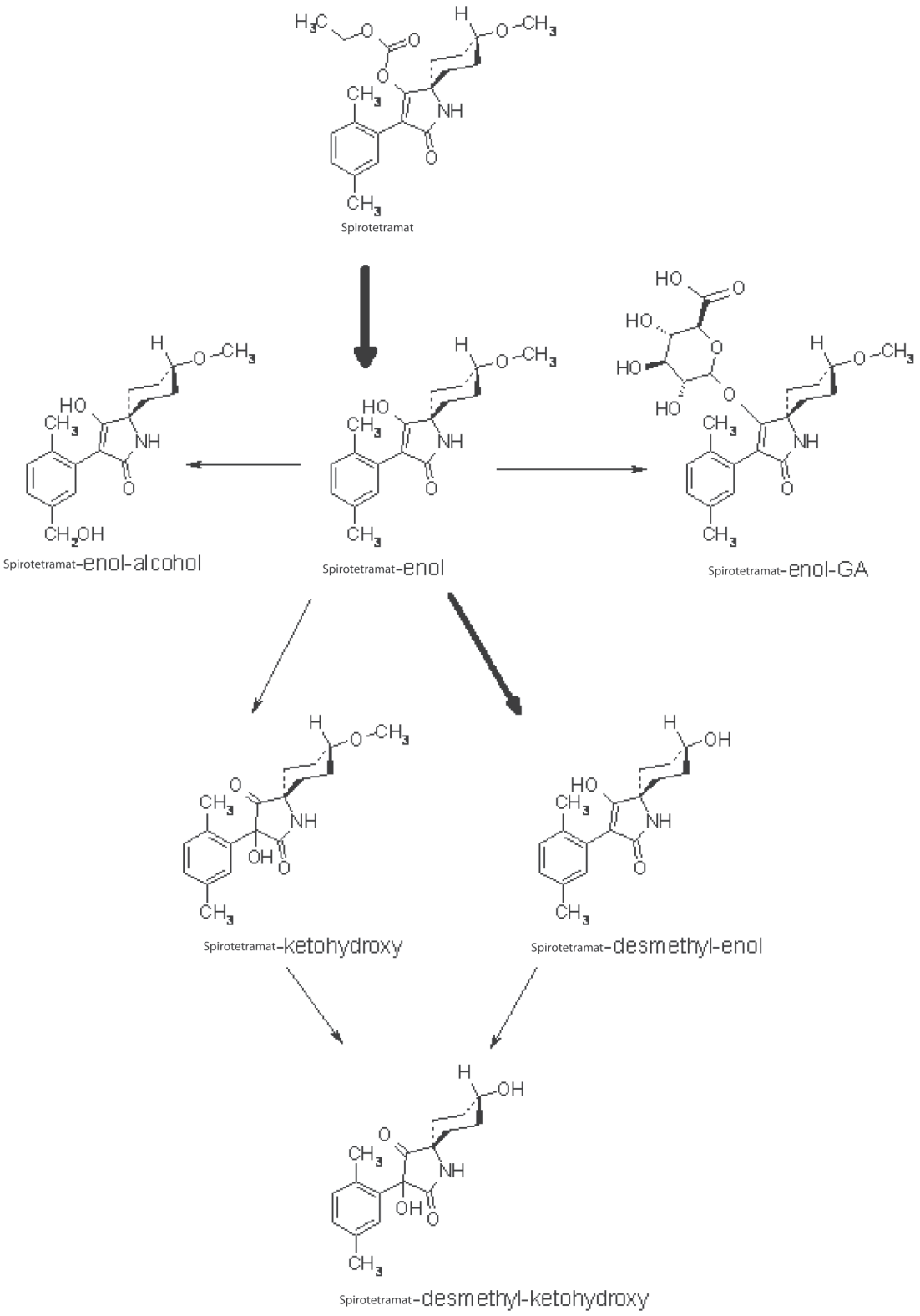

Figure 2. Proposed metabolic pathway of spirotetramat in rats

Urinary excretion was rapid in the groups receiving the lower dose, being almost complete within the first 24 h after administration. About 92% of the total recovered radiolabel (equivalent to 112% of the administered dose) was excreted via the urine. About 8% of the total recovered radiolabel (equivalent to 10% of the administered dose) was detected in faeces of rats given the lower dose, 24 h after dosing. Excretion was considerably slower in the rats at the higher dose than in those at the lower dose. Only 27% of the administered dose was excreted via the urine 24 h after dosing and 18% via the faeces, respectively. Most of the radiolabel was recovered from the gastrointestinal tract.

At sacrifice, the highest values for total radioactive residue (TRR) were measured in the organs and tissues sampled 1 h after administration (Table 8). Values declined rapidly to less than 0.05 mg/kg

Table 7. Balance of radioactivity in excreta, plasma, organs and tissues of male rats (n = 4) given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat

	Mean recovery of radiolabel (% of administered dose)					
	Dose (mg/kg bw)					
	2			1000		
Specific radioactivity [kBq/mg]	3670	3670	3670	7.34	7.34	7.34
Time-point (hours after dosing)	1 h	8 h	24 h	1 h	8 h	24 h
Urine						
0–1 h	12.5	-—	-—	1.6	-—	-—
0–4 h	-—	20.1	37.3	-—	4.9	6.0
4–8 h	-—	45.6	39.4	-—	10.9	9.4
8–24 h	-—	-—	35.3	-—	-—	11.4
Urine, total	12.5	65.7	111.9	1.6	15.8	26.8
Faeces	-—[a]	-—[a]	9.5	-—[a]	-—[a]	17.8
Blood						
Erythrocytes	1.4	0.3	0.003	0.3	0.2	0.1
Plasma	3.5	1.0	0.003	0.5	0.4	0.1
Organs						
Liver	22.9	9.2	0.067	1.2	0.9	0.4
Kidney	4.8	1.5	0.005	0.3	0.2	0.1
Testis	0.4	0.2	0.002	0.1	0.1	< 0.1
Organs, total	33.1	12.3	0.080	2.4	1.8	0.7
Skin	12.7	3.2	0.031	3.0	2.4	0.7
Carcass	28.3	10.6	0.097	5.9	6.1	1.8
Gastrointestinal tract	32.1	8.6	0.205	85.9	72.9	47.0
Total, body	106.2	34.6	0.413	97.1	83.1	50.2
Balance	118.7	100.3	121.8	98.7	98.9	94.8

From Klempner (2006b)

[a] For rats killed at 1 h and 8 h, the faeces were included with the gastrointestinal tract.

within 24 h in rats at the lower dose. The decline was slow after the higher dose; there was even a slight increase in testes and carcass from 1 h to 8 h after dosing.

In rats given the lower dose, parent compound was not detected in any sample or tissue. Spirotetramat-enol was the main metabolite in all samples. Spirotetramat-desmethyl-enol was the second most prominent metabolite in urine, plasma and testes, whereas in liver and kidney, spirotetramat-ketohydroxy was found to be the second most prominent metabolite. Spirotetramat-ketohydroxy was only present at trace concentrations in the plasma, testes and urine. Spirotetramat-enol-glucuronic acid (GA), spirotetramat-enol-alcohol and spirotetramat-desmethyl-ketohydroxy were detected at low concentrations and are therefore of minor importance. Twenty-four hours after dosing, residues in tissues and plasma were too low for quantification of metabolites. Identification rates were high, being in the range of 89–100%. The metabolic profiles for urine were in

Table 8. Total radioactive residues in organs and tissues of male rats (n = 4) given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat

Sample	Mean concentration of total radioactive residues (mg active substance equivalents/kg)					
	Dose (mg/kg bw)					
	2	2	2	1000	1000	1000
Specific radioactivity [KBq/mg]	3670	3670	3670	7.34	7.34	7.34
	1 h	8 h	24 h	1 h	8 h	24 h
Erythrocytes	1.723	0.412	0.003	199.2	147.9	47.1
Plasma	4.527	1.242	0.004	352.5	271.4	102.0
Liver	11.740	5.073	0.024	315.2	245.9	109.0
Kidney	11.220	3.517	0.011	307.9	221.7	85.3
Testes	0.668	0.342	0.004	66.5	77.4	28.5
Skin	0.978	0.259	0.003	119.2	93.4	31.7
Carcass	0.906	0.355	0.003	97.8	103.3	33.2
	Dose-normalized concentration (mean values)					
Erythrocytes	0.951	0.220	0.002	0.212	0.156	0.045
Plasma	2.498	0.663	0.003	0.376	0.287	0.098
Liver	6.487	2.701	0.013	0.336	0.260	0.105
Kidney	6.197	1.873	0.006	0.328	0.235	0.082
Testis	0.369	0.182	0.002	0.071	0.082	0.028
Skin	0.540	0.138	0.001	0.127	0.099	0.031
Carcass	0.500	0.190	0.002	0.104	0.110	0.032
	Radioactivity as % of dose administered (mean values)					
Erythrocytes	1.42	0.34	0.003	0.28	0.23	0.06
Plasma	3.52	0.98	0.003	0.52	0.42	0.14
Liver	22.92	9.23	0.067	1.21	0.86	0.38
Kidney	4.83	1.48	0.005	0.26	0.18	0.07
Testis	0.42	0.24	0.002	0.08	0.09	0.04
Skin	12.71	3.22	0.031	2.97	2.37	0.71
Carcass	28.30	10.55	0.097	5.87	6.10	1.81

From Klempner (2006b)

good agreement with the results for rats at the lower dose in the study of absorption, distribution, metabolism and excretion.

Similar results were obtained for rats at the higher dose. Parent compound was not detected in any sample or tissue. Spirotetramat-enol was the main metabolite in all samples. Spirotetramat-desmethyl-enol was the second most prominent metabolite in the urine, plasma and organs. Significant proportions of spirotetramat-ketohydroxy were present in the liver and kidney only. Spirotetramat-enol-GA, spirotetramat-enol-alcohol and spirotetramat-desmethyl ketohydroxy were detected at low levels and were of minor importance. Identification rates were high, being in the range of 95–100%. A summary of the metabolites identified in the urine is shown in Table 9. The metabolites identified in plasma and organs of rats at the lower dose and higher dose, respectively, are summarized in Table 10 and Table 11.

Table 9. Metabolites identified in the urine of male rats given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat

Metabolite		Concentration of metabolite (% of administered dose)					
		Dose (mg/kg bw)					
		2			1000		
Sampling interval		0–1 h	0–8 h	0–24 h[a]	0–1 h	0–8 h	0–24 h[a]
1	Unknown	ND	ND	0.07	ND	ND	ND
2	Spirotetramat-enol-GA	0.04	0.40	0.55	ND	0.12	0.25
3	Unknown	ND	0.09	0.16	ND	ND	0.02
4	Unknown	ND	0.12	0.17	ND	ND	0.08
5	Unknown	ND	0.22	0.36	ND	ND	0.07
6	Unknown	ND	0.07	0.12	ND	ND	0.04
7	Unknown	ND	ND	0.07	ND	ND	0.03
8	Unknown	ND	0.10	0.13	ND	ND	ND
9	Unknown	ND	0.13	0.20	ND	ND	ND
10	Unknown	ND	ND	0.02	ND	ND	ND
11	Spirotetramat-enol-alcohol	0.26	0.81	1.15	ND	0.16	0.33
12	Spirotetramat-desmethyl-enol	1.26	20.50	33.09	0.15	4.18	10.63
13	Unknown	0.10	0.78	1.17	ND	0.16	0.29
14	Spirotetramat-desmethylketohydroxy	ND	0.13	0.20	ND	ND	0.03
16	Spirotetramat-enol	10.72	42.12	74.07	1.46	11.15	14.99
18	Spirotetramat-ketohydroxy	0.07	0.26	0.37	ND	ND	0.05
Identified		12.4	64.2	109.4	1.6	15.6	26.3
Unknown [b]		0.1	1.5	2.5	ND	0.2	0.5
Total		12.5	65.7	111.9	1.6	15.8	26.8

From Klempner (2006b)
GA, glucuronic acid; ND, not detected.
[a] Calculated sum of pooled urine from 0–4 h, 4–8 h and 8–24 h.
[b] Characterized based on their retention time in high-performance liquid chromatography (HPLC).

The first and most important metabolic reaction was the cleavage of the ester bond of the side-chain yielding the spirotetramat-enol. The demethylation of the cyclohexyl-*O*-methyl group to the respective alcohol (spirotetramat-desmethyl-enol) was a further important metabolic reaction, as well as the hydroxylation in the azaspiro ring of spirotetramat-enol, resulting in spirotetramat-ketohydroxy. Other metabolic reactions, such as conjugation of the spirotetramat-enol with glucuronic acid and oxidation of one of the methyl groups of the phenyl ring forming the spirotetramat-enol-alcohol were of minor importance.

In summary, in rats given the lower dose, absorption, distribution and excretion were rapid. Excretion was mainly renal and was nearly complete 24 h after dosing. The residues in plasma and organs declined rapidly from the maximum value at 1 h after dosing to low residues in plasma 24 h after administration. For all time-points, the residues in liver and kidney were distinctly higher than in plasma. This finding may indicate that metabolites are transferred by active transport mechanisms from plasma to the excretory organs. The residues in the testes, carcass and skin were distinctly lower than in plasma, showing the rapid excretion of the residues and the lack of accumulation of the compound. The metabolic profiles in urine were similar to those found in the study of absorption, distribution, metabolism and distribution. The ratio of the two main metabolites (spirotetramat-enol and spirotetramat-desmethyl-enol) in urine was about 2 : 1. Spirotetramat-desmethyl-enol was found at lower proportions in plasma and organs with spirotetramat-enol/spirotetramat-desmethyl-enol ratios

Table 10. Total radioactive residues of metabolites in the plasma, liver, kidney and testes of male rats given a single oral dose of [azaspirodecenyl-3-^{14}C]-spirotetramat at 2 mg/kg bw

Metabolite	Percentage of total radioactive residues[a]							
	Plasma		Liver		Kidney		Testes	
	1 h	8 h	1 h	8 h	1 h	8 h	1 h	8 h
Spirotetramat-enol-GA	ND	ND	0.1	0.2	0.4	0.4	0.1	ND
Spirotetramat-enol-alcohol	ND	ND	0.5	0.4	0.2	ND	0.2	ND
Spirotetramat-desmethyl-enol	2.3	3.6	7.3	6.8	2.3	3.6	2.5	3.3
Unknown	ND	ND	0.7	0.7	0.3	0.3	ND	ND
Spirotetramat-desmethyl-ketohydroxy	ND	ND	1.2	0.7	0.6	0.9	ND	ND
Unknown	ND	ND	0.3	0.2	0.5	0.4	ND	ND
Spirotetramat-enol	97.1	96.4	72.1	79.1	73.3	74.4	94.8	83.8
Unknown	ND	ND	1.0	0.3	1.7	1.1	ND	ND
Spirotetramat-ketohydroxy	0.6	ND	13.2	8.1	20.0	18.7	2.3	2.4
Unknown	ND	ND	0.1	ND	0.3	ND	ND	ND
Unknown	ND	ND	ND	ND	ND	ND	ND	9.4
Unknown	ND	ND	ND	ND	ND	ND	ND	0.7
Subtotal	100.0	100.0	96.5	96.5	99.5	99.7	99.9	99.4
Identified	100.0	100.0	94.4	95.3	96.8	97.9	99.9	89.4
Unknown [b]	ND	ND	2.1	1.2	2.7	1.8	ND	10.0
Not analysed/solids	—	—	3.5	3.5	0.5	0.3	0.1	0.6
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

From Klempner (2006b)

GA, glucuronic acid; ND, not detected.

[a] Values for total radioactive residues mg of equivalents of active substance/kg are presented in Table 8.

[b] Characterized on the basis of their retention time in high-performance liquid chromatography (HPLC).

Table 11. Total radioactive residues of metabolites in plasma, liver, kidney and testes of male rats given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat at 1000 mg/kg bw

Metabolite	Percentage of total radioactive residuesa											
	Plasma			Liver			Kidney			Testes		
	1 h	8 h	24 h	1 h	8 h	24 h	1 h	8 h	24 h	1 h	8 h	24h
Spirotetramat-enol-GA	ND	ND	ND	0.5	1.2	1.0	0.5	1.0	1.1	ND	ND	ND
Spirotetramat-enol-alcohol	ND	ND	ND	0.7	0.8	ND	0.4	ND	ND	ND	ND	ND
Spirotetramat-desmethyl-enol	3.3	7.5	6.8	11.6	14.7	13.0	4.9	13.1	11.5	2.6	6.4	8.7
Spirotetramat-desmethyl-ketohydroxy	ND	ND	ND	1.0	1.7	1.8	1.1	3.4	3.6	ND	ND	ND
Spirotetramat-enol	96.7	92.5	93.2	76.4	72.0	74.1	75.4	64.9	65.9	94.9	91.7	89.3
Unknown	ND	ND	ND	ND	ND	ND	1.2	1.3	1.2	ND	ND	ND
Spirotetramat-ketohydroxy	ND	ND	ND	5.5	6.8	6.6	16.3	16.0	16.2	2.2	1.4	1.1
Subtotal	100.0	100.0	100.0	95.6	97.1	96.4	99.7	99.7	99.5	99.7	99.5	99.1
Identified	100.0	100.0	100.0	95.6	97.1	96.4	98.6	98.5	98.4	99.7	99.5	99.1
Unknown [b]	ND	ND	ND	ND	ND	ND	1.2	1.3	1.2	ND	ND	ND
Not analysed/solids	—	—	—	4.4	2.9	3.6	0.3	0.3	0.5	0.3	0.5	0.9
Total:	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

From Klempner (2006b)

GA, glucuronic acid; ND, not detected.

[a] Values for total radioactive residues in mg of equivalents of active substance/kg are given in Table 8.

[b] Characterized on the basis of their retention time in high-performance liquid chromatography (HPLC).

of 10 : 1 to 40 : 1. This demonstrates that spirotetramat-desmethyl-enol was excreted more rapidly than spirotetramat-enol. The highest proportions of spirotetramat-desmethyl-enol were detected in liver, where the compound is formed by metabolic transformation of spirotetramat-enol. The proportions in plasma, kidney and testis were comparable and significantly lower than in liver. Spirotetramat-ketohydroxy was found as a prominent metabolite in the liver and kidney, but was only present at trace concentrations in the plasma, testes and urine.

In rats at the higher dose, absorption and excretion were slower and lower than at the lower dose. Only 27% of the administered dose was renally excreted 24 h after administration. The residues in the plasma were slightly higher than in the liver and kidney. This may result from the saturation of the active transport mechanisms at high concentrations, resulting in a more uniform distribution of the compound in the body. The decline of residues was slower than in rats at the lower dose. As observed in rats at the lower dose, the residues in the testes, carcass and skin were lower than in the plasma. Plasma and all organs showed slow depletion of radioactive residues. The metabolism was similar to that found in rats at the lower dose, with the exception that spirotetramat-desmethyl-enol was found at higher proportions in rats at the higher dose. As in rats at the lower dose, spirotetramat-desmethyl-enol was found at higher proportions in the urine (the ratio of spirotetramat-enol/spirotetramat-desmethyl-enol was 1.5 : 1) than in plasma and organs (ratios of 5 : 1 to 35 : 1) due to the rapid excretion of this metabolite. The highest percentages of spirotetramat-desmethyl-enol were detected in the liver as well as in kidney. The percentages in the plasma, kidney and testes were lower and comparable. Significant percentages of spirotetramat-ketohydroxy were present in the liver and kidney only.

On the basis of all the results, the Meeting concluded that due to the saturation of active transport mechanisms in the excretory organs after administration of higher doses, the depletion of residues and the excretion via the urine and faeces is slow, with a potential for accumulation in the body after repeated high doses. Moreover it can also be inferred that the pharmacokinetic parameter in rats given doses of 2 or 100 mg/kg bw are not significantly different, although they are in rats at 1000 mg/kg bw. It is possible that there is also saturation of absorption from the gastrointestinal tract in rats at at 1000 mg/kg bw. The study complied with GLP and a statement of QA was provided (Klempner, 2006b).

In a study to investigate the distribution pattern of spirotetramat and its metabolites, male (age 9 weeks) and female (age 12–13 weeks) Wistar Hsd/Cpb:WU rats were given a single dose of azaspirodecenyl-3-^{14}C-labelled spirotetramat (purity, > 98%) at a target dose of 3 mg/kg bw by oral gavage. Quantitative whole-body autoradiography (QWBA) using a radioluminography (RLG) technique allowed visualization of selective enrichments of total radioactivity, particularly for certain parts of organs or tissues that are difficult or impossible to sample during the sectioning of the animal, rendering the determination of the concentration of radiolabel in situ arduous. The data were obtained over 7 days (males) and 5 days (females) after dosing. Eight males and eight females were killed 1, 4, 8, 24, 48, 72, 120 and 168 h after dosing. The study complied with GLP and a statement of QA statement was provided.

Spirotetramat was readily absorbed from the gastrointestinal tract, distributed among almost all organs and tissues and excreted rapidly within approximately 24 h (females) or 48 h (males). Excretion was mainly renal. Only a minor amount (approximately 3–6%) was excreted via the faeces. No radiolabel was detected in the expired air, showing the stability of the labelling position in the molecule. QWBA revealed fast absorption and distribution of the test compound with peak values observed within 1 h after dosing. Among the organs, tissues and fluids analysed quantitatively, the highest concentrations of equivalents were observed in the liver, kidney and blood. Moderate peak concentrations were found in the lungs and myocardium, brown fat, skin, the glands and the reproductive organs. Lower concentrations were found in all other organs and tissues. The lowest peak

concentrations were found in the spinal cord, the brain and the eye. From peak values, concentrations of radiolabel declined by several orders of magnitude to below the limit of detection for all organs and tissues within 48 h in males and within 24 h in females. Only in the liver of males were very low residues (slightly above the limit of quantitation) detected at 72 h after dosing. Based on these results, the distribution of radiolabel and corresponding metabolites in the organs and tissues of male and female rats is considered to be sufficiently understood. The TRR values for the organs and tissues in male and female rats, respectively, are shown in Tables 12 and 13 (Klempner, 2006c).

1.2 Metabolism in vitro

A comparative study of metabolism in vitro in male rats, mice and humans revealed differences in the proportions of metabolites formed by the different species. This study, which complied with GLP and for which a QA statement was provided, used Liverbeads™ –immobilized hepatocytes entrapped within an alginate matrix. The study was carried out with spirotetramat at two concentrations

Table 12. Distribution of radiolabel in organs and tissues of male rats given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat at 3 mg/kg bw

Organ/tissue	Total radioactive residues (µg active substance equivalents/g wet weight)							
	Time of sacrifice (h)							
	1	4	8	24	48	72	120	168
Blood	2.711	1.285	0.277	< LOQ	—	—	—	—
Liver	7.437	5.439	1.173	0.017	< LOQ	0.006	< LOQ	< LOD
Renal cortex	10.635	4.811	1.429	0.008	< LOD	—	—	—
Renal medulla	12.723	7.614	2.391	0.008	< LOD	—	—	—
Brown fat	1.247	0.626	0.177	—	—	—	—	—
Perirenal fat	—	0.076	0.043	< LOD	—	—	—	—
Skeletal muscle	0.658	0.331	0.064	—	—	—	—	—
Myocardium	1.611	0.738	0.177	< LOD	—	—	—	—
Lung	1.097	0.501	0.196	< LOD	—	—	—	—
Spleen	0.537	0.255	0.065	< LOD	—	—	—	—
Pancreas	0.591	0.306	0.068	< LOD	—	—	—	—
Bone marrow	0.681	0.298	0.088	—	—	—	—	—
Testes	0.509	0.404	0.088	< LOD	—	—	—	—
Brain	0.102	0.056	0.012	< LOD	—	—	—	—
Spinal cord	0.106	0.058	0.011	—	—	—	—	—
Pitutary gland	0.966	0.599	0.101	—	—	—	—	—
Pineal body	1.023	0.479	0.106	—	—	—	—	—
Adrenal gland	2.005	0.683	0.168	—	—	—	—	—
Thymus	0.579	0.236	0.053	< LOD	—	—	—	—
Thyroid gland	1.412	0.639	0.142	—	—	—	—	—
Salivary gland	1.222	0.506	0.100	—	—	—	—	—
Nasal mucosa	0.439	0.319	0.061	—	—	—	—	—
Skin	0.955	0.572	0.106	—	—	—	—	—
Vitreous body (eye)	0.146	0.037	0.024	—	—	—	—	—

From Klempner (2006c)
< LOD, less than limit of detection; < LOQ, less than limit of quantitation.

i.e. 50 µmol/l and 520 µmol/l. At the lower concentration, analysis of the Liverbeads™ samples using liquid chromatography/mass spectrometry (LC/MS) revealed five different metabolites of spirotetramat in mice, four in rats and three in humans. Spirotetramat itself was not detected in the Liverbeads™ from any species tested. The major metabolites detected in the mouse were spirotetramat-enol and spirotetramat-enol-GA, occurring at 66% and 30%, respectively. The glucurono-conjugation of the main metabolite spirotetramat-enol appeared to be a major route in the in-vitro degradation (detoxification) of spirotetramat in the mouse. Spirotetramat-enol-alcohol, spirotetramat-desmethyl-enol and spirotetramat-ketohydroxy were detected at a very low level in the mouse (1–2%). In the rat, the main metabolites were spirotetramat-enol and spirotetramat-desmethyl-enol, occurring at 87% and 7% respectively. Spirotetramat-enol-alcohol and spirotetramat-ketohydroxy represented 4% and 3% respectively. The spirotetramat-enol-GA (enol-glucuronide) was not detected in vitro in the rat. The major metabolites detected in humans were spirotetramat-enol and spirotetramat-enol-GA

Table 13. Distribution of radiolabel in organs and tissues of male rats given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat at 3 mg/kg bw

Organ/tissue	Total radioactive residues (µg active substance equivalents/g wet weight)						
	Time of sacrifice (h)						
	1	4	8	24	48	72	120
Blood	1.195	0.365	0.088	—	—	—	—
Liver	4.497	1.318	0.397	< LOD	—	—	—
Renal cortex	5.148	1.489	0.438	—	—	—	—
Renal medulla	7.306	2.624	0.913	—	—	--	—
Brown fat	0.530	0.116	0.037	—	—	—	—
Perirenal fat	0.108	0.053	0.010	—	—	—	—
Skeletal muscle	0.247	0.070	0.018	—	—	—	—
Myocardium	0.749	0.198	0.052	—	—	—	—
Lung	0.823	0.116	0.043	—	—	—	—
Spleen	0.241	0.068	0.017	—	—	—	—
Pancreas	0.281	0.074	0.021	—	—	—	—
Bone marrow	0.268	0.089	0.020	--	—	—	—
Ovary	0.595	0.111	0.028	—	—	—	—
Uterus	0.759	0.170	0.045	—	—	—	—
Brain	0.047	0.013	< LOQ	—	—	—	—
Spinal cord	0.051	0.014	< LOQ	—	—	—	—
Pitutary gland	0.476	0.134	0.032	—	—	—	—
Pineal body	0.504	0.122	0.038	—	—	—	—
Adrenal gland	0.877	0.216	0.065	—	—	—	—
Thymus	0.214	0.066	0.015	—	—	—	—
Thyroid gland	0.567	0.162	0.036	—	—	—	—
Salivary gland	0.578	0.147	0.031	—	—	—	—
Nasal mucosa	0.172	0.074	0.014	—	—	—	—
Skin	0.564	0.140	0.032	—	—	—	—
Vitreous body (eye)	0.060	0.040	0.009	—	—	—	—

From Klempner (2006c)
< LOD, less than limit of detection; < LOQ, less than limit of quantitation.

(enol-glucuronide), but their relative abundance was different from that observed in the mouse, occurring at 92% and 6%, respectively. Spirotetramat-desmethyl-enol was present at a very low level (1%); spirotetramat-enol-alcohol and spirotetramat-ketohydroxy were not detected in humans.

At the higher concentration, no additional metabolites were detected beyond those seen in low concentrations in the rat, mouse and human Liverbeads™. The low degree of metabolism observed in this experiment could be indicative of a saturation of the biotransformation enzymatic system.

At the lower concentration, spirotetramat was thus completely metabolized in the liver cells from all species and no parent compound was detected at the end of the incubation. Spirotetramat-enol was the first and most prominent metabolite, accounting for 66–92% of total metabolites. The metabolic profile among the three species at low concentration showed marked differences. In the rat, the spirotetramat-enol was further metabolized by oxidation reactions to spirotetramat-desmethyl-enol (oxidative demethylation), spirotetramat-enol-alcohol (oxidation of aromatic methyl group) and spirotetramat-ketohydroxy (oxidation of the azaspirodecenyl moiety). Oxidation products accounted for about 14%. Conjugation was not detected as an in-vitro metabolic transformation. The general picture of the in-vitro metabolic pathway in the rat was very similar to in-vivo metabolism in the male rat. The same metabolites were detected with spirotetramat-enol and spirotetramat-desmethyl-enol as the two most important degradation products, although the proportions of metabolites were different in vivo and in vitro. In the mouse, oxidative degradation of spirotetramat-enol was detected as a minor metabolic reaction in vitro only (4% of oxidation products). Conjugation to spirotetramat-enol-GA was very prominent with the conjugate accounting for about 30%. Human liver cells showed an in-vitro metabolism that was more similar to that found in the mouse than in the rat. Conjugation to spirotetramat-enol-GA (6%) was more prominent than oxidative transformation, which was only detected as a minor transformation (1%). The results of this study are summarized in Table 14 (Totis, 2006).

1.3 Modelling of physiologically-based pharmacokinetics

Physiologically-based pharmacokinetic (PBPK) simulations were carried out with the commercially available software PK-Sim (Bayer Technology Services GmbH). PK-Sim is based on a generic whole-body PBPK model which describes the uptake and distribution of organic substances

Table 14. Relative distribution of the metabolites in hepatocytes of mice, rats and humans

Species	Metabolite (percentage of administered dose)					
	Spirotetramat-enol-glucuronide (M = 477)	Spirotetramat-enol alcohol (M = 317)	Spirotetramat-desmethyl-enol (M = 287)	Spirotetramat-enol (M = 301)	Spirotetramat-keto hydroxy (M = 317)	Spirotetramat (M = 373.45)
Spirotetramat, 50 µmol/l						
Rt (min)	15.2–15.4	25.4–25.8	35.5–36.4	45.6–45.8	47.1	59.2
Rat	0	4	7	87	3	0
Mouse	30	1	1	66	2	0
Human	6	0	1	92	0	0
Spirotetramat, 520 µmol/l						
Rat	0	0	0	100	0	0
Mouse	9	1	0	89	1	0
Human	2	0	0	98	0	0

From Totis (2006)
M, mass; Rt, retention time.

after oral or intravenous administration. The experimental data used in the model are shown in Table 15.

The model showed that the behaviour of spirotetramat in male rats is well described by physiology-based simulations. It is possible to find a single parameterization of the PBPK model in such a way that observed plasma concentrations, their nonlinearity in dose, organ concentrations and metabolism and excretion can be described by the simulation with excellent agreement with experimental data. Strongly increased concentrations of spirotetramat in the liver and kidney compared with the plasma and other tissues, as observed by QWBA (quantitative whole-body autoradiography), indicate the presence of active-transport processes for the uptake of spirotetramat metabolites into these tissues. Moreover, the renal excretion rate of spirotetramat-enol can only be explained by active tubular secretion into the urine, because the physicochemical properties yield a glomerular filtration rate that is much smaller than the observed rate of excretion. It was shown that saturation of the renal transport processes is highly likely to be responsible for the experimentally observed nonlinearity of plasma concentrations of spirotetramat at doses of 2 mg/kg bw and 100 mg/kg bw. While the available experimental data do not indicate which of the two processes, uptake or excretion, is most critical, it is reasonable to assume that in reality there is simultaneous saturation of both processes, as unbound concentrations comparable to typical binding constants (in the pmol range) for transport proteins are reached or exceeded in the plasma as well as the kidney cells.

The PBPK simulations allowed extrapolation to doses higher than those investigated experimentally in studies of sbsorption, distribution, metabolism and excretion. Calculations were carried out for doses up to 1000 mg/kg bw, covering the range that has been studied in toxicological experiments. It was found that the saturation of the active-transport processes responsible for transferring metabolites from plasma to excretory organs leads to a strong change in the shape of the plasma concentration curve at the highest doses. This has in turn a significant impact on pharmacokinetic parameters, describing the systemic exposure to a compound. While the dose-normalized maximum concentrations show only a immoderate increase with dose, this increase is rather high for the area under the concentration curve. The AUC_{norm} is about five times higher at 1000 mg/kg bw than at 2 mg/kg bw. This means that the increase in overall systemic exposure is five times higher than expected in the usually assumed case of dose linearity. An even more pronounced change was found for the peak/trough ratio $[C_{max}/C_{(24h)}]$. This parameter decreases by a factor of approximately 500, reaching values as low as 5–6 at 1000 mg/kg bw. Such low peak/trough ratios are indicative of a potential continuous rise of systemic concentrations upon repeated daily administration. In fact, such a rise in body burden was found for doses above 500 mg/kg bw in simulations of daily administration for

Table 15. Experimental data used for a physiologically-based pharmacokinetic model with spirotetramat

Title	Data used	Reference
[Azaspirodecenyl-3-14C]-spirotetramat: absorption, distribution, excretion and metabolism in the rat	Plasma concentrations (total radioactivity) at doses of 2 mg/kg bw and 100 mg/kg bw. Metabolite spectrum in excreta at doses of 2 mg/kg bw and 100 mg/kg bw	Klempner (2006b)
[Azaspirodecenyl-3-14C]spirotetramat: distribution of the total radioactivity in male and female rats determined by quantitative whole-body autoradiography (QWBA) including determination of the total radioactivity in excreta and exhaled 14CO2	Tissue concentrations (total radioactivity) in peripheral organs	Klempner (2006a)
[Azaspirodecenyl-3-14C]-spirotetramat: comparison of the in vitro metabolism in Liverbeads™ from rat, mouse and human.	Fraction of desmethyl-enol detected after incubation with spirotetramat at 50 µmol/l for 4 h	Totis (2006)

From Schmitt (2006a)

4 weeks. At 1000 mg/kg bw, the mean daily concentrations increased about twofold with time until a steady state was reached after about 75 days. This led to an even more marked non-linearity of the AUC, with a sevenfold increase of AUC_{norm} between 2 mg/kg bw and 1000 mg/kg bw, compared with fivefold after a single dose.

A sensitivity analysis also revealed that under certain circumstances peak/trough ratios considerably less than 5 will be obtained. This is the case if either the fraction of dose absorbed from the gut is higher or the metabolic rate for the transition of enol to desmethyl-enol is lower at high doses than in the present simulations. The actual values of the properties might generally differ from those of the simulation because they rely on parameter values estimated with some uncertainty. An assessment of the dependence of the disproportional increase in systemic exposure on the peak/trough ratio showed that a distinct change in behaviour occurs between ratios of 5 and 3. Below that range, the moderate temporal increase of systemic exposure described above changes into a strong one, with concentrations increasing continuously over the 4 weeks investigated here. This leads to systemic exposures (AUC) to the active substance at doses that are about 20 times higher than those extrapolated linearly from values at the lowest dose. Although this case was not predicted here even for the highest dose of 1000 mg/kg bw, it cannot be excluded that it actually occurs. A sensitivity analysis revealed that within the uncertainty range of estimated model parameters, peak/trough ratios that are even lower than 3 could be obtained. This is the case if the binding constant of spirotetramat to the hepatic-uptake transport protein is actually lower than estimated here, a case that is reasonable, but needs further experimental data to be confirmed or rejected. The disproportionate increase in plasma concentration after repeated administration of spirotetramat might have an impact on systemic exposure after high doses in toxicological studies. However without further supporting data, the simulations at doses greater than 100 mg/kg bw should be viewed with caution, since the PBPK model was developed using experimental data only up to a dose of 100 mg/kg bw (Schmitt, 2006a).

A more refined PBPK model using the results of the study of absorption, distribution, metabolism and excretion (Klempner, 2006a) and the organ metabolism study (Klempner, 2006b) predicted a distinct, disproportionate increase of the body burden by spirotetramat-enol and spirotetramat-desmethyl-enol after repeated administration of very high doses of spirotetramat due to saturation of active transport process for this substance. Using the most recent experimental information about tissue concentrations in liver and kidney at the highest dose and the concentrations of the metabolite desmethyl-enol, parameterization of the PBPK-model for spirotetramat could be improved significantly. The results of the PBPK-simulations show a good agreement with observed data for the time-course of the plasma and tissue concentrations of spirotetramat-enol and spirotetramat-desmethyl-enol as well as for the excreted amounts. In most cases the calculated data show deviations from those observed within or below a factor of two. For the dose of 1000 mg/kg, calculated enol concentrations were four–seven-fold higher than those determined experimentally. No explanation for this discrepancy could be found, although several hypotheses were tested. However, other results that depend on the spirotetramat-enol plasma concentration (e.g. the excreted amounts of spirotetramat-enol and spirotetramat-desmethyl-enol or the spirotetramat-desmethyl-enol plasma and tissue concentrations) are in good agreement with the experimental data. Moreover, it could be shown that the saturation of the transport processes involved in the excretion is predominantly responsible for the decreases in peak/trough ratio at high doses that were experimentally confirmed for 1000 mg/kg bw.

For the application of the model in simulations of scenarios not investigated experimentally it is, however, important that, despite the discrepancy in absolute concentrations found at high doses, the general pharmacokinetic behaviour with the strong reduction of $C_{max}/C_{(24h)}$ with increasing dose is well represented by the calculations. The reduction of the peak/trough ratio is the main reason for the strong rise of concentrations predicted for repeated administration of doses larger than 300 mg/kg bw. While for the dose of 100 mg/kg bw per day no long-term increase in plasma concentration

was observed, this is clearly the case at 500 mg/kg bw per day. At 300 mg/kg bw, there is only a slight increase of about a factor of two over 4 weeks. Simulations of repeated daily administration of spirotetramat for 4 weeks revealed that mean daily plasma concentrations of spirotetramat-enol remained constant over time after doses of 10 or 100 mg/kg bw per day, but rising plasma concentrations were seen after dosing with 300 mg/kg bw per day due to the beginning of saturation of the uptake process into liver and kidney cells. It is predicted that mean daily plasma concentrations of spirotetramat-desmethyl-enol also remain constant after doses of 10 or 100 mg/kg bw per day, increase linearly with time at daily doses of 300 or 500 mg/kg bw per day and increase nearly 100-fold after daily administration of 1000 mg/kg bw per day. As was hypothesized by the authors of the previously described PBPK-modelling study the disproportionate increase in plasma concentration of the metabolites after repeated administration of spirotetramat might have an impact on systemic exposure after high doses in toxicological studies (Schmitt, 2006b).

2. Toxicological studies

2.1 Acute toxicity

(a) Oral toxicity

In a study of acute oral toxicity, groups of five fasted female Wistar rats were given spirotetramat (purity, 93.5%) at a dose of 2000 mg/kg bw by gavage. All the rats survived and gained weight during the study. No clinical signs of toxicity were observed, nor were there any findings at necropsy. The median lethal dose (LD_{50}) was > 2000 mg/kg bw (Eigenberg, 2004a).

(b) Dermal toxicity

In a study of dermal toxicity, groups of five male and five female Wistar rats were given spirotetramat (purity, 93.5%) at a dose of 2000 mg/kg bw applied dermally. All the rats survived and gained weight during the study. Clinical signs observed on days 0–3 were: red stained-nose, wetness-urogenital area, yellow-stained urogenital area, and red-coloured skin on the back. There were no findings on necropsy. The dermal LD_{50} for male and female rats was > 2000 mg/kg bw (Eigenberg, 2004b).

(c) Exposure by inhalation

In a study of exposure by inhalation, groups of five male and five female Wistar rats were exposed to spirotetramat (purity, 96.5%) at mean solid aerosol concentrations of 1.10 or 4.18 mg/l air by nose-only exposure for 4 h followed by a 2-week observation period. Attempts were made to make the aerosols generated respirable to rats. With regard to the respirability of the aerosol generated, internationally recognized recommendations such as SOT (Society of Toxicology, 1992) were fulfilled, i.e. the median mass aerodynamic diameter (MMAD) was 3.7–5.1 μm (geometric standard devaition, GSD 2.3).

Exposure to spirotetramat at concentrations of up to 4.18 mg/l air did not result in mortality. The following clinical signs were observed: ungroomed hair-coat, piloerection, bradypnoea, laboured breathing, dyspnoea, breathing sounds, **reddened** nostrils, nasal discharge (serous), **red** nostrils **with** encrustations, **red encrustations in** nose/snout region:, stridor, reduced motility, limpness, high-legged gait, impaired reflexes, hypothermia, and decreased body weights. The duration of signs was governed by respiratory effects indicative of irritation of the lower and upper respiratory tract and resolved towards the beginning of the second week after exposure. Findings on necropsy were unremarkable. The median lethal concentration (LC_{50}) of spirotetramat was > 4.18 mg/l air (Pauluhn, 2002).

(d) Dermal irritation

In a study of acute dermal irritation, three male rabbits were given 500 mg of spirotetramat (purity, 96.5%) applied dermally under a patch to the shaved intact dorsal skin. After a 4-h exposure period, the patch was removed and the skin sites were evaluated. Scores were taken 60 min, 24 h, 48 h and 72 h after patch removal. None of the three rabbits showed any test substance-related lesions. There were no systemic intolerance reactions. Spirotetramat is not a skin irritant (Leuschner, 2002a).

(e) Ocular irritation

To examine the effect of spirotetramat on rabbit eyes, three male rabbits were each given a single application of 100 mg of spirotetramat (purity, 96.5%) into the conjunctival sac of the right eye. Corneal opacity (grade1) was observed in all rabbits 24 h to 6 days after instillation and in two rabbits up to 7 days after instillation. Irritation of the iris (grade 1) was observed in all rabbits (rabbit No. 1, 24 h to 6 days after instillation; rabbit No. 2, 72 h to 5 days after instillation; and rabbit No. 3, 24 h to 5 days after instillation). Conjunctival redness (grade 1) was observed in all rabbits 1 h to 72 h after instillation and in Nos 2 and 3 until 4 days after instillation. Conjunctival chemosis (grade 1) was noted in rabbit No. 3 24 h and 48 h after instillation. There were no systemic intolerance reactions. Spirotetramat is an eye irritant (Leuschner, 2002b).

(f) Dermal sensitization

Guinea-pigs

In a maximization test that complied with OECD guideline No. 406, EC guideline 96/54/EC (22nd adaptation of guideline 67/548/EEC) and health effects test guideline OPPTS 870.2600, 20 female guinea-pigs were given spirotetramat (purity, 96.5%; formulated in polyethylene glycol 400 to yield a suspension) at the following concentrations: intradermal induction, 5%; topical induction, 50%; challenge, 25%. Two additional guinea-pigs were used for dose-finding and the control group comprised 10 guinea-pigs. Challenge with spirotetramat at 25% produced skin effects (grade 1–3) in 18 out of 19 guinea-pigs (95%). No skin effects were seen in guinea-pigs in the control group. Under the conditions of the maximization test and with respect to the evaluation criteria, spirotetramat exhibits a skin-sensitization potential (Vohr, 2002).

In a Buehler epicutaneous patch test that complied with OECD guideline No.406, EC guideline 96/54/EC (22nd adaptation of guideline 67/548/EEC and health effects test guideline OPPTS 870.2600, 20 female guinea-pigs were given spirotetramat (purity, 97.2%; formulated in polyethylene glycol 400 to yield a suspension or a paste) at the following concentrations: first to third induction**:**, 71%; challenge**:**71%. The control group comprised 10 guinea-pigs. Two additional guinea-pigs were used for dose-finding for the challenge concentration. There were no skin effects after induction or challenge in either the control group or the group receiving spirotetramat. The Meeting concluded that spirotetramat has no skin-sensitization potential (Vohr, 2004).

Mice

The local lymph-node assay (LLNA) was used to assess dermal contact sensitization in mice treated with spirotetramat. Groups of five female CBA/J mice were treated with spirotetramat (purity, 97.2%) at a concentration of 1%, 2.5%, 5% or 10%. Additional groups were treated with isoeugenol, the positive control, at a concentration of 0.5%, 1%, 2.5% or 5%. One additional group received dimethylformamide, the vehicle, only. The test substances were applied on the external surfaces of each

ear (i.e. 50 µl per mouse) for three consecutive days (days 0, 1 and 2) at the appropriate concentration. On day 5, the cell proliferation in the local lymph nodes was measured by incorporation of tritiated methyl-thymidine and the values obtained were used to calculate proliferation indices.

No mortality and no clinical signs of toxicity were observed during the study. No cutaneous reactions were observed in any group. The proliferation indices for spirotetramat were 3.4, 4.3, 5.4 and 5.9 at concentrations of 1%, 2.5%, 5% and 10%, respectively. The proliferation indices for the positive control were 0.8, 1.3, 1.8 and 3.4 at concentrations of 0.5%, 1%, 2.5% and 5%, respectively. The skin sensitization potential of spirotetramat was approximately five times that of isoeugenol, the positive control (Esdaile, 2004).

2.2 Short-term studies of toxicity

Mice

In a 4-week dose range-finding study that did not comply with GLP, groups of five male CD-1 mice were fed diets containing spirotetramat (purity, 97%) at a concentration of 0, 500 or 5000 ppm, equivalent to 0, 136.5 and 1415 mg/kg bw per day. The method of calculating compound intake (mg/kg bw per day) was not reported

There was no mortality or any treatment-related clinical findings, apart from diminished food intake compared with the control group (500 ppm, –19%; 5000 ppm, –16%) and no other toxicological findings were noted.

Table 16. Acute toxicity of spirotetramat

Species	Strain	Sex	Route	LD50 (mg/kg bw)	LC50 (mg/l air)	Other effects	Reference
Rat	Wistar	Female	Oral	> 2000	—	—	Eigenberg (2004a)
Rat	Wistar	Male and Female	Dermal	> 2000	—	—	Eigenberg (2004b)
Rat	Wistar	Male and female	Inhalation (4-h, nose only) (MMAD was 3.7–5.1 pm, solid aerosol)*	—	> 4.18	—	Pauluhn (2002)
Rabbit	Himalayan	Male	Dermal irritationa	—	—	Not an irritant	Leuschner (2002a)
Rabbit	Himalayan	Male	Ocular irritationa	—	—	Irritant	Leuschner (2002b)
Guinea-pig	SPF strain Hsd Poc:DH	Female	Dermal sensitization (maximization)[a]	—	—	Exhibits a skin-sensitization potential	Vohr (2002)
Guinea-pig	SPF strain Crl:HA	Female	Skin sensitization (Buehler patch test)[a]	—	—	No evidence of skin sensitization	Vohr (2004)
Mice	CBA/J	Female	Skin sensitization effects (local lymph node assay)[a]	—	—	Skin-sensitizing potential	Esdaile (2004)

[a] The test material used for inhalation, eye and primary irritation and other sensitization studies is a *cis*-isomer of the TGAI test material used for the acute oral and acute dermal studies.
MMAD, mass median aerodynamic diameter.

The no-observed-adverse-effect level (NOAEL) was 5000 ppm, equivalent to 1415.2 mg/kg bw per day, on the basis of pathology and the organ investigated **(liver/gall bladder, adrenal glands, testes and epididymides)** (Schladt, 2001).

In a study that complied with GLP and for which a statement of quality assurance (QA) was provided, groups of 15 male and 15 female CD-1(ICR)/BR mice were given diets containing spirotetramat (purity, 93.1–96.5%) at nominal dietary concentrations of 0, 70, 350, 1700 or 7000 ppm, equal to 0, 13, 60, 300 and 1305 mg/kg bw per day for males and 0, 6, 72, 389 and 1515 mg/kg bw per day for females, for approximately 14 weeks. The mice were aged approximately 10 weeks at the initiation of the study. The objectives of this study were to obtain a toxicological profile for spirotetramat under conditions of prolonged and repeated exposure in the mouse, to establish doses for a subsequent lifetime exposure study and also to design a study to assess the oncogenic potential in this animal.

After approximately 14 weeks of continuous dietary exposure to the test substance, no toxicologically relevant response was observed.

The NOAEL was 7000 ppm, equal to1305 mg/kg bw per day, on the basis of lack of findings at any dose tested (Wahle, 2005a).

Rats

In a study of dermal administration, which complied with GLP and for which a statement of QA was provided, groups of 10 male and 10 female Wistar Hanover CRL:WI(GLX/BRL/HAN)IGS BR rats were given spirotetramat (purity, 97.6–98.5%) dermally at a dose of 0, 100, 300 or 1000 mg/kg per day for 28 or 29 days. The rats were aged approximately 8–10 weeks at the initiation of study. The test substance was held in contact with the skin for a minimum of 6 h per day for five consecutive days per week for 4 weeks. The bandage and tape were removed each day and the application site was gently wiped with water-dampened gauze and then with dry gauze to remove as much test substance residue as feasible without damaging the skin. The resulting mean dose was approximately 700 mg/kg bw per day at the highest dose. During the dosing period, clinical observations were conducted daily and body weights were measured weekly. Ophthalmic examinations were performed once before administration of the test substance and during week 4 of the study. Clinical chemistry and haematology analyses were performed on all rats during week 3 of the study, except for prothrombin time and thromboplastin time which were analysed during week 4. A gross necropsy was performed, organ weights were taken and tissues were examined microscopically.

Since no treatment-related effects were observed in males or females at any dose, the NOAEL was 1000 mg/kg bw per day, the highest dose (Eigenberg, 2006a).

In a 4-week study that did not comply with GLP, groups of five female Hsd/Win:WU rats were diets containing spirotetramat (purity, 98.2%) at a concentration of **0**, 500 or 5000 ppm (equal to **0**, 47.3 and 501.8 mg/kg per day) for 4 weeks.

The intake of test compound was proportional to the dietary concentration.

No clinical signs of toxicity were observed and survival was unaffected by treatment with spirotetramat. Values for body weight, body-weight gain and food intake of treated rats were comparable to those for rats in the control group. Clinical chemistry examination revealed a non-dose-related decrease in triglyceride concentrations in rats at 500 and 5000 ppm.The liver weight of treated rats was comparable to that of rats in the control group. Gross pathology and histopathology showed no treatment-related effects. The liver-cell proliferation assay showed no relevant increase in cell proliferation or in nuclear-area values for treated rats. The study author concluded that a 4-week treatment with spirotetramat slightly influenced lipid metabolism in female rats. However,

this finding was **not** considered to be toxicologically. The results of the present study were also supported by the findings in CD-1 mice in which dietary exposure to spirotetramat resulted in no treatment-related effects in male mice up to a dietary concentration of 5000 ppm (Krotlinger et al., 1998).

In a short-term feeding study that complied with GLP and for which a statement of QA was provided, groups of male and female Wistar Hanover rats (Crl:WI[Glx/BRL/Han]IGS BR; aged approximately 10 weeks) were given diets containing spirotetramat (purity, 93.1–96.5%) at nominal dietary concentrations of 0, 150, 600, 2500 or 10 000 ppm for approximately 14 weeks. The mean daily intake of spirotetramat over approximately 14 weeks at nominal dietary concentrations of 150, 600, 2500 or 10 000 ppm was equal to 9, 36, 148 and 616 mg/kg bw per day for males and 11, 46, 188 and 752 mg/kg bw per day for females, respectively. The control group and group at the highest dose contained 20 males and 20 females, while all other groups contained 10 females and 10 females. To assess the reversibility of any effects observed during the exposure period, 10 males and 10 females from the control group and the group at the highest dose were placed on control diet for the remainder of the study (approximately 4 weeks). The rats were aged approximately 10 weeks at the initiation of study.

Body weight and food consumption determinations as well as a detailed clinical examination of each rat were conducted weekly throughout the study. Observations for moribundity and mortality were performed once daily. Standard haematological, clinical chemistry and urine-analysis endpoints were evaluated from blood drawn via the orbital sinus (while under light anaesthesia with IsoFlo®; isoflurane); and urine collected just before the respective termination of both the exposure and recovery periods. Selected hepatic-enzyme activities were also measured. Ophthalmological examinations were conducted on all acclimatized rats before exposure, and then again on all surviving rats immediately before the respective termination of both the exposure and recovery periods of the study. All rats were examined post mortem.

At 10 000 ppm, a decrease in the rate of body-weight gain of 17.6% was noted in males only; a corresponding decrease of 8.4% was noted in absolute body weight after 14 weeks. Organ-weight changes were limited to a slight decrease in absolute testicular weight. Histopathological considerations included an increased incidence of minimal to severe abnormal spermatozoa and hypospermia in the epididymis and minimal to moderate tubular degeneration in the testis. The average incidence and severity (in parentheses) of treatment-related microscopic findings in testes and epididymis is shown in Table 17.

Table 17. Average incidence and severitya of treatment-related microscopic findings in testes and epididymis of rats given diets containing spirotetrmat for 14 weeks

Finding	Dietary concentration (ppm)						
	0	150	600	2500	10 000	0	10 000
						Recovery	Recovery
Epididymis, abnormal spermatozoa	0	—	0	0	9* (1.8)	0	1 (1.0)
Epididymis, hypospermia	0	—	0	0	5* (2.6)	0	1 (5.0)
Testes, tubular degeneration	0	—	0	0	5* (2.0)	0	1 (2.0)
Testes, vacuolization	0	—	0	0	5* (1.6)	0	0

From Wahle (2005a)

***** Significantly different from controls, $p < 0.05$.

[a] Figures in parentheses indicate average severity, graded from 1 (minimal) to 5 (severe).

An increased incidence of minimal to slight accumulation of alveolar macrophages in the lungs (males, 2, 4, 0, 5, 9; females, 1, 0, 0, 1, 7) was noted in both sexes and was statistically significant at the highest dose. The effects on all parameters showed a degree of reversibility by the end of the recovery period and many parameters had fully reverted to control values. The effects on testes and epididymis (including sperm) were reversible in most animals after cessation of treatment. No effects attributable to exposure to spirotetramat at 150, 600 and 2500 ppm were observed.

The lowest-observed-adverse-effect level (LOAEL) was 10 000 ppm, equal to 616 mg/kg bw per day, on the basis of decrease in body-weight gain in males, structural changes in the testes, and structural changes in the lungs of males and females. The NOAEL was 2500 ppm, equal to 148 mg/kg bw per day, on the basis of decreased body-weight gain in males, structural changes in the testes, abnormal spermatozoa and hypospermia and tubular degeneration and structural changes in the lungs (males and females) at 10 000 ppm, equal to 616 mg/kg bw per day (Wahle, 2005a).

In a 1-year study of oral toxicity, groups of 25 male and 25 female Wistar Hanover (Crl:WI[Glx/BRL/Han]IGS BR) rats (age approximately 9–10 weeks) were given diets containing spirotetramat (purity, 97.5–98.5%) at nominal dietary concentrations of 0, 250, 3500 or 7500 ppm (males)/12 000 ppm (females) for approximately 1 year. The mean daily intake of the test substance (mg spirotetramat/kg bw per day) over approximately 1 year at nominal dietary concentrations of 250 ppm, 3500 ppm or 7500ppm (males)/12 000 ppm (females) respectively, was equal to 13, 189 and 414 for males and 18, 255 and 890 for females. All test diets (including control) were available for consumption ad libitum at all times. The concentration of spirotetramat in the diet, as well as the homogeneity and stability of spirotetramat as a dietary admixture was confirmed.

Body weight and food consumption determinations were conducted weekly for 13 weeks and once per month (at either 4 or 5 weeks, depending on the number of days in the month) thereafter; detailed clinical examinations, including general open-field observations, of each rat were conducted weekly throughout the study. Observations for moribundity and mortality were performed at least once daily. During month 12, the last 10 rats of each sex per dose were subjected to a functional observational battery (FOB) test to assess motor activity, grip strength and sensory reactivity to stimuli of different types (e.g. visual, auditory and proprioceptive stimuli). Standard haematological, clinical chemistry and urine-analysis end-points were evaluated from blood (fasted; drawn via the orbital sinus while under light anesthesia with IsoFlo®; isoflurane) and urine collected at approximately 3, 6, and 12 months during the study. Ophthalmological examinations were conducted on all acclimatized rats before exposure, and then again on all surviving rats just before termination. All rats were given a post-mortem examination, which included documenting and saving all gross lesions, weighing designated organs, and collecting representative tissue specimens for histopathological evaluation.

At a dietary concentration of 250 ppm, no effects attributable to exposure to spirotetramat were observed.

At 3500 ppm there was an increase in the incidence of minimal to slight accumulation of alveolar macrophages in the lungs in males only. No other exposure-related effects were observed in this group.

At the highest dietary concentration, 7500 ppm for males and 12 000 ppm for females, a decline in body weight of 6.6% was noted in females, based on final live body weight (compared with controls). However male body weight was unaffected. Clinical observations included an increased incidence of yellow and brown staining in females, generally located in the perigenital area and tail. Gross observations consisted of an increased incidence of discoloration of the lung in females. Slightly increased liver weight was found in both sexes. Histopathological considerations included a statistically significantly increased incidence of minimal to slight accumulation of alveolar

macrophages in the lungs in both sexes. An increased incidence of exfoliated germ cells/debris was observed in the epididymis of males at 7500 ppm (3 out of 25 vs 0 out of 24 in controls). Abnormal spermatozoa were also observed in males at the highest dose (2 out of 25 vs 0 out of 25 in controls). Although the increases were not statitistically significant, they were considered to be toxicologically relevant. Microscopic findings are summarized in Table 18.

The NOAEL was 250 ppm, equal to 13.2 mg/kg bw per day, on the basis of an increased in the incidence of accumulation of alveolar macrophages in the lungs of males at 3500 ppm. The studies complied with GLP and a statement of QA was provided (Wahle, 2005b).

Dogs

In a 4-week dose range-finding study that complied with GLP and for which a statement of QA was provided, groups of two male and two female beagle dogs (aged 5–6 months) were given diets containing spirotetramat (purity, 93.5–96.5%) at a concentration of 0, 100, 400, 1600 or 6400 ppm, equal to 0, 3, 13, 42 and 104 mg/kg bw per day for males and 0, 3, 12, 70 and 127 mg/kg bw per day for females. Clinical observations and food consumption were measured daily, while body weights were measured weekly. Ophthalmological, neurological and heart and liver examinations were performed before exposure and before sacrifice. Clinical chemistry and haematological parameters were measured before exposure and on day 7 and on day 23, while urine analysis was conducted before exposure, then on day 9 and 24. At study termination, liver UDP-GT activity was measured, and gross necropsy, organ weight and histopathology were peformed.

In the group at 6400 ppm, the highest dose, body weight and food consumption were decreased. There were compound-related clinical findings at this dose, including emaciation and loss of weight. Concentrations of calcium and albumin were decreased, secondary to emaciation, and there was also a decrease in thymus weight in this group. Microscopic changes observed at this dose included atrophy of the parotid salivary gland (one male), exacerbated sexual immaturity (one male) and thymic involution (one male and both females).

Although there were compound-related decreases in the concentrations of the thyroid hormones triiodothyronine (T3), thyroxin (T4) and thyroid-stimulating hormone (TSH) in the groups at 400, 1600 and 6400 ppm (Table 19), these alterations were not considered to be biologically significant owing to the lack of accompanying abnormalities in thyroid weights or histological appearance of the thyroid gland in these dogs. The hormonal alterations were not considered to be of sufficient

Table 18. Incidence and average severity (figures in parenthesis) of microscopic findings in rats fed diets containing spirotetramat for 1 year

Parameter	Incidence (severity[a])			
	Dietary concentration (ppm)			
	0	250	3500	7500/12 000
Males (n = 25)				
Lungs, macrophages, alveolar	1 (1.0)	2 (1.0)	6 (1.0)*	11 (1.2)*
Testes, abnormal spermatozoa	0	0	0	2 (1.5)
Epididymides, exfoliated germ cells/debris	0	0	0	3 (2.0)
Females (n = 25)				
Lungs, macrophages, alveolar	5 (1.0)	3 (1.0)	2 (1.0)	21 (1.3)*

From Wahle (2005b)

* $p < 0.05$.

[a] Figures in parentheses indicate average severity, graded from 1 (minimal) to 5 (severe).

magnitude to produce biologically significant effects in the thyroid endocrine axis after 28 days of exposure.

The NOAEL was 1600 ppm, equal to 42 mg/kg bw per day, on the basis of decreases in body weight, decreased concentrations of calcium, albumin, T4, T3 and TSH, and decreased thymus weight and involution at 6400 ppm.

Table 19. Serum concentrations of thyroid hormones in male dogs (n = 2) given diets containing spirotetramat for 4 weeks

Dietary concentration (ppm)		Serum concentration of hormone (ng/ml)		
		−11 days	7 days	23 days
T3				
0	Mean	1.0	1.0	0.9
	SD	0.0	0.0	0.1
100	Mean	0.9	1.0*	0.9
	SD	0.0	0.0	0.1
400	Mean	0.9	0.8*	0.7
	SD	0.2	0.0	0.1
1600	Mean	1.1	0.7*	0.6
	SD	0.6	0.1	0.3
6400	Mean	0.9	0.4*	0.4
	SD	0.1	0.0	0.1
T4				
0	Mean	2.6	1.8	1.5
	SD	0.8	0.3	0.3
100	Mean	2.3	1.7	1.5
	SD	0.1	0.5	0.3
400	Mean	2.1	1.2	0.9*
	SD	0.1	0.7	0.1
1600	Mean	2.4	0.8	0.6*
	SD	0.7	0.3	0.0
6400	Mean	2.6	0.3	0.3*
	SD	0.2	0.2	0.3
TSH				
0	Mean	0.32	0.24	0.24
	SD	0.20	0.13	0.19
100	Mean	0.21	0.16	0.10
	SD	0.13	0.13	0.07
400	Mean	0.31	0.22	0.13
	SD	0.22	0.25	0.15
1600	Mean	0.21	0.09	0.12
	SD	0.01	0.06	0.04
6400	Mean	0.16	0.07	0.06
	SD	0.01	0.04	0.06

From Eigenberg (2004c)
SD, standard deviation; T3, triiodothyronine; T4, thyroxin; TSH, thyroid stimulating hormone.
* $p < 0.05$.

In a study that complied with GLP and for which a QA statement was provided, groups of four male and four female beagle dogs (aged 7–8 months) were given diets containing spirotetramat (purity, 97.6–97.8%) at a concentration of 0, 150, 300, 1200 or 4000/2500 ppm, corresponding to intakes of 5, 9, 33 and 81 mg/kg bw per day for males and 6, 10, 32 and 72 mg/kg bw per day for females (not including the highest dose), for 92–95 days. The group at the highest dose was given spirotetramat at 4000 ppm for 2 weeks, but this dose was subsequntly lowered to 2500 ppm owing to excessive

Table 20. Serum concentrations of thyroid hormones in female dogs (n = 2) given diets containing spirotetramat for 4 weeks

Dietary concentration (ppm)		Serum concentration of hormone (ng/ml)		
		−11 days	7 days	23 days
T3				
0	Mean	0.8	1.1	1.0
	SD	0.0	0.3	0.1
100	Mean	0.7	0.7	0.8
	SD	0.1	0.3	0.1
400	Mean	0.8	0.9	0.9
	SD	0.1	0.2	0.3
1600	Mean	1.1*	1.0	1.0
	SD	0.0	0.0	0.0
6400	Mean	0.8	0.5	0.4*
	SD	0.1	0.1	0.0
T4				
0	Mean	3.5	1.9	1.9
	SD	0.6	1.1	0.7
100	Mean	2.9	1.6	1.7
	SD	0.3	0.2	0.0
400	Mean	2.3	0.9	1.0
	SD	0.4	0.2	0.4
1600	Mean	3.5	1.2	1.4
	SD	0.6	0.2	0.1
6400	Mean	2.2	0.4	0.3*
	SD	0.7	0.2	0.0
TSH				
0	Mean	0.34	0.34	0.24
	SD	0.12	0.05	0.09
100	Mean	0.09	0.17	0.13
	SD	0.08	0.04	0.00
400	Mean	0.25	0.20	0.22
	SD	0.12	0.16	0.12
1600	Mean	0.24	0.19	0.29
	SD	0.13	0.13	0.19
6400	Mean	0.12	0.03	0.02
	SD	0.02	0.01	0.01

From Eigenberg (2004c)
SD, standard deviation; T3, triiodothyronine; T4, thyroxin; TSH, thyroid stimulating hormone.
*$p < 0.05$

weight loss. Clinical observations were conducted daily, food consumption was measured daily and body weights were taken weekly. Ophthalmological examinations were performed before exposure and before sacrifice. Clinical chemistry, haematology and urine-analysis measurements were taken once before exposure and during study weeks 4, 8 and 13. A gross necropsy was performed, organ weights were measured and tissues were examined microscopically.

No dogs were died or were sacrificed in extremis in this study.

During the first 2 weeks of the study, there was a compound-related reduction in body weight in the group at 4000 ppm. After week 2, when the dose of 4000 ppm was reduced to 2500 ppm, there was no compound-related effect on body weight. Although the females in this group began to gain body weight after the dietary concentration of test material was reduced, it was evident that they did not recover completely since the mean body weight at termination was still below the value before exposure. During the clinical assessments, one of these females was described as thin. Food-consumption values for this group were also depressed throughout the study.

Decline in values for erythrocyte parameters (erythrocyte count, haemoglobin concentration and erythrocyte volume fraction) were seen in females receiving spirotetramat at 2500 ppm on day 58; this decline continued throughout the remainder of the study and the difference between the values of these parameters in the group at 2500 ppm and the control group was statistically significant at day 84.

Gross examinations revealed that the thymus of one female at 2500 ppm was atrophied, or "reduced in size" as described in the histopathology report.

Compound-related decreases in concentrations of thyroid hormones were observed in the groups at 1200 and 2500 ppm. However, the alterations were not considered to be of a sufficient magnitude to produce biologically significant effects (i.e. no change in thyroid weight, no microscopic thyroid changes and no compensating increase in TSH).

The NOAEL was 1200 ppm, equal to 32 mg/kg bw per day, on the basis of body-weight loss and haematological effects in females at 4000/2500 ppm (Eigenberg, 2005).

In a short-term study that complied with GLP for which a QA statement was provided, groups of four male and four female beagle dogs (age 7–8 months) were given diets containing technical-grade spirotetramat (purity, 97.6–98.5%) at a concentration of 0 (concurrent vehicle control), 200, 600 or 1800 ppm, equivalent to 0, 6, 20 and 55 mg/kg bw per day for males, 0, 5, 19 and 48 mg/kg bw for females, respectively, for 1 year. Clinical observations were made daily. Food consumption was measured daily and body weights were taken weekly. Clinical chemistry, urine analysis and full blood count, including differentials, were performed for all dogs once before administration of the test substance. After initiation of dosing, haematological, clinical chemistry and urine-analysis data were collected from all dogs during study weeks 13, 25, 38 and 51. During study week 22, blood was collected from all dogs for evaluation of T4, T3 and TSH.. Ophthalmic examinations were performed before exposure and before sacrifice. A gross necropsy was performed, organ weights were measured and tissues were examined microscopically. The analysis of the results revealed that notable clinical findings, like dehydration, swelling, decreased activity and reactivity, seizures and ataxia were observed in a male at 1800 ppm. There were no unscheduled mortalities during the study. No compound-related effect on food consumption and body weight were observed. Haematologically, there were no toxicologically significant findings. There was a compound-related decrease in T4 for males and females at all doses and a decrease in T3 for males at all doses and for females at 1800 ppm. Two males showed a slight reduction in the size of the peripheral thyroid follicles that was considered to be compound-related. Thyroid follicular cells within the affected follicles in these two males did not differ from those of the controls. Despite the reduced serum concentrations of T3 and T4 at some time-points, no changes in thyroid weight and no compensating increases in TSH were seen at ≤ 600 ppm. Therefore

the decreases in thyroid hormones at ≤ 600 ppm were not considered to be toxicologically significant. The changes in serum concentrations of T4 and T3 are presented in Tables 21 and 22, respectively.

Gross necropsy and organ weights revealed a reduced thymus size and dilated brain in males at 600 and 1800 ppm. Thymus involution was graded as mild in one male at 600 ppm and as moderate in one male at 1800 ppm. Brain ventricular dilatation was also noted in females, but only at 600 ppm. Brain ventricular dilatation was seen at 600 ppm in one male (mild) and one female (moderate) as well as at 1800 ppm in one male (moderate); mild axonal degeneration was detected in one female at 1800 ppm. The brain ventricular dilatation was not accompanied by any clear histopathological

Table 21. Serum concentrations of thyroxin (T4) in dogs given diets containing spirotetramat for up to 1 year

Dietary concentration (ppm)	Mean serum concentration (µg/dl)					
	Day –5	Day 96	Day 155	Day 180	Day 271	Day 357
Males						
0	2.14	2.35	2.17	2.13	1.86	1.84
200	2.20	0.99	1.30	0.87	0.94	1.16
600	2.33	0.89	1.14	0.70	0.78	1.02*
1800	2.74	0.53*	1.01*	0.48*	0.33**	0.58**
Females						
0	2.44	1.83	2.56	2.18	1.80	2.88
200	2.57	1.19	1.76	1.38	1.23	1.79*
600	2.63	0.93*	1.24**	1.32	0.90*	1.11**
1800	2.37	0.69**	1.12**	0.51**	0.58**	0.85**

From Eigenberg (2006b)
* $p \leq 0.05$; ** $p \leq 0.01$

Table 22. Serum concentrations of triiodothyronine (T3) in dogs given diets containing spirotetramat for up to 1 year

Dietary concentration (ppm)	Mean serum concentration (µg/dl)					
	Day –5	Day 96	Day 155	Day 180	Day 271	Day 357
Males						
0	0.89	0.79	0.71	0.82	0.64	0.65
200	0.87	0.61	0.60	0.64**	0.49	0.57
600	0.91	0.68	0.62	0.61**	0.50	0.67
1800	0.95	0.48*	0.52*	0.52**	0.43*	0.46*
Females						
0	0.80	0.70	0.69	0.85	0.61	0.73
200	0.80	0.73	0.62	0.75	0.58	0.62
600	0.78	0.69	0.66	0.82	0.63	0.63
1800	0.87	0.62	0.59	0.61	0.50	0.61

From Eigenberg (2006b)
* $p \leq 0.05$; ** $p \leq 0.01$.

alteration. Organ weights were not affected by treatment. Mean thyroid absolute and relative organ weights were not statistically different from those of the controls. Mean absolute heart weight was statistically significantly lower ($p < 0.05$) at 1800 ppm in males; however, there was no significant difference in mean relative weight and correlative histopathological changes were not found. Absolute and relative thymus weights were low (although not statistically significantly) in males only at 600 ppm. Histopathology revealed that two males at 1800 ppm had a slight reduction in the size of the peripheral thyroid follicles. The Meeting concluded that the reduced serum concentrations of T3 and T4 at 600 ppm were not an adverse effect as these changes were inconsistent, and there was no TSH feedback response, no clinical findings indicating hypothyroidism, and no morphological changes in the thyroid.

The NOAEL was 200 ppm, equal to 5 mg/kg bw per day, on the basis of involution of the thymus. This NOAEL is conservative in view of the equivocal changes in thyroid hormones, and the brain ventricular dilatation of uncertain significance seen at 600 ppm (Eigenberg, 2006b).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity for which statements of compliance with GLP and QA were provided, groups of 55 male and 55 female CD-1 (CD-1 [ICR]/BR) mice (age approximately 9 weeks) were given diets containing spirotetramat (purity, 97.4–98.5%) at nominal dietary concentrations of 0, 70, 1700 or 7000 ppm for approximately 18 months. The highest dietary concentration of 7000 ppm was reduced to 6000 ppm from week 12 of the study in order to achieve an average intake of active ingredient of approximately 1000 mg/kg bw per day throughout the 18 months of exposure. The mean daily intake of spirotetramat over approximately 18 months at nominal dietary concentrations of 70, 1700 or 7000 ppm, respectively, was equal to 10.9, 263 and 1022 mg/kg bw per day for males and 13.7, 331 and 1319 mg/kg bw per day for females.

All test diets (including control) were consumed ad libitum at all times. The concentration of spirotetramat in the diet and the homogeneity and stability of spirotetramat as a dietary admixture were confirmed.

Body weight and food consumption determinations were conducted weekly for 13 weeks and once per month (at either 4 or 5 weeks, depending on the number of days in the month) thereafter; detailed clinical examinations of each animal were conducted weekly throughout the study. Observations for moribundity and mortality were performed at least once daily. Standard differential leukocyte and erythrocyte morphology end-points were evaluated from blood drawn via the orbital sinus of unfasted mice at approximately 12 and 18 months. All mice were given a post-mortem examination, which included documenting and saving all gross lesions, weighing designated organs, and collecting representative tissue specimens for histopathological evaluation.

After approximately 18 months of continuous dietary exposure to the test substance, the mice showed no evidence of a compound-induced toxicological response at any dose up to and including the limit dose. No evidence of a compound-induced neoplastic response was observed in any tissue examined.

The NOAEL was 7000 ppm, equal to 1022 mg/kg bw per day, on the basis of lack of findings at this, the highest dose tested (Wahle, 2006b).

Rats

In a study of carcinogenicity for which statements of compliance with GLP and QA were provided, groups of 55 male and 55 female Wistar Han rats (Crl:WI[Glx/BRL/Han]IGS BR) (age approximately 8 weeks) were given diets containing spirotetramat (purity, 97.4–98.5%) at nominal

dietary concentrations of 0, 250, 3500 or 7500 ppm (males)/12 000 ppm (females) for approximately 2 years. The mean daily intake of spirotetramat (mg spirotetramat/kg bw per day) over approximately 24 months at nominal dietary concentrations of 250, 3500 or 7500 (male)/12 000 (female) ppm, respectively, were 12.5, 169 and 373 mg/kg bw per day for males and 16.8, 229 and 823 mg/kg bw per day for females.

All test diets (including control) were consumed ad libitum at all times. The concentration of spirotetramat in the diet and the homogeneity and stability of spirotetramat as a dietary admixture were confirmed.

Body weight and food consumption determinations were conducted weekly for 13 weeks and once a month (at either 4 or 5 weeks, depending on the number of days in the month) thereafter; detailed clinical examinations of each rat were conducted weekly throughout the study. Observations for moribundity and mortality were performed at least once daily. Standard differential leukocyte and erythrocyte morphology end-points were evaluated from blood drawn via the orbital sinus from unfasted rats at approximately 12, 18 and 24 months. All rats were given a post-mortem examination, which included documenting and saving all gross lesions, weighing designated organs, and collecting representative tissue specimens for histopathological evaluation.

At a dietary concentration of 250 ppm, no effects attributable to exposure to spirotetramat were observed.

At dietary concentrations of 3500 ppm and greater there were decreases in absolute kidney weight and an increased incidence of renal tubular dilatation in males (0; 0; 8*; 19*, out of 55 rats, respectively, out of the 55 rats in each group; * $p < 0.05$) and females (0, 0, 16*, 42*, respectively, out of the 55 rats in each group; * $p < 0.05$).

At dietary concentrations of 7500 (males)/12000 (females) ppm, a decline in body weight of 10% and 14% was noted in males and females, respectively, based on final live body weight measured during week 103 (compared with controls). Clinical observations included an increased incidence of "body, scaly" (generally limited to the hind limbs) and yellow and brown staining in males and females (staining being generally located in the perigenital area and/or on the tail and being less prevalent in males). Gross observations were limited to an increased incidence of small discoloured and/or raised zones on the lungs of females. Organ-weight changes included decreased absolute kidney weight and increased relative lung weight in males and females.

Histopathological findings were noted in the kidneys, lungs, testes and epididymis, and the bile duct. In the kidney, findings included increases in the incidence of renal tubular dilatation in males at 3500 ppm and greater (0, 0, 8*, 19*, respectively, out of the 55 rats in each group; * $p < 0.05$) and females (0, 0, 16*, 42*, respectively, out of the 55 rats in each group; * $p < 0.05$). In the lungs, an increased incidence of alveolar macrophage accumulation and a complex of changes described as interstitial pneumonia were noted that was statistically significant at the highest dose. Both findings were described as a continuum in terms of morphological change (correlating with the gross lung observations observed in females at 12 000 ppm) and were evaluated together (males: 29, 32, 36, 47*; females: 24, 27, 32, 55*, respectively, out of the 55 rats in each group; * $p < 0.05$). Macrophage accumulation without additional change was coded as such. Interstitial pneumonia was diagnosed with the presence of one or more of the following lesions: presence of lymphocytes, cholesterol clefts, interstitial thickening of the alveolar septae by connective tissue, or increased alveolar pneumocytes and presence of occasional extravasation of erythrocytes (micro haemorrhage). The lung lesions described above were focal or multifocal in distribution and involved a very small overall portion of the lung tissue; most of the lung tissue was characterized as normal. The changes were of uncertain significance, possibly indicative of effects on the immune system at 7500 ppm. The effects on the lungs of males and females are shown in Table 23 and Table 24, respectively.

In the testes, an increased incidence of a generally slight morphological testicular change was noted (more subtle than distinct tubular degeneration), characterized by a depletion, asynchrony, and degeneration of latter-stage spermatids. In the epididymis, an increased incidence of immature/exfoliated germ cells/debris was observed in the lumen contents of the head, body and tail, which correlates with the testicular change. No morphological changes were observed in the anatomical structure of the epididymis tissue. These results are presented in Table 24.

In the bile duct, an increased incidence of hyperplasia/fibrosis with associated minimal periportal mononuclear-cell infiltrate was noted in females.

The NOAEL was 250 ppm, equivalent to 12.5 mg/kg bw per day, on the basis of a decrease in body-weight gain and structural changes in the kidneys at 3500 ppm. No evidence of a compound-induced neoplastic response was observed in any tissue examined (Wahle, 2006a).

Table 23. Lung effects in rats fed diets containing spirotetramat for 2 years

Effect	Dietary concentration (ppm)			
	0	250	3500	7500 (males)/12 000 (females)
Males				
No. of ratsa	40	33	44	35
Mean lung weight (g) ± SD	2.245 ± 0.309	2.362 ± 0.504	2.290 ± 0.426	2.420 ± 0.503
Mean relative lung weight ± SD	0.366 ± 0.059	0.402 ± 0.093	0.377 ± 0.074	0.435 * ± 0.111
No. of tissues examinedb	55	55	55	55
No. of tissues in which no abnormalities were detected	16	15	15	5
Abnormality:c				
Epithelialization	3 (2.0)	1 (1.0)	3 (2.3)	3 (2)
Alveolar macrophages	7 (1.3)	17 * (1.2)	17 * (1.4)	3 (1.0)
Granulomatous inflammation	3 (1.7)	1 (3.0)	3 (2.0)	6 (1.7)
Interstitial pneumonia	22 (1.6)	15 (1.7)	19 (1.7)	44 * (2.3)
Females				
No. of rats	38	39	37	42
Mean lung weight (g) ± SD	1.949 ± 0.478	1.899 ± 0.533	1.852 ± 0.392	1.934 ± 0.302
Mean relative lung weight	0.527 ± 0.138	0.492 ± 0.146	0.479 ± 0.111	0.600* ± 0.101
No. of tissues examined	55	55	55	55
No. abnormalities detected	27	25	17	0
Abnormality:a				
Epithelialization	—	—	2 (1.0)	—
Alveolar macrophages	20 (1.3)	14 (1.1)	18 (1.1)	3 (1.7)
Granulomatous inflammation	—	—	—	—
Interstitial pneumonia	4 (1.5)	13* (1.4)	14* (2.1)	52* (3.4)

From Wahle (2006a)

[a] Excluding ill or dead rats.

[b] All rats, including ill or dead rats.

[c] Figures in parentheses indicate average severity, graded from 1 (minimal) to 5 (severe).

* $p < 0.05$

2.4 Genotoxicity

Spirotetramat was evaluated for genotoxicity in a battery of studies of genotoxicity (five studies in vitro and three studies in vivo) (Table 25). All studies complied with GLP and QA statements were provided.

On the basis of the results of these studies, the Meeting concluded that spirotetramat has no mutagenic or genotoxic properties either in vivo or in vitro.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a dose range-finding study of reproductive toxicity, which complied with GLP and for a QA statement was provided, groups of 10 male and 10 female Wistar Hanover rats (age 14 weeks) were fed diets containing spirotetramat (purity, 97.5–97.8%) at nominal dietary concentrations of 0, 200, 500, 6000 or 10 000 ppm. The mean daily intake of spirotetramat is summarized in Table 26. All test diets (including control) were consumed ad libitum and the homogeneity and stability of spirotetramat as a dietary admixture was confirmed.

Body weight and food consumption were measured and detailed clinical examinations were conducted weekly throughout the study. Multiple reproductive parameters were evaluated. All rats were given a post-mortem examination that included documenting and saving all gross lesions, weighing designated organs and collecting representative tissue specimens for histopathological evaluation and sperm analysis. Additional investigations performed included developmental landmarks (vaginal patency and preputial separation) of the F_1 offspring, sperm analysis on F_1 males aged 8–9 weeks, histopathology of the epididymis and testes of the P-generation males, F_1 male weanlings aged 21 days, and F_1 males aged 8–9 weeks.

At the highest dietary concentration (10 000 ppm), no effects were observed on body weight, food consumption, or clinical observations among the P generation. Statistically significant declines (compared with the control group) in the absolute and relative weight of the cauda epididymis were observed. No pregnancies occurred and there were no offspring at this dietary concentration. Females did not have implantation sites. Sperm analysis revealed significant declines in the motility

Table 24. Microscopic findings in testes and epididymis of rats fed diets containing spirotetramat for 2 years

Findinga	Dietary concentration (ppm)			
	0	250	3500	7500
Testis				
No. of tissues examined	55	55	55	55
Degeneration	12 (3.4)	8 (3.5)	10 (2.9)	6 (3.2)
Spermatid degeneration, depletion, asynchrony	0	0	0	9 (1.2)*
Epididymis				
No. of tissues examined	55	55	54	54
Germ cell, exfoliated/debris	6 (2.2)	10 (1.9)	6 (1.8)	31 (2.5)*

From Wahle (2006a)

[a] Average severity is indicated in parentheses, graded from 1 (minimal) to 5 (severe).

* Statistically significant, $p < 0.05$.

Table 25. Results of studies of genotoxicity with spirotetramat

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium strains* TA1535, TA100, TA1537, TA98 and TA102	16–1581 µg/plate in DMSO	93.5–96.5	Negative ± S9	Herbold (2002a)
Reverse mutation[a]	*S. typhimurium* TA1535, TA100, TA1537, TA98 and TA102	16–1581 µg /plate in DMSO	95.2	Negative ± S9	Herbold (2006a)
Chromosomal aberration[b]	Chinese hamster V79 cells	10–50 µg/ml –S9 in DMSO 20–80 µg/ml +S9 in DMSO	93.5–96.5	Weakly positive	Herbold (2002b)
Chromosomal aberration	Chinese hamster V79 cells	0, 70 µg/ml –S9 in DMSO 0, 120 µg/ml +S9 in DMSO	98.6	Negative ± S9	Herbold (2003)
Forward mutation	HRPT locus in V79 cells (Chinese hamster lung cells)	2.5–80 µg/ml –S9 in DMSO 20–140 µg/ml +S9 in DMSO	93.5–96.5	Negative ± S9	Herbold (2002c)
In vivo					
Micronucleus formation	NMRI male mice (femoral marrow)	Two intraperitoneal doses at 0, 125, 250 or 500 mg/kg bw; sampling at 24 h after last dose	93.5–96.5	Negative	Herbold (2002d)
Chromosomal aberration	NMRI mice (bone marrow)	Single dose at 125, 250 or 500 mg/kg bw; sampling at 24 h and 48 h (highest dose) after last dose	92.7–93.2	Negative	Herbold (2003)
Unscheduled DNA synthesis	Male rat hepatocytes (Wistar)	A single oral dose at 0, 1000 or 2000 mg/kg bw Hepatocytes were prepared 4 h or 16 h after dosing.	92.7–93.2	Negative	Brendler-Schwaab (2003)

DMSO, dimethyl sulfoxide; S9, 9000 × g supernatant from rodent liver

[a] A repeat study.

[b] With one exception, cultures treated at the highest concentration showed statistically significant increases in numbers of metaphases with aberrations, with and without metabolic activation. However, these increases were very weak and may therefore have been induced by an impurity. In addition, metaphase quality at higher concentrations interfered with scoring, thus it may also be possible that the increased rates of chromosomal aberration were caused by a secondary non-genotoxic mechanism that was also responsible for reduced metaphase quality.

Table 26. Mean daily intake of spirotetramat by the parental generation in a dose range-finding study of reproductive toxicity in rats fed diets containing spirotetramat

Study phase	Mean daily intake (mg/kg bw per day)[a]			
	Dietary concentration (ppm)			
	200	500	6000	10 000
Males				
Premating	10.5	27.8	320.1	537.9
Females				
Premating	12.8	31.4	384.1	645.7
Gestation	12.9	31.9	393.9	—[b]
Lactation	26.7	74.4	831.0	—[b]

From Young (2006)

[a] Individual values were based on the means for each particular phase for each generation.

[b] No pregnancies occurred in the group at 10 000 ppm.

and percentage progression of sperm. A decline in epididymal counts was observed with no effect on testicular counts. There was an increase in the occurrence of morphologically abnormal sperm. Micropathology showed abnormal sperm in the epididymis (10 out of 10) and cauda epididymis (9 out of 10). The abnormal sperm consisted of what appeared to be the retention of the residual body to the tail of the spermatozoa, the severity of this change being graded as minimal to moderate. The results of sperm analysis/histopathology of epididymis and testes are shown in Table 27.

At 6000 ppm, parental females showed non-significant trends in body-weight declines during lactation. There were no treatment-related effects on food consumption or clinical observations, nor on of the reproductive parameters assessed. Among the F_1 offspring, a significant decline in pup weight occurred by day 21 (–13.9%) with total body-weight gain being significantly reduced (–14.7%) when compared with the control group. F_1 animals aged 8–9 weeks showed statistically significant declines in body weight. Slight declines in the motility and percentage progression of sperm were observed. There was an increase in the occurrence of morphologically abnormal sperm. Micropathology showed abnormal sperm in the epididymis (4 out of 15) and cauda epididymis (4 out of 14). The severity of the changes observed was considere to be minimal to moderate.

At dietary concentrations of 500 ppm and 200 ppm, no treatment-related effects were noted in the parental adults or the F_1 offspring.

Table 27. Effects on histopathology of the testes and epididymis and on sperm parameters in a dose range-finding study of reproductive toxicity in rats fed diets containing spirotetramat

Effect	Generation	Dietary concentration (ppm)				
		0	200	500	6000	10 000
Sperm analysis						
Motility (%)	P	74	74	79	75	31
	F1	90	90	88	76	
Progression (%)	P	57	54	58	52	14
	F1	69	64	63	56	
Testicular count (No./g)	P	99	128	107	111	98
	F1	82	75	73	73	
Epididymal count (No./g)	P	986	733	798	944	464
	F1	504	486	507	463	
Abnormal sperm a (No./200)	P	0.7	0.7	0.5	0.8	95.5
	F1	2.8	1.5	1.3	20.2	
Histopathology						
Abnormal epididymal sperm cells	P	0/10	ND	ND	0/10[b]	10/10
	F1	1/17	ND	ND	4/15	
Abnormal epididymal sperm cells (cauda)	P	0/10	ND	ND	0/10[b]	9/9
	F1	1/17	ND	ND	4/14	
Testes, degeneration	P	0/10	ND	ND	1/10[b]	0/10
	F1	0/17	ND	ND	0/15	

From Temerowski (2008)

Figures in italic type were considered to be treatment-related, although no statistical test for significance was used.

ND, not detected

[a] Presenting as amorphous heads.

[b] One P-generation male at 6000 ppm had degenerative testes and no sperm development, but this was considered to be not treatment-related. A single F_1 interim male control aged 8–9 weeks had a moderate number of abnormal sperm cells within epididymis and cauda epididymis).

The NOAEL for parental toxicity in P-generation males was 6000 ppm, equal to premating doses of 320.1 mg/kg bw per day, on the basis of reduced cauda epididymal weights and effects on sperm at 10 000 ppm, equal to 537.9 mg/kg bw per day. The highest dietary concentration (10 000 ppm) caused treatment-related effects on sperm cells of parental males and included decreased sperm motility and progression, decline in epididymal sperm count and increase in abnormal sperm in the epididymis and the cauda epididymis.

The NOAEL for parental toxicity in P-generation females was 500 ppm, equal to doses during lactation of 74.4 mg/kg bw per day, on the basis of slight declines in body-weight gain during lactation in the P-generation females at 6000 ppm,equal to 831 mg/kg bw per day.

The NOAEL for reproductive toxicity in P-generation males was 6000 ppm, equal to premating doses of 320.1 mg/kg bw per day, on the basis of decreased sperm motility and progression, decreased epididymal counts and increase in abnormal sperm in the epididymis and the cauda epididymis at 10 000 ppm, equal to 538 mg/kg bw per day. Other than the lack of pregnancies in females at 10 000 ppm, which was attributed to the sperm effects in males, there were no treatment-related reproductive effects observed in P-generation females during this pilot study.

The NOAEL for offspring toxicity was 500 ppm, equal to 74.4 mg/kg bw per day, on the basis of decreased body weights on day 21 of lactation at a dietary concentration of 6000 ppm, equal to 831 mg/kg bw per day. Decreases in pup weight corresponded with maternal body-weight decreases.

The NOAEL for male parental toxicity in F_1 males aged 8–9 weeks was 500 ppm on the basis of decreased terminal body weights at 6000 ppm.

The NOAEL for male reproductive toxicity in F_1 males aged 8–9 weeks was 500 ppm on the basis of decreased sperm motility and progression and an increase in abnormal sperm in the epididymis and the cauda epididymis at 6000 ppm (Young, 2006**a**).

In a two-generation study of reproductive toxicity, whichcomplied with GLP and for which a QA statement was provided, groups of 30 male and 30 female Wistar Hanover (Crl:WI[Glx/BRL/Han]IGS BR) rats (age 9–10 weeks) were fed diets containing spirotetramat (purity, 97.4–98.5%) at nominal doses of 0, 250, 1000 or 6000 ppm. The rats were exposed to the treated feed throughout the entire study. The parental animals (P and F_1 generations) were given test diets for 10 weeks before mating. Mating was accomplished by co-housing one female with one male for up to 14 consecutive days, with exceptions noted in the Protocol Amendments and Deviations. Approximately four to six rats from each treated group were co-housed daily beginning on the first day of the mating phase and continuing until all rats had been co-housed. During the mating phase, vaginal smears were taken each morning and examined for the presence of sperm and/or internal vaginal plug. Females found to be inseminated were placed in a polycarbonate nesting cage. The day on which insemination was observed in the vaginal smear was designated day 0 of gestation for that female. In order to evaluate females that may have been inseminated without exhibiting sperm in the vaginal smear or an internal vaginal plug, all remaining females were placed in polycarbonate nesting cages after the 14-day mating period. Selection of F_1 pups for evaluation of preputial separation and vaginal patency was made when the pups were aged 21 days. The F_2-generation pups were maintained until age 21 days.

Analysis of homogeneity, stability and achieved concentrations was reported. Mean daily intake of spirotetramat is shown in Table 28.

The exposure durations for rats in both generations were as follows: P-generation males, 16 weeks; P-generation females, 16–18 weeks; F_1-males, 24 weeks; F_1-females, 26_28 weeks; F_2-pups, 21 days.

Mortality checks were performed twice daily (morning and afternoon) during the working week and once daily at weekends and during holidays. Cage-side observations assessed mortality,

moribundity, behavioral changes, signs of difficult or prolonged delivery, and overt toxicity. In the event that possible clinical sign was observed during the cage-side evaluation, the rat was removed from the cage and a detailed assessment was conducted. A detailed evaluation of clinical signs was conducted once per week throughout the entire in-life phase of the study. Body weights and food consumption were measured and fresh feed provided once per week for males and females during the 10-week premating period with exceptions stated in the Protocol Amendments and Deviations. During the mating period and until sacrifice, body weights for the males were measured once per week. Body weights were also measured during the mating period for unmated females. Also during the mating period, fresh feed was provided for males and unmated females once per week, but food consumption was not measured. During gestation, dam body weights were measured on days 0, 6, 13 and 20, and fresh feed was provided and food consumption measured once per week. During lactation, dam body weights were measured on days 0, 4, 7, 14 and 21. Fresh feed was provided and food consumption measured once per week, with the exception of week 1 when food consumption was measured twice (days 0–4 and 4–7). The estrous cycle (determined by examining daily vaginal smears) was characterized for all P- and F_1-generation females during the 3 weeks before mating. Additionally, the estrous cycle stage was determined for all females immediately before termination. For all P- and F_1-generation males at termination, sperm was collected from one testes and one epididymis for enumeration of homogenization-resistant spermatids and cauda epididymal sperm reserves, respectively. In addition, an evaluation of morphology and motility was performed on sperm sampled from the distal portion (closest to the urethra) of the vas deferens. Sperm motility and counts were conducted using the IVOS (Integrated Visual Operating Systems, 1998). Morphology and testis counts were conducted on the control group and the group at the highest dose and in all treated groups for epididymal counts for the first generation. Morphology and counts were conducted on all treated groups for the second generation. Litters were examined after delivery and pups were sexed,

Table 28. Mean daily intake of spirotetramat in a two-geenration study in rats fed diets containing spirotetramat

Study phase	Mean daily intake (mg/kg bw per day)a		
	Dietary concentration (ppm)		
	250	1000	6000
Males			
P generation:			
Premating	17.2	70.7	419.3
F1-generation:			
Premating	19.3	79.5	486.7
Females			
P generation:			
Premating	20.0	82.5	484.7
Gestation	19.6	76.7	467.4
Lactation	39.4	162.9	895.7
F1 generation:			
Premating	21.7	90.3	539.5
Gestation	17.8	69.8	434.7
Lactation	39.4	161.0	930.6

From Young (2006)

[a] Individual values were based on the means for each particular phase for each generation.

examined for gross abnormalities and the number of stillborn pups and live pups recorded. Pup weight and external alterations were recorded on postnatal days 0, 4, 7, 14 and 21.

The analysis of the results showed that no treatment-related mortalities or clinical observations were observed at any dietary concentration tested in either generation. There were no treatment-related adverse effects at 250 ppm or 1000 ppm in either generation.

At 6000 ppm, the following observations were made (statistical significances are based on comparisons with the control group). In the P generation, males showed declines in body-weight gain during premating and females exhibited statistically significant declines in food consumption during lactation (days 0–21). F_1 offspring showed declines in pup weight (day 21) and pup-weight gain (days 4–21), which were considered to be secondary to maternal effects noted during lactation. Male and female F_1 pups (combined) aged 21 days were also reported to show an increase in relative brain weight and a decrease in absolute spleen weight, which were also considered to be secondary to pup-weight decreases.

At 6000 ppm, no compound-related effects were observed on the reproductive performance of the P generation.

F_1-generation adult males at 6000 ppm exhibited decreases in body weight from days 14–70 of premating, as well as declines in overall body-weight gain. F_1-generation females, during the premating phase, displayed decreases in overall body-weight gain when compared with the control group. Females also exhibited decreases in body-weight throughout gestation and in body weight and food consumption throughout lactation. In males and females, gross pathology and micropathology revealed terminal body-weight declines and microscopic changes in the kidney, consisting of dilated tubules with occasional proteinaceous material.

F_2 offspring at 6000 ppm also showed declines in pup weight (day 7–21) and pup-weight gain (days 4–21), which were considered to be secondary to maternal effects noted during lactation. Also observed in the pups aged 21 days (combined males and females) and considered to be secondary to pup-weight decreases was a decrease in absolute brain, thymus and spleen weight.

There were morphological effects on sperm (presenting as amorphous sperm heads) was noted in the F_1 generation; this was consistent with in the results of the dose range-finding study described above (Young, 2006). Variation in susceptibility was observed, as 9 out of 30 males exhibited a minimal effect (at least one amorphous sperm head was noted out of 200 viewed), but only one outlying male was affected to the extent that it compromised fertilizing capabilities. This same male showed abnormal sperm in the epididymis. Overall fertility in the group at this dose was not affected. No other compound-related reproductive findings were observed. The results of sperm analysis/histopathology of epididymis and testes are shown in Table 29.

The NOAEL for male parental toxicity was 1000 ppm, equal to premating doses of 70.7 mg/kg bw per day or 79.5 mg/kg bw per day in P- or F_1-generation males, respectively, on the basis of body-weight gain decreases in P-generation males at 6000 ppm, equal to 419.3 mg/kg bw per day, and body-weight gain decreases, decreased terminal body weights and increased renal multifocal tubular dilatation in F_1-generation males at 6000 ppm, equal to 486.7 mg/kg bw per day.

The NOAEL for female parental toxicity was 1000 ppm, equal to premating doses of 82.5 mg/kg bw per day or 90.3 mg/kg bw per day in P- or F_1-generation females, respectively, on the basis of reduced food consumption during lactation in P-generation females at 6000 ppm, equal to 484.7 mg/kg bw per day, and reduced food consumption during lactation, reduced body weights (end of premating), body-weight gain decreases (premating), decreased terminal body weights and increased renal multifocal tubular dilatation in F_1-generation females at 6000 ppm, equal to 539.5 mg/kg bw per day.

The NOAEL for reproductive toxicity was 1000 ppm in males, equal to premating doses of 79.5 mg/kg bw per day in the F_1 generation, on the basis of abnormal sperm cell morphology in F_1-generation males at 6000 ppm, equal to 486.7 mg/kg bw per day, which included one rat with abnor-

mal epididymal sperm cells and pronounced abnormal spermatozoa leading to compromised fertilizing capabilities and other animals (9 out of 30) showing one to four amorphous sperm heads out of 200 sperm cells viewed. Reproductive performance was not impaired at 6000 ppm in either generation.

The NOAEL for reproductive toxicity in females was 6000 ppm, equal to premating doses of 484.7 mg/kg bw per day or 539.5 mg/kg bw per day in the P or F_1 generation, respectively, on the basis of the absence of effects at 6000 ppm on mean duration of the estrus cycle, number of normally cycling females, mating fertility and gestation indices, mean duration of gestation, mating performance, vaginal opening, and litter parameters determined at birth (pup weight, total number of pups born, stillborn pups, viability index on lactation day 0, sex ratio and mean litter size).

The NOAEL for offspring toxicity was 1000 ppm on the basis of decreased body weights on day 21 of lactation and decreased body-weight gain in F_1 pups and decreased body weights on days 14 and 21 of lactation and decreased body-weight gain in F_2 pups at 6000 ppm. Pup-weight declines corresponded with maternal body weight and food consumption declines (Young, 2006).

(b) Developmental toxicity

Rats

In a pilot study of developmental toxicity that did not comply with standard GLP, groups of seven inseminated female Wistar Hsd Cpb:WU rats were given spirotetramat (purity, 98.8%) at a

Table 29. Effects on histopathology of the testes and epididymis and on sperm parameters in a two-generation study of reproductive toxicity in rats fed diets containing spirotetramat

Effect	Generation	Dietary concentration (ppm)				
		0	250	1000	6000	Historical controls
Sperm analysis						
Motility (%)	P	84	83	84	85	76–90
	F1	81	83	84	80	—
Progression (%)	P	59	59	61	62	48–68
	F1	57	60	59	56	—
Testicular count (No./g)	P	90	—	—	87	64–81
	F1	88	90	90	79	—
Epididymal count (No./g)	P	623	563	482*	472*	234–431
	F1	517	490	520	497	—
Abnormal sperma (No./200)	P	2.0	—	—	2.8	0.8–3.1
	F1	2.9	2.6	1.9	8.9	—
Histopathology						
Abnormal epididymal sperm cells	P	0/30	—	—	0/30	—
	F1	0/30	—	—	1/30	
Abnormal epididymal sperm cells (cauda)	P	0/30	—	—	0/30	—
	F1	0/30	—	—	1/30	
Testes, degeneration	P	0/30	—	—	0/30	—
	F1	0/30	—	—	0/30	

From Young (2006)

[a] Presenting as amorphous heads: one male at 250 ppm with aspermia (considered to be not treatment-related), animal evaluated owing to gross necropsy findings (Temerowski, 2006).

* $p < 0.05$.

Figures in italic type are considered to be treatment-related.

daily oral dose of 0, 50, 200, 800 or 1000 mg/kg bw per day (dose volume, 10 ml/kg bw) by gavage in 0.5% aqueous carboxymethylcellulose from day 6 to day 19 post coitum. The groups at 200 mg/kg bw per day and 1000 mg/kg bw per day comprised 11 rats and 8 rats, respectively. For better clarification of toxic effects, seven additional females were added later to the group at 1000 mg/kg bw per day. The fetuses were delivered by caesarean section on day 20 post coitum. Investigations were performed on general tolerance of the test compound and its effect on intrauterine development.

Treatment-related effects included maternal effects at 800 mg/kg bw per day (body-weight loss, impaired body-weight development) and at 1000 mg/kg bw per day (respiratory findings, piloerection, body-weight loss, impaired body-weight gain, increased urination, light-coloured faeces). Effects on intrauterine development could not be completely excluded at 200 mg/kg bw per day (possible marginal reduction in fetal weight), and were also evident at 800 mg/kg bw (reduced placental and possibly fetal weight, retarded ossification, wavy ribs). Effects were clearly observed at 1000 mg/kg bw per day (distinctly reduced fetal and placental weight, necrotic placental borders, retarded ossification, wavy and 14th ribs and possible marginal increase in the incidence of common malformations) (Klaus, 2001).

In a study of developmental toxicity that complied with GLP and for which a QA statement was provided, groups of 25 inseminated female Wistar Hsd Cpb:WU rats were given spirotetramat (purity, 99.0% *cis* isomer) at a daily oral dose of 0, 20, 140 or 1000 mg/kg bw per day by gavage in 0.5% aqueous carboxymethylcellulose from day 6 to day 19 post coitum. The fetuses were delivered by caesarean section on day 20 of gestation. Investigations were performed on general tolerance of the test compound and its effect on intrauterine development. The study was performed in compliance with international guidelines (OECD guideline No.414, 2001; US EPA Health Effects Test Guidelines OPPTS 870.3700, 1998; Japanese MAFF guidelines of 2000 as amended in 2001and EEC Commission Directive 88/302/EEC, 1988).

Mortality, appearance and behaviour were not affected by treatment with spirotetramat at doses of up to 1000 mg/kg bw per day. Treatment-related effects at 1000 mg/kg bw per day comprised impaired feed intake after start of treatment and up to the end of study, transient marginal body-weight loss together with impaired body-weight gain and reduced final body weight and carcass weight. Light-coloured faeces were observed in the group at 1000 mg/kg bw per day, although this finding might be related to the large amount of white test substance given.

Necropsy revealed no treatment-related findings at doses of up to 1000 mg/kg bw per day. A marginal reduction in placental weight together with a more distinct reduction in fetal weight was observed in the group at 1000 mg/kg bw per day. External, visceral and skeletal evaluation of fetuses revealed a slightly increased number of fetuses and litters with generally common non-specific malformations at the maternally toxic dose of 1000 mg/kg bw per day. A potential of spirotetramat to induce a specific type of malformation was not deduced from these findings. Overall incidence and type of malformations at 20 and 140 mg/kg bw per day did not indicate a treatment-related effect. External fetal deviations were not observed in this study and the incidence and type of visceral deviations of fetuses was not affected by treatment with spirotetramat at doses of up to 1000 mg/kg bw per day. Effects on food consumption and body-weight gain were, however, observed at 140 mg/kg bw per day.

Skeletal, including cartilaginous, tissue evaluation of fetuses revealed retarded ossification at 1000 mg/kg bw per day, together with an increased incidence of skeletal variations (wavy ribs, 14th ribs, combined osseous and cartilaginous findings) (Table 30). Furthermore, retarded ossification of single localizations and increased incidence of wavy ribs were seen in the groups at 140 and 20 mg/kg bw per day, without a clear dose–response relationship. On the basis of the results of an additional supplementary study with spirotetramat (described below; Klaus, 2004a), which revealed no indication for a treatment-related increase in the incidence of wavy ribs at doses up to 140 mg/kg bw per

day and no evidence for effects on degree of ossification at up to 140 mg/kg bw per day, the Meeting excluded the possibility of a treatment-related effect on ossification and incidence of wavy ribs at a dose up to 140 mg/kg bw per day in the present study.

The NOAEL for systemic maternal toxicity was 140 mg/kg bw per day on the basis of treatment-related effects at 1000 mg/kg bw per day (impaired feed intake, transient marginal body-weight loss together with impaired body-weight gain and reduced final body weight and carcass weight).

The NOAEL for intrauterine development was 140 mg/kg bw per day on the basis of ossification at 1000 mg/kg bw per day, together with an increased incidence of skeletal variations (wavy ribs, 14th ribs, combined osseous and cartilaginous findings) (Klaus, 2004b).

On the basis of the results of a study of developmental toxicity in rats given spirotetramat at a dose of 20, 140 or 1000 mg/kg bw per day described above in Klaus (2004b) in which equivocal retarded ossification of single localizations and equivocal increased incidence of wavy ribs were seen at 140 and 20 mg/kg bw per day without a clear dose–response relationship, an additional study of developmental toxicity was performed for clarification of results. The study complied with GLP and

Table 30. External visceral and skeletal malformations in fetuses in a study of developmental toxicity in rats given spirotetramat by gavage

Malformationa	Dose (mg/kg bw per day)			
	0	20	140	1000
Cleft palate	—	—	—	1
Microphthalmia (eye rudiment flat/eyeball reduced in size/eye socket reduced in size), unilateral	1	1	—	1
Anophthalmia (eye rudiment flat/eyeball missing/eye socket reduced in size), unilateral	1	1	—	—
Upper jaw shortened, macroglossia, domed head, all bones of fore-and hindlimbs, of skull and vertebral column dysplastic	—	—	1	—
Lobe of thyroid gland absent	3 (2)	—	1	—
Atrial septal defect of the heart	1	1	—	1
Coarctation of aortic arch between left carotid and left subclavian arteries, ascending aorta reduced in size, left subclavian artery arises from descending aorta	—	—	—	1
Dysplasia of forelimb bones (scapula, humerus, radius and/or ulna)	1	2 (2)	—	4 (4)
Supernumerary lumbar vertebra	—	—	—	1
First sacral vertebral arch has the shape of a lumbar vertebral arch in the osseous and the cartilaginous part and the cartilaginous part is not fused with the cartilaginous processus transversus of the secnd sacral vertebral arch, pelvis shifted caudally	—	—	—	3 (3)
Number of fetuses per group	247	301	253	270
Number of fetuses with malformations	7	5	2	12
Malformed fetuses per group (%)	2.83	1.66	0.79	4.44
Number of litters per group	20	24	23	22
Number of litters with malformations	4	4	2	9
Malformed litters per group (%)	20.00	16.67	8.70	40.91

From Klaus (2004b)

[a] The number of litters affected is given in parentheses.

a QA statement was provided. Groups of 25 inseminated female Wistar rats were given spirotetramat (purity, 99.1%) as a daily oral dose at 0, 10, 35 or 140 mg/kg bw per day by gavage in 0.5% aqueous carboxymethylcellulose from day 6 to day 19 post coitum. The fetuses were delivered by caesarean section on day 20 of gestation. Investigations were performed on general tolerance of the test compound, including haematology, clinical chemistry, liver weight and histopathology of liver, as well as the effect of spirotetramat on intrauterine development.

Treatment-related maternal effects with respect to mortality, clinical signs including appearance and amount of excreta (urine, faeces), food intake, body-weight development, haematology parameters, liver weight, necropsy findings and histopathology of liver were not observed at doses of up to 140 mg/kg bw per day. Treatment-related effects on reproductive parameters, i.e. gestation rate, post-implantation loss, litter size, placental weight and appearance, fetal weight and fetal sex distribution were not evident at doses of up to 140 mg/kg bw per day. The incidence and type of fetal malformations were unaffected by treatment at doses of up to 140 mg/kg bw and a teratogenic potential of spirotetramat at doses of up to 140 mg/kg bw per day was not evident. Meaningful fetal external or visceral deviations (findings other than malformations) were not evident at doses of up to 140 mg/kg bw per day. Evaluation for degree of fetal skeletal ossification and incidence of skeletal variations, including evaluation of cartilaginous structures, revealed no clear evidence for treatment-related effects at doses of up to 140 mg/kg bw per day. The incidence of wavy ribs was not affected at doses of up to 140 mg/kg bw per day. Summarizing and evaluating all data investigated, the following no-observed adverse-effect-levels (NOAEL) were determined:

The NOAEL for maternal toxicity was 140 mg/kg bw per day. The NOAEL for developmental toxicity was 140 mg/kg bw per day (Klaus, 2004a).

Rabbits

In a pilot study of developmental toxicity that was not conducted in accordance with standard GLP, groups of three mated female Himalayan rabbits were given spirotetramat as a daily oral dose at 0, 5, 25, 100, 160, 250 or 500 mg/kg bw per day by gavage in 0.5% carboxymethylcellulose in demineralized water from day 6 to day 28 post coitum. The fetuses were delivered by caesarean section on day 29 post coitum. Investigations were performed on the general tolerance of the test compound and its effect on intrauterine development (including external and visceral evaluation of the fetuses).

One female at 500 mg/kg bw per day died on day 10 post coitum. The remaining two females of this group and one female at 250 mg/kg bw per day were killed for humane reasons on day 10 or 18 post coitum (500 mg/kg bw per day) or day 23 post coitum (250 mg/kg bw per day), presenting wounds in the region of the head, throat or forelimbs. Another female at 250 mg/kg bw per day was killed after abortion on day 24 post coitum. Hypoactivity (lying on side) on a single day occurred in one female in each of the groups at 500 and 250 mg/kg bw per day as well as in the female that aborted in the group at 250 mg/kg bw per day, which also showed laboured breathing and convulsions. Cold ears, severely decreased or no feed intakes and distinct to severe body-weight loss (up to 343 g) also occurred in females at 500 and 250 mg/kg bw per day.

Necropsy revealed hardened fatty tissue in the abdominal cavity and at the border of the pancreas and round depressions in the gastric mucosa in one female at 500 mg/kg bw per day; an enlarged gall bladder in one female and gaseous contents in the stomach and intestine as well as haemorrhages in the renal capsule in an additional female at 250 mg/kg bw per day. Cold ears occurred for several days in all females at 160 mg/kg bw per day. Feed intakes were transiently distinctly to severely decreased in two females at 160 mg/kg bw per day, these rabbits also showing moderate to distinct body-weight loss (191 g and 273 g). Gross necropsy did not reveal treatment-related findings at doses of up to 160 mg/kg bw per day.

The gestation rate in the group at 250 mg/kg bw per day was decreased by one abortion and by one total resorption. Owing to this abortion, total resorption, early sacrifice or death of all females in

the groups at 250 and 500 mg/kg bw per day, the evaluation of the remaining reproduction parameters was limited to doses up to and including 160 mg/kg bw per day. The resorption rate and the number of fetuses as well as fetal sex distribution were unaffected at doses up to and including 160 mg/kg bw per day. Placental and fetal weights in the group at 160 mg/kg bw per day were slightly decreased when compared with the control group, which was, however, most likely to be caused by the incidentally higher litter sizes in the group at 160 mg/kg bw per day rather than a treatment-related effect. A definitive assessment is, however, not possible owing to the low number of females in this pilot study.

The isolated malformations seen in one fetus at 100 mg/kg bw per day (malposition of forelimb) and one fetus in each group at 25 and 160 mg/kg bw per day (cardiac ventricular septal defect with/without truncus arteriosus) are considered incidental as these malformations are known to be common findings in the strain of rabbits used. Thus maternal toxicity was evident at 160 mg/kg bw per day and was severe at doses of 250 mg/kg bw per day and above including death or sacrifice in moribund condition. The abortion and the total resorption at 250 mg/kg bw per day were considered to be caused by maternal toxicity rather than being a specific effect on reproduction. Developmental toxicity (slightly decreased fetal weights) could not be excluded at 160 mg/kg bw per day as this dose revealed maternal toxicity.

The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of body-weight loss, impaired body-weight development, decreased food consumption and cold ears at 160 mg/kg bw per day. The NOAEL for developmental toxicity was 100 mg/kg bw per day on the basis of marginally reduced fetal and placental weights at 160 mg/kg bw per day. No detailed raw data were provided in the study report (Holzum, 2001).

In a study of developmental toxicity that complied with GLP and for which a QA statement was provided, groups of 22 pregnant Himalayan (CHBB:HM) rabbits were given spirotetramat (purity, 98.9%) as a daily oral dose at 0, 10, 40 (24 rabbits) or 160 (30 rabbits) mg/kg bw per day by gavage in 0.5% aqueous carboxymethyl cellulose from day 6 to 28 post coitum. Stability, homogeneity and dose concentrations were confirmed analytically. On day 29 of gestation, the fetuses were delivered by caesarean section.

One female at 160 mg/kg bw per day was found dead after having shown reddish excretion and soft faeces and five females had to be sacrificed in moribund condition after having shown severely reduced to zero feed intake, severe body-weight loss, cold ears, alopecia, reduced or no faeces, diarrhoea (in one female) and soft and light-coloured faeces, reddish excretion, decreased water intake and urination and discoloured urine most probably indicating concentration of urine. Females that had to be sacrificed in moribund condition showed fluid and/or gaseous contents in the caecum, discoloured liver and mottled gall bladder at necropsy. Abortion was observed most likely as a consequence of maternal toxicity in two other females at 160 mg/kg bw per day and in one female at 40 mg/kg bw per day that showed clinical symptoms, impaired feed intake and body-weight loss before abortion; i.e. symptoms comparable to those of the females that had to be sacrificed in moribund condition. The remaining females at 160 mg/kg bw per day had cold ears, alopecia and soft, mucoid and light-coloured faeces. The abortion in the group at 40 mg/kg bw per day was considered to be incidental. Post-implantation loss in the remaining females, and the number of fetuses as well as placental weight and appearance, fetal weight and fetal sex distribution were unaffected by treatment at doses of up to 160 mg/kg bw per day.

Fetal malformations seen were different in type, scattered between the different doses and revealed the highest total incidence of malformations in the group at 10 mg/kg bw per day, lying well within the normal range of scattering for the strain of rabbits used and showing no dose-dependency. Malformations of cartilaginous parts of ribs, cardiac ventricular septal defects and malpositioned forelimbs (one of the most common malformations in the rabbit strain used) seen in a number of

fetuses were not dose-related and single malformations that appeared only once in the group at 160 mg/kg bw per day (domed head together with encephalomeningocele, cleft palate, microphthalmia) were different in type and comparable with data on historical controls. Thus a teratogenic effect of spirotetramat was excluded at doses of up to 160 mg/kg bw per day. Apart from a possibly increase in the incidence of fetuses with distinct liver lobulation in the group at 160 mg/kg bw per day, a treatment-related effect on fetal external and visceral deviations (findings other than malformations) was not found at doses of up to 160 mg/kg bw per day. Fetal skeletal including cartilaginous tissue evaluation revealed **no** treatment-related effects at a dose up to 160 mg/kg bw per day. Findings for the progeny (abortions at 40 and 160 mg/kg bw per day, distinct fetal liver lobulation at 160 mg/kg bw per day) only occurred at doses with signs of distinct (40 mg/kg bw per day) to severe maternal toxicity.

The NOAEL for systemic maternal toxicity was 40 mg/kg bw per day on the basis of abortion, clinical signs, impaired food and water consumption and body-weight loss at 160 mg/kg bw per day (the LOAEL). The NOAEL for developmental toxicity was is 160 mg/kg bw per day, the highest dose tested (Klaus, 2004c).

2.6 Special studies

(a) Study screening for acute oral neurotoxicity

In a study screening for acute oral neurotoxicity, which complied with GLP and for which a QA statement was provided, groups of 12 male and 12 female nonfasted young adult Wistar Crl:WI(Glx/BRL/Han) IGS BR rats (age 9 weeks) were given spirotetramat (purity, 97.8–98.5%) as a single oral dose at 0 (vehicle), 200, 500 or 2000 mg/kg bw by gavage in 0.5% methylcellulose/0.4% Tween 80 in deionized water (adjusted to pH 4 with acetic acid to enhance stability) and administered in a dosing volume of 10 ml/kg. Since there were compound-related effects at 200 mg/kg bw, a follow-up study was conducted under the same conditions and providing nominal doses of 0 (vehicle), 50, 100 and 500 mg/kg bw to verify the findings at 500 mg/kg bw. The following observations and measurements were included in the initial study: mortality checks, clinical observations, body-weight measurements, a functional observational battery (FOB), automated measurements of activity (figure-eight maze), a gross necropsy, brain weight, measurements and microscopic examination of skeletal muscle, peripheral nerves, eyes (with optic nerves), and tissues from the central nervous system. Observations and measurements included in the follow-up study consisted of mortality checks, clinical observations, body-weight measurements, a FOB and figure-eight maze.

On the basis of analytical results, the actual doses of spirotetramat for the initial study were 0, 182, 515 and 1930 mg/kg bw for males and females and actual doses for the follow-up study were 0, 47.4, 99.8 and 523 mg/kg bw for males and females.

No compound-related deaths occurred at any dose.

In the initial study, compound-related clinical signs were evident at all doses. Urine stains were observed at all doses and were considered to be related to treatment. Perianal staining was observed in males at the two higher doses (one in each group) but not at the lowest dose or in females at any dose. These signs were evident on day 0 and generally resolved within 1–4 days after treatment. For the follow-up study, urine stains were evident in females at 500 mg/kg bw, but not at lower doses or in males at any dose. This sign was first observed on day 0 and resolved within 1–4 days after treatment. Body weight was not affected by treatment in males or females at any dose.

For the FOB in the initial study, urine stains were observed in males (one each) at the lowest and intermediate dose. There were no findings related to treatment observed in males at the highest dose or females at any dose. In the follow-up study, there were no treatment-related findings at any dose in males or females. Measures of motor and locomotor activity in the initial study were

significantly reduced in males at the highest dose (48% and 64%, respectively) and females (40% and 65%, respectively). At 500 mg/kg bw, motor activity was reduced (32%) and locomotor activity was significantly reduced (44%) in males only. Locomotor activity was slightly reduced (29%) in males at the lowest dose. In addition, motor and locomotor activity was reduced in males at several time-points at 500 and 2000 mg/kg bw and for locomotor activity in males at the lowest dose. A similar trend was evident in females at 500 and 2000 mg/kg bw (locomotor activity only at the intermediate dose), but the difference from controls was not statistically significant. In the follow-up study, motor and locomotor activity was slightly reduced (21% and 30%, respectively) in males at 500 mg/kg, but not at lower doses or in females at any dose. Motor and locomotor activity was reduced at several time-points in both sexes at 500 mg/kg bw.

There were no compound-related effects on days 7 or 14 after treatment and habituation was not affected by treatment on any day, at any dose. There were no compound-related gross lesions in males or females at any dose. Brain weight was not affected by treatment in males or females at any dose. Compound-related microscopic lesions were not evident in males or females at the highest dose.

To summarize, an acute oral dose of spirotetramat produced evidence of toxicity in males and females at all doses (200, 500 and 2000 mg/kg bw) in the initial study. Evidence of toxicity was limited to clinical signs (urine staining at all doses, perianal staining in males at the intermediate and highest dose) and decreased activity in the figure-eight maze (females at the intermediate and highest dose and males at all doses) beginning on the day of treatment and with complete recovery by day 7. There was no evidence of neurotoxicity at any dose and there were no compound-related gross or microscopic lesions at a limit dose of 2000 mg/kg bw. A follow-up study confirmed findings in the initial study at 500 mg/kg bw, with no compound-related effects at lower doses. The results of these studies are summarized in Tables 31–37.

The NOAEL was 100 mg/kg bw (Gilmore et al., 2005).

(b) Mechanism of action

Two mechanistic studies were conducted to identify the time of onset and location of the first visible effects on the rat testes, and to determine the metabolite responsible for the testicular/sperm toxicity attributable to spirotetramat.

Table 31. Clinical observations in initial and follow-up studies of acute neurotoxicity in rats given spirotetramat by gavage

Observation [a]	Dose (mg/kg bw)					
	0	50	100	200	500	2000
Males (n = 12)						
Urine stain						
Initial study	0	—	—	1 (days 0–1, 7) [a]	3 (day 0)	5 (days 0–2)
Follow-up study	0	0	0	—	0	—
Perianal stain						
Initial study	0	—	—	—	1 (day 0)	1 (day 0)
Females (n = 12)						
Urine stain						
Initial study	0	—	—	1 (day 0)	6 (days 0–1)	6 (days 0–3)
Follow-up study	0	0	0	—	6 (days 0–3)	—

From Gilmore et al. (2005)

[a] Range of days on which the observation was made is given in parentheses.

In a study to identify the primary target cell(s) of spirotetramat in male rat testes and epididymis that complied with GLP and for which a QA statement was provided, groups of 32 male rats (Crl:WI[Glx/BRL/Hans]IGS BR strain)(aged approximately 13 weeks) were given spirotetramat (purity, 97.2%) at a dose of 0 (vehicle only) or 1000 mg/kg bw per day by gavage in an aqueous solution of 0.5% methylcellulose 400 for 3, 10, 21 or 41 days (eight rats per scheduled sacrifice time). Clinical signs were recorded daily, body weight and food consumption was measured at least weekly. A detailed physical examination was performed once during the acclimatization phase and weekly throughout the study. Serial sacrifices were performed on days 3, 10, 21 and 41, approximately 4 h after the last dosing. All rats were necropsied, selected organs weighed and a range of tissues was taken, fixed and examined microscopically. In addition, after sacrifice, sperm from the right epididymis was collected for sperm enumeration and morphology assessment.

There was one treatment-related death of a rat at 1000 mg/kg bw per day on day 31. This rat showed only increased salivation on days 17 and 21 and a slightly reduced body-weight gain between days 21 and 28. Several of the remaining rats showed clinical signs of toxicity on skin or fur (lesion, piloerection, generalized or localized soiled fur), movement or behaviour (reduced motor activity,

Table 32. Mean motor and locomotor activity for initial study of acute neurotoxicity in rats given spirotetramat by gavage

Test day	Mean total activity counts per session ± standard deviation			
	Dose (mg/kg bw)			
	0	200	500	2000
Males (n = 12)				
Motor activity:				
Day −7	416 ± 219	318 ± 121	385 ± 138	383 ± 135
Day 1	516 ± 182	546 ± 420	350 ± 141 (−32%)a	270* ± 83 (−48%)[a]
Day 7	493 ± 170	573 ± 70	409 ± 79	459 ± 128
Day 14	410 ± 118	429 ± 125	346 ± 103	374 ± 96
Locomotor activity:				
Day −7	265 ± 172	202 ± 93	239 ± 91	238 ± 93
Day 1	312 ± 140	222 ± 102 (−29%)[a]	174* ± 80 (−44%)[a]	113* ± 39 (−64%)[a]
Day 7	307 ± 126	270 ± 65	233 ± 45	271 ± 75
Day 14	236 ± 82	227 ± 83	175 ± 43	221 ± 80
Females (n = 12)				
Motor activity:				
Day −7	508 ± 169	399 ± 166	567 ± 136	539 ± 116
Day 1	491 ± 142	443 ± 162	444 ± 110	294* ± 135 (−40%)[a]
Day 7	520 ± 246	450 ± 294	530 ± 130	477 ± 160
Day 14	409 ± 160	494 ± 190	428 ± 209	469 ± 177
Locomotor activity:				
Day −7	329 ± 108	220* ± 122	363 ± 117	345 ± 81
Day 1	292 ± 90	262 ± 112	251 ± 104	102* ± 47 (−65%)[a]
Day 7	294 ± 105	256 ± 170	315 ± 76	261 ± 88
Day 14	239 ± 108	303 ± 163	263 ± 153	240 ± 95

From Gilmore et al. (2005)

[a] Percentage decrease compared with control values is shown in parentheses.

* Significantly different from control, $p \leq 0.05$.

hyper-reactivity, tremors), mouth (increased salivation), respiration (noisy, coughing) and general appearance (general pallor, wasted, hunched posture) for a limited period. A mean body-weight loss was observed between days 1 and 10 and a body weight remained static between days 10 and 15. Thereafter, mean body-weight gain per day was slightly lower than values for the controls. As a consequence, mean body weight progressively decreased throughout the study from 2% after 3 days of treatment to 12% at study termination on day 41 ($p < 0.01$ or $p < 0.05$), compared with controls. Mean food consumption was decreased by 22% ($p < 0.01$) from days 1–8, when compared with the control group. Subsequently, mean food consumption was slightly reduced by 6–10% from days 8–22, or was comparable to the control values thereafter.

Table 33. Mean motor activity in an initial study of acute neurotoxicity in rats given spirotetramat by gavage

Interval	Mean motor activity (No. of movements/10 min interval) ± standard deviation							
	Dose (mg/kg bw)							
	0	200	500	2000	0	200	500	2000
	Males				Females			
Day −7								
1	96 ± 27	87 ± 15	93 ± 27	90 ± 15	104 ± 19	92 ± 29	116 ± 13	106 ± 20
2	80 ± 48	76 ± 31	80 ± 20	78 ± 37	89 ± 29	78 ± 50	107 ± 30	93 ± 19
3	77 ± 49	68 ± 39	80 ± 30	85 ± 25	98 ± 46	73 ± 42	104 ± 31	100 ± 24
4	65 ± 44	65 ± 44	58 ± 29	65 ± 26	87 ± 48	65 ± 35	107 ± 35	94 ± 25
5	58 ± 49	19 ± 21	52 ± 47	43 ± 38	70 ± 37	46 ± 50	80 ± 41	88 ± 35
6	39 ± 28	4* ± 97	22 ± 31	22 ± 31	61 ± 49	45 ± 43	53 ± 44	59 ± 43
Day 1								
1	115 ± 31	118 ± 48	91 ± 29	80* ± 13	118 ± 36	114 ± 21	105 ± 24	76 ± 30
2	105 ± 34	109 ± 88	73 ± 22	64 ± 55	95 ± 49	86 ± 27	78 ± 30	50 ± ± 26
3	113 ± 48	97 ± 67	55* ± 22	34* ± 17	93 ± 36	73 ± 40	76 ± 24	50 ± 31
4	82 ± 36	97 ± 86	54 ± 29	28* ± 19	79 ± 35	70 ± 38	74 ± 30	33 ± 29
5	65 ± 47	75 ± 103	41 ± 27	37 ± 24	63 ± 28	52 ± 42	52 ± 22	49 ± 40
6	36 ± 36	50 ± 75	35 ± 34	26 ± 18	43 ± 38	49 ± 39	60 ± 29	35 ± 28
Day 7								
1	120 ± 38	124 ± 32	117 ± 22	121 ± 21	126 ± 37	132 ± 51	143 ± 34	138 ± 23
2	98 ± 29	87 ± 27	80 ± 16	86 ± 17	105 ± 29	98 ± 57	115 ± 31	108 ± 39
3	87 ± 36	104 ± 82	77 ± 15	76 ± 29	81 ± 34	73 ± 52	93 ± 19	85 ± 37
4	68 ± 40	146 ± 275	67 ± 26	72 ± 35	63 ± 27	53 ± 60	70 ± 33	66 ± 36
5	63 ± 31	56 ± 24	40 ± 29	61 ± 43	50 ± 33	48 ± 52	67 ± 62	46 ± 40
6	57 ± 39	56 ± 30	28 ± 23	44 ± 36	96 ± 223	47 ± 50	43 ± 37	34 ± 29
Day 14								
1	115 ± 36	122 ± 27	115 ± 29	107 ± 24	121 ± 46	136 ± 44	124 ± 60	128 ± 42
2	83 ± 30	90 ± 24	76 ± 17	81 ± 10	88 ± 37	108 ± 45	92 ± 51	101 ± 39
3	78 ± 24	77 ± 21	65 ± 34	73 ± 20	70 ± 33	86 ± 46	69 ± 38	77 ± 48
4	59 ± 34	61 ± 30	41 ± 18	45 ± 17	52 ± 34	69 ± 53	58 ± 43	59 ± 40
5	42 ± 27	38 ± 33	27 ± 32	30 ± 20	43 ± 26	47 ± 30	49 ± 37	57 ± 44
6	33 ± 21	41 ± 35	21 ± 24	39 ± 34	36 ± 27	49 ± 18	36 ± 45	48 ± 32

From Gilmore et al. (2005)
* $p \leq 0.05$.

Sperm analysis after 3 or 10 days of treatment revealed no treatment-related changes in the numbers of spermatozoa (absolute and relative to the epididymis weight) or in the frequency of all types of abnormal spermatozoa. After 21 days, the numbers of spermatozoa were still comparable to the control values, but the frequency of all types of abnormal spermatozoa was slightly increased in the treated group (6.4% vs 2.5% in the control group, $p < 0.01$). After 41 days of treatment, the absolute and relative numbers of spermatozoa were markedly decreased by 77% and 68% ($p < 0.01$), respectively, compared with controls. In association, changes in sperm morphology were more marked,

Table 34. Mean locomotor activity in an initial study of acute neurotoxicity in rats given spirotetramat by gavage

Interval	Mean locomotor activity (No. of movements/10 min interval) ± standard deviation							
	Dose (mg/kg bw)							
	0	200	500	2000	0	200	500	2000
	Males (n = 12)				Females (n = 12)			
Day −7								
1	62 ± 24	57 ± 14	63 ± 23	60 ± 12	70 ± 17	55 ± 18	77 ± 15	70 ± 13
2	51 ± 39	46 ± 24	46 ± 14	48 ± 25	55 ± 21	44 ± 36	69 ± 28	54 ± 14
3	52 ± 39	44 ± 27	52 ± 22	52 ± 21	64 ± 32	43 ± 34	64 ± 26	60 ± 23
4	42 ± 34	41 ± 29	34 ± 19	42 ± 19	59 ± 35	36 ± 25	71 ± 31	66 ± 24
5	38 ± 36	11 ± 14	31 ± 29	25 ± 24	45 ± 24	25 ± 26	51 ± 27	55 ± 23
6	19 ± 18	3 ± 6	13 ± 20	11 ± 16	36 ± 27	18 ± 21	31 ± 29	40 ± 29
Day 1								
1	76 ± 21	65 ± 23	54* ± 15	45* ± 14	78 ± 19	70 ± 12	62 ± 20	35 ± 16
2	64 ± 30	44 ± 24	34* ± 14	19* ± 9	53 ± 22	51 ± 20	47 ± 27	18 ± 11
3	68 ± 38	44* ± 24	28* ± 16	14* ± 10	51 ± 21	43 ± 24	44 ± 22	14 ± 8
4	50 ± 29	39 ± 26	29 ± 22	13* ± 11	45 ± 21	39 ± 26	41 ± 23	11 ± 8
5	34 ± 30	18 ± 17	15* ± 14	12* ± 14	39 ± 20	32 ± 30	26 ± 15	15 ± 12
6	20 ± 25	13 ± 19	14 ± 16	9 ± 7	26 ± 27	28 ± 27	30 ± 18	9 ± 6
Day 7								
1	84 ± 9	86 ± 25	74 ± 16	78 ± 10	86 ± 27	85 ± 32	99 ± 25	84 ± 19
2	61 ± 24	50 ± 22	45 ± 13	50 ± 9	72 ± 23	57 ± 38	74 ± 26	63 ± 28
3	54 ± 30	43 ± 12	46 ± 12	45 ± 18	56 ± 26	39 ± 27	58 ± 20	45 ± 21
4	41 ± 27	39 ± 14	36 ± 16	43 ± 25	36 ± 18	29 ± 34	38 ± 27	34 ± 20
5	38 ± 21	29 ± 14	20 ± 16	35 ± 28	26 ± 23	23 ± 25	26 ± 22	21 ± 19
6	30 ± 27	24 ± 15	13 ± 11	21 ± 21	18 ± 20	23 ± 30	20 ± 17	15 ± 15
Day 14								
1	76 ± 21	72 ± 18	66 ± 14	71 ± 21	82 ± 35	92 ± 41	89 ± 47	82 ± 29
2	48 ± 22	48 ± 13	38 ± 11	46 ± 7	49 ± 24	67 ± 37	60 ± 40	57 ± 22
3	43 ± 18	40 ± 16	32 ± 16	41 ± 19	41 ± 20	52 ± 41	41 ± 26	40 ± 21
4	31 ± 22	31 ± 19	18 ± 9	27 ± 14	25 ± 22	35 ± 39	32 ± 28	26 ± 19
5	21 ± 15	18 ± 21	12 ± 14	16 ± 13	24 ± 16	29 ± 23	25 ± 21	14 ± 14
6	17 ± 15	19 ± 22	9 ± 11	20 ± 21	18 ± 19	28 ± 12	17 ± 23	21 ± 22

From Gilmore et al. (2005)
* $p \leq 0.05$.

Table 35. Mean motor and locomotor activity during the follow-up study of acute neurotoxicity in rats given spirotetramat by gavage

Test day	Mean total activity counts per session ± standard deviation			
	Dose (mg/kg per day)			
	0	50	100	500
Males (n = 12)				
Motor activity:				
Day −7	404 ± 160	460 ± 100	383 ± 100	382 ± 160
Day 1	373 ± 124	387 ± 111	349 ± 97	296 ± 74 (−21%)
Locomotor activity:				
Day −7	276 ± 128	323 ± 82	242 ± 76	240 ± 104
Day 1	257 ± 100	274 ± 84	228 ± 73	179 ± 50 (−30%)
Females (n = 12)				
Motor activity:				
Day −7	474 ± 222	483 ± 109	539 ± 162	443 ± 151
Day 1	440 ± 178	485 ± 192	470 ± 189	375 ± 175
Locomotor activity:				
Day −7	319 ± 171	306 ± 97	350 ± 112	312 ± 122
Day 1	293 ± 131	323 ± 142	321 ± 152	236 ± 131

From Gilmore et al. (2005)

[a] Percentage decrease compared with control values is shown in parentheses.

Table- 36. Mean motor activity in the follow-up study of acute neurotoxicity in rats given spirotetramat by gavage

Interval	Mean motor activity (No. of movements/10 min interval) ± standard deviation							
	Dose (mg/kg bw)							
	0	50	100	500	0	50	100	500
	Males (n = 12)				Females (n = 12)			
Day –7								
1	96 ± 32	105 ± 18	101 ± 16	104 ± 37	98 ± 23	97 ± 16	115 ± 18	103 ± 19
2	85 ± 30	107 ± 27	77 ± 17	87 ± 32	82 ± 32	89 ± 24	92 ± 30	80 ± 21
3	75 ± 24	98 ± 21	67 ± 25	73 ± 36	84 ± 45	94 ± 31	88 ± 35	82 ± 39
4	65 ± 43	68 ± 39	60 ± 32	49 ± 31	76 ± 48	94 ± 28	93 ± 37	75 ± 41
5	47 ± 40	48 ± 34	45 ± 30	40 ± 34	73 ± 47	58 ± 29	78 ± 28	61 ± 38
6	36 ± 30	35 ± 36	34 ± 24	29 ± 31	60 ± 50	52 ± 34	72 ± 38	42 ± 35
Day 1								
1	92 ± 28	95 ± 23	81 ± 15	84 ± 22	129 ± 50	119 ± 29	118 ± 22	87* ± 32
2	82 ± 26	78 ± 20	74 ± 16	58 ± 19	99 ± 36	119 ± 27	93 ± 21	77 ± 25
3	75 ± 20	72 ± 32	58 ± 30	47 ± 21	77 ± 37	87 ± 41	88 ± 35	63 ± 38
4	55 ± 21	60 ± 20	55 ± 25	38 ± 22	59 ± 43	71 ± 46	62 ± 48	58 ± 38
5	41 ± 25	49 ± 22	51 ± 24	34 ± 18	45 ± 35	48 ± 40	63 ± 54	40 ± 34
6	29 ± 24	32 ± 30	31 ± 24	35 ± 29	32 ± 29	41 ± 48	46 ± 37	50 ± 43

From Gilmore et al. (2005)

* Significantly different from controls, $p \leq 0.05$.

as the frequency of total abnormal spermatozoa reached 72.0% in the treated group compared with 3.8% in the control group ($p < 0.01$).

At necropsy, mean terminal body weight was comparable to values for the control group at sacrifice on day 3, but was reduced by 6–12% at days 10, 21 and 41. No toxicologically-relevant changes were noted in the testes, epididymis or prostate weights at days 3, 10 or 21. At day 41, the prostate gland weight was unaffected by treatment, but mean absolute and relative testes and epididymis weights were statistically significantly reduced by 11–26%. At the macroscopic examination, no treatment-related gross pathology changes were detected after 3, 10 and 21 days of treatment. However, after 41 days, small epididymis was observed in all rats, together with a small prostate gland in 2 out of 7 rats and small and/or soft testis in 6 out of 7 rats.

Microscopic examination revealed adverse treatment-related changes in the seminiferous tubules and epididymis after 21 days of treatment. In the testes, marked degenerating elongating spermatids (steps 9–14 of the maturation cycle) were found in 8 out of 8 rats treatedwith spirotetramat, together with multinucleated giant spermatids in 2 out of 8 rats and moderate to marked degenerating round spermatids in 4 out of 8 rats. These morphological findings identified after 21 days of treatment are consistent with a treatment-related effect in round spermatids or late stage spermatocytes. In the epididymis, slight to moderate increase of intraluminal abnormal aberrant cells were found in all treated rats after 21 days of treatment, as a consequence of degenerating spermatids observed in the testis. At final sacrifice on day 41, slight to severe degenerating elongating spermatids (steps 9–14) were found in 7 out of7 treated rats, together with loss of elongating spermatids (steps 9–19) in 5 out of 7 treated rats. Multinucleated giant spermatids in 4 out of 7 rats and marked degenerating round spermatids (around steps 7–8) in 5 out of 7 rats were also observed. These morphological findings in the testes confirm what was observed after 21 days of treatment and may be consistent with a

Table 37. Mean locomotor activity in the follow-up study of acute neurotoxicity in rats given spirotetramat by gavage

Interval	Mean locomotor activity (No. of movements/10 min interval) ± standard deviation							
	Dose (mg/kg bw)							
	0	50	100	500	0	50	100	500
	Males (n = 12)				Females (n = 12)			
Day −7								
1	73 ± 28	81 ± 17	73 ± 15	70 ± 17	68 ± 19	65 ± 13	76 ± 13	77 ± 16
2	56 ± 28	78 ± 27	49 ± 15	60 ± 27	57 ± 25	55 ± 21	57 ± 22	53 ± 17
3	51 ± 22	72 ± 22	46 ± 19	48 ± 24	56 ± 37	64 ± 23	55 ± 24	57 ± 34
4	44 ± 33	45 ± 26	36 ± 21	31 ± 22	54 ± 38	60 ± 25	64 ± 28	52 ± 30
5	30 ± 30	28 ± 24	26 ± 20	20 ± 24	48 ± 31	34 ± 23	54 ± 22	45 ± 32
6	21 ± 21	20 ± 26	13 ± 13	10 ± 17	37 ± 36	28 ± 24	43 ± 21	29 ± 26
Day 1								
1	73 ± 23	76 ± 18	62 ± 12	61 ± 14	99 ± 39	89 ± 27	88 ± 20	64* ± 25
2	57 ± 23	57 ± 16	50 ± 15	37 ± 15	71 ± 28	79 ± 21	64 ± 20	48 ± 20
3	51 ± 19	53 ± 25	37 ± 22	30 ± 17	53 ± 29	58 ± 33	61 ± 29	39 ± 28
4	38 ± 19	42 ± 15	32 ± 18	23 ± 14	33 ± 30	45 ± 37	42 ± 39	33 ± 27
5	22 ± 18	31 ± 17	28 ± 16	16 ± 10	23 ± 27	28 ± 28	44 ± 40	22 ± 22
6	16 ± 15	17 ± 19	17 ± 16	12 ± 10	15 ± 15	23 ± 29	22 ± 22	30 ± 28

From Gilmore et al. (2005)
* $p \leq 0.05$.

treatment-related effect in round spermatids. As a consequence of degenerating spermatids observed in the testis,a marked increase of intraluminal aberrant cells associated with a moderate to marked oligospermia were found in the epididymis in all treated rats on day 41. In conclusion, spirotetramat at 1000 mg/kg bw per day over at least 21 days induced treatment-related effects in round spermatids or late stage spermatocytes and subsequent changes in the testis and epididymis (Kennel, 2005).

The testicular/sperm toxicity attributed to spirotetramat was investigated using the enol metabolite of spirotetramat. The structure of spirotetramat consists of two parts – an enol entity and an acyl chain. The enol is the major metabolite of spirotetramat in rats whereas the acyl chain of spirotetramat aids the penetration of spirotetramat into the plant and is cleaved once in the plant. As the structure of the acyl chain resembles that of the established testicular toxicant, methoxy acetic acid, it was proposed that this chain may be responsible for the testicular toxicity of spirotetramat. However, as this moiety is chemically unstable, the enol metabolite of spirotetramat was investigated. This study did not comply with GLP nor was a QA statement provided.

Groups of five male rats (strain Rj:WI(IOPS HANS) (aged 12–13 weeks) were given the metabolite spirotetramat-enol (ourity, 96.8; a beige powder) at a dose of 0 (vehicle only) or 800 mg/kg bw per day by gavage in 0.5% methylcellulose in a dose volume of 5 ml/kg for 21 days. Clinical signs and body weights were recorded daily. Food consumption was measured weekly. A detailed physical examination was performed once during the acclimatization period and weekly throughout the study. All rats were sacrificed 4 h after the last of 21 doses. All rats were necropsied and selected organs were excised and weighed. Both testes and the left epididymis from each rat were fixed and examined microscopically. Sperm from the right epididymis were collected for sperm enumeration and morphological assessment. The dose of 800 mg/kg bw per day of spirotetramat-enol used in this study was chosen as it is equivalent to a dose of 1000 mg/kg bw per day of the parent compound, spirotetramat, which has been used in a previous investigative study described above (Kennel, 2005) in which testicular and sperm toxicity were observed. The exposure period selected was 21 days, as significant testicular and sperm effects were first recorded at this time-point in the previous study investigating the parent compound.

As clinical signs of toxicity, body-weight effects and a treatment-related death were attributd to administration of the parent compound, a preliminary test was performed to evaluate the toxicity of spirotetramat-enol, the results of which were not described.

Oral exposure to spirotetramat-enol for 21 days did not induce any treatment-related mortality. However, a range of clinical signs was observed, particularly in the later stages of the study. These signs included localized soiled fur (anogenital and mouth regions), increased salivation and reduced motor activity. A mean body-weight loss was observed on days 2 ($p \leq 0.01$, when compared with controls), 14, 16, 18 ($p \leq 0.01$ when compared with controls) and 20. The mean body-weight gain on all other days of study was similar to control values. This resulted in the overall body-weight gain being only 22% of that of the controls. This was not, however, statistically significant due to the large standard deviations for both groups. The mean body weight was consistently, though not statistically significantly reduced compared with controls, from 1.3% on day 2 to 7% on the last day of dosing. Mean food consumption was reduced by 14.2% ($p \leq 0.05$) during days 1–8 and by 8.5% during days 8–15. The mean food consumption was similar to control values during the last week of exposure.

At necropsy, the mean terminal body weight of the treated group was lower (–8%, not statistically significant) when compared with controls. The mean relative testes weights were statistically significantly higher ($p \leq 0.05$), but this change mainly correlated with the lower terminal body weights. Microscopic examination of the seminiferous tubules and the epididymis revealed a treatment-related change after 21 days of treatment. Sloughing of germ cells associated with degenerating elongating spermatids was found in all rats treated with spirotetramat-enol. In the left epididymis, exfoliated germ cells were found in all treated rats, as a consequence of degenerating spermatids observed in the testes. Sperm analysis revealed a slight increase in the absolute numbers of spermatozoa (5%, not

statistically significant) whereas there was a slight decrease in the relative (to the epididymal weight) numbers (7.9%, not statistically significant) after 21 days of treatment. The frequency of total abnormal spermatozoa was significantly increased ($p \leq 0.05$) after 21 days of treatment (14.9% vs 3.2% in the control group). Most of these abnormalities were either isolated heads of normal morphology or spermatozoa with a normal head but possessing an abnormal mid-piece.

In conclusion, oral exposure of male rats to spirotetramat-enol at a dose of 800 mg/kg bw for 21 days induced treatment-related effects in the testes and epididymis and induced an increase in the frequency of spermatozoa with an aberrant morphology. The effects observed in this study are similar, both in terms of observations and magnitude of responses, to those recorded for a previous study in which the parent compound, spirotetramat, was assessed under similar conditions (Kennel, 2005). Based on the results of this study, the Meeting concluded that the testicular/sperm toxicity of the spirotetramat is unlikely to be due to its acyl chain but rather to its major metabolite i.e. spirotetramat-enol (Tinwell, 2006).

(c) Studies with metabolites

(i) Acute toxicity

Studies of acute oral toxicity have been carried out with a number of metabolites, as summarized in Table 38.

(ii) Genotoxicity

Studies of genotoxicity have been carried out with a number of metabolites, as summarized in Table 39.

Table 38. Acute oral toxicity with metabolites of spirotetramat in female Wistar rats

Metabolite	Strain	Purity (%)	LD50 (mg/kg bw)	Reference[a]
Spirotetramat-cis-ketohydroxy	HsdCpb:Wu	98.7	> 2000	Schuengel (2005a)
Spirotetramat-desmethyl-ketohydroxy	HsdCpb:Wu	94.6	> 2000	Schuengel (2006a)
Spirotetramat-mono-hydroxy	HsdCpb:Wu	98.41	> 2000	Schuengel (2005b)
Spirotetramat-di-hydroxy	HsdCpb:Wu	94.5.	> 2000	Schuengel (2006b)

[a] All studies complied with good laboratory practice and statements of quality assurance were provided.

Table-39. Studies of genotoxicity with metabolites of spirotetramat in vitro

Metabolite	End-point	Test object	Concentration/dose	Purity (%)	Result	Reference[a]
Spirotetramat-*cis*-ketohydroxy	Point mutation	*S. typhimurium* TA1535, TA100, TA1537, TA98 and TA102	≤ 1581 µg per plate ± S9	98.7	Negative	Herbold (2005a)
Spirotetramat-desmethyl-ketohydroxy	Point mutation	*S. typhimurium* TA1535, TA100, TA1537, TA98 and TA102	≤ 500 µg per plate ± S9	94.6	Negative	Wirnitzer (2006)
Spirotetramat-mono-hydroxy	Point mutation	*S. typhimurium* TA1535, TA100, TA1537, TA98 and TA102	≤ 5000 µg per plate ± S9	98.41	Negative	Herbold (2005b)
Spirotetramat-di-hydroxy	Point mutation	*S. typhimurium* TA1535, TA100, TA1537, TA98 and TA102	≤ 5000 µg per plate ± S9	94.5	Negative	Herbold (2006b)

S9, 9000 × *g* supernatant from rodent liver

[a] All studies complied with good laboratory practice and statements of quality assurance were provided.

(iii) [Azaspirodecenyl-3-^{14}C]- spirotetramat-enol-glucoside: study of absorption, distribution, excretion and metabolism in the rat

Spirotetramat-enol-glucoside was a main metabolite of spirotetramat in a study of the metabolism of spirotetramat in lettuce and hence was included in the plant-residue method (Hass et al., 2004). Spirotetramat-enol-glucoside was detected in fruiting and leafy crops in European residue trials (unpublished results). Because it was an uncommon metabolite in the study of absorption, distribution, excretion and metabolism in rats, an additional study was conducted in order to investigate the absorption, distribution, excretion and metabolism of spirotetramat-enol-glucoside in male rats. The study complied with GLP and a QA statement was provided.

One male rat was given a single dose of spirotetramat-enolglucoside (radiolabelled with ^{14}C in the 3-position of the spiro-ring of the molecule) at a target dose of 0.1 mg/kg bw by oral gavage in aqueous saline solution. Plasma, urine and faeces were collected at various times after dosing. Skin, gastrointestinal tract and carcass were sampled at sacrifice.

Of the administered dose, 98.3% was recovered from measurement of the total radioactivity in urine and faeces as well as in skin, gastrointestinal tract and carcass at sacrifice, as shown in Table 40.

The radiolabelled spirotetramat-enol-glucoside was rapidly absorbed from the gastrointestinal tract of the male rat. Absorption started immediately after oral dosing. Excretion was rapid and was almost complete by 24 h after dosing. The radiolabelled residues in skin, gastrointestinal tract and carcass of the rat were determined at sacrifice, 48 h after dosing. A negligible amount of radiolabel was found in skin and gastrointestinal tract. Thequantity of radiolabel in the carcass amounted to 1% of the administered dose.

The test item and its metabolites were identified in this study by reverse-phase HPLC. The identification rate was high and amounted to 93% of the administered dose. Spirotetramat-enol was the main metabolite in excreta (about 64% of the administered dose). Minor metabolites were spirotetramat-desmethyl-enol (about 5%) and spirotetramat-ketohydroxy (about 3%). Unchanged spirotetramat-enol-glucoside was detected in excreta (about 21% of the administered dose), while 20.7% of the administered dose was found in the faeces. Only one minor, polar, metabolite (0.5% of the administered dose) in the faeces was not identified (Klempner, 2006d).

(iv) [Azaspirodecane-3-14C] spirotetramat-ketohydroxy: study of absorption, distribution, excretion and metabolism in the rat

Spirotetramat-ketohydroxy is a metabolite of spirotetramat that was found in the studies of metabolism in target crops (apple, lettuce and cotton), and identified as a main metabolite in cotton (Sur et

Table 40. Recovery of radiolabel from a rat given a single dose of spirotetramat-enolglucoside by gavage

Sample	Recovery (% of administered dose)
Urine	53.3
Faeces	43.7
Total excreted	97.0
Skin	0.09
Sum of organs	0.99
Body without gastrointestinal tract	1.08
Gastrointestinal tract	0.11
Total body	1.19
Balance	98.3

From Klempner (2006d)

al., 2005). Spirotetramat-ketohydroxy was also detected in a confined rotational-crop study, where it was the main metabolite in Swiss chard and turnip roots. In the study described here, which complied with GLP and for which a QA statement was provided, a group of four male Wistar rats was given a single dose of spirotetramat-ketohydroxy (radiolabelled with ^{14}C at the carbon at position 3 in the azaspirodecane ring (radiochemical purity > 99%) at a target dose of 2 mg/kg bw by oral gavage in aqueous Tragacanth®. The rats were killed 48 h after dosing. Total radioactivity, including parent compound and/or metabolites, was determined in the excreta (urine and faeces) as well as in organs and tissues. Investigations on metabolites were performed with selected samples of urine and with faecal extracts.

About 99% of the administered dose was recovered from the urine and faeces and in organs and tissues. Radiolabelled spirotetramat-ketohydroxy was rapidly absorbed from the gastrointestinal tract of male rats, absorption starting immediately after oral dosing. Although no exact value for the absorption rate could be derived from these observations, it was assumed that a high proportion of the administered dose was absorbed and systemically available, in view of this behaviour and the fact that the test item was intensively metabolized showing the same metabolic pattern in the urine and faeces.

Excretion was rapid and was almost complete 24 h after dosing. Of the total recovery of 98.6%, 54.5% of the dose was excreted via the urine and 44.1% via the faeces.

The radiolabelled residues in the organs and tissues of the rats were determined at sacrifice, 48 h after dosing. Negligible amounts of radiolabel were found in the skin, liver and gastrointestinal tract, showing that the elimination of the compound-related radioactivity was nearly complete. The concentrations of residues in all other organs and tissues were very low (< 0.01 ppm, or < LOD).

For elucidation of the metabolism, urine samples and faecal extracts were analysed by HPLC with radiodetection and by LC/MS and LC/MS-MS. The identification rate was high, amounting to 86% of the administered dose. The test item was detected as a minor quantity of < 1% of the administered dose in faeces only. Spirotetramat-ketohydroxy was completely metabolized, forming numerous metabolites. The first and most important metabolic reaction was the oxidative demethylation of the cyclohexyl-*O*-methyl group to the respective alcohol (spirotetramat-desmethylketohydroxy). The two isomers of spirotetramat-desmethyl-ketohydroxy were the main components in excreta accounting for 15% of the administered dose. All other identified metabolites were further degradation products of spirotetramat-desmethyl-ketohydroxy. Most were different mono-, di- and tri-oxygenated metabolites leading in a first metabolic transformation to the corresponding hydroxy metabolites. A second oxidative transformation (formal loss of two hydrogen atoms) gave the corresponding keto-metabolites with or without additional double-bond formation. The oxygenated metabolites were classified in three groups to simplify evaluation and quantification: mono-hydroxy, di-hydroxy and tri-hydroxy metabolites of spirotetramat-desmethyl-ketohydroxy. About 63% of the dose comprised the various different mono- and di-hydroxy metabolites of spirotetramat-desmethyl-ketohydroxy. Tri-hydroxy metabolites were of minor importance. Only one metabolite of this group was detected, accounting for about 3% of the administered dose. Conjugation with e.g. glucuronic acid and sulfate, was detected for few metabolites only and at low quantities. Cleavage of the azaspirodecane ring of spirotetramat-desmethylketohydroxy was found to a minor extent, as shown by two metabolites: spirotetramat-desmethyl-mandelic acid-amide and spirotetramat-desmethyl-glyoxyilic amide. The two metabolites comprised ≤ 2.8% of the administered dose. Only five minor metabolites (all < 1.6% of the administered dose) were not identified, but were characterized by their HPLC elution behaviour (Klempner, 2006e).

(d) Comparative study of in-vitro dermal absorption of [^{14}C]spirotetramat in human and rat skin

In a study that complied with GLP and for which a QA statement was provided, **t**he dermal penetration of [^{14}C]spirotetramat in a soluble concentrate (SC240) formulation through rat and

human dermatomed skin was investigated at nominal concentrations of 240, 1.5 and 0.05 mg/ml, for the highest, intermediate and lowest dose formulations, respectively. The mean percentage of [^{14}C]spirotetramat considered to be directly absorbed over 24 h from the formulation providing the highest dose was 0.10% and 0.29% for human and rat skin, respectively. The mean percentage of [^{14}C]spirotetramat considered to be potentially absorbable over 24 h from the formulation providing the highest dose was 0.41% and 2.90% for human and rat skin, respectively, yielding a factor difference of 7.1 between the two species for the undiluted product. For the formulation providing the intermediate dose, the mean percentage directly absorbed was 0.07% and 1.49% for human and rat skin, respectively. For the formulation providing the intermediate dose, the mean percentage potentially absorbable was 1.41% and 11.76% for human and rat skin, respectively, yielding a factor difference of 8.3 between the two species for the spray dilution. For the formulation providing the lowest dose, the mean percentage directly absorbed was 0.24% and 6.99% for human and rat skin, respectively. For the formulation providing the lowest dose, the mean percentage potentially absorbable was 11.47% and 16.32% for human and rat skin, respectively, yielding a factor difference of 1.4 between the two species for the spray dilution (Capt, 2006).

3. Observations in humans

3.1 Assessment of skin sensitization in workers handling spirotetramat

Spirotetramat, which is known from experiments in laboratory animals to be a dermal sensitizer, has caused two proven cases of type-4 sensitization (allergic contact dermatitis) in workers handling the undiluted active ingredient.

In 2004, two cases of type-4 skin sensitization were observed and confirmed by patch testing in two workers handling the active ingredient spirotetramat during development. One worker had been mixing active ingredient into the animal feed for toxicology testing, and the other had prepared test formulations containing active ingredient. In both cases, the facial skin was involved, and in both cases the use of personal protective equipment was not satisfactory. Symptoms were reddening, itching, swelling and eczema of the facial skin in the preorbital area.

To rule out further cases among employees and to possibly improve protection, a questionnaire survey was performed in nine countries. The partipants included staff involved in research, development, industrial operations and health care of the manufacturer. Participation (which was voluntary in nature) was satisfactory (175 out of 269 workers; 65%). The questionnaire ruled out sensitization to spirotetramat in 170 out of of 175 employees (97%). Five employees (3%) required further assessment owing to vague indications of possible sensitization. In three of these cases, sensitization could be ruled out by an occupational physician; in a fourth case sensitization was ruled out by an occupational dermatologist. Patch testing for sensitization to spirotetramat was proposed in one case, but the employee declined this offer. The joint judgement of the two physicians involved was that sensitization was unlikely, yet not definitely ruled out. Although no further cases of sensitization have been detected, technical and personal protective-equipment protection must be maintained. Occupational medical surveillance for exposed workers should continue (Steffens, 2005).

3.2 Occupational medical experiences with spirotetramat

Occupational medical surveillance of 12 workers exposed to spirotetramat, performed yearly on a routine basis as reported by the manufacturer and not directly related to exposures, did not reveal any unwanted effects in the workers except the two previously mentioned proven cases of type-4 sensitization. The medical examinations included history, full physical examination with orientating neurological status (reflexes, sensibility and coordination) and skin status. The technical

examination and laboratory investigation included lung function, electrocardiogram/ergometry, vision testing, audiometry, chest X-ray, sonography (if necessary); blood sugar random (BSR), full blood count, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, glucose, creatinine, cholesterol, and urine status. All the workers used personal safety measures to the manufacturers' standards, full mask with filter ABEK-P3, protective gloves for chemicals, chemical-resistant suit and safety glasses (Kehrig, 2006).

3.3 Literature survey

A number of literature databases were investigated concerning the following issues in regard to spirotetramat:

- Reports on clinical cases and poisoning incidents;
- Epidemiological studies;
- Clinical signs and symptoms of poisoning.

None of the databases contained publications on incidents of human poisoning with spirotetramat. No negative effects were reported (Temerowski, 2006).

As spirotetramat is a new active substance, there are no studies, published papers or further reports available.

Comments

Biochemical aspects

After oral administration at a dose of 2 or 100 mg/kg bw, spirotetramat was rapidly absorbed in rats. The extent of absorption in the single low-dose test was 95%. The maximum plasma concentration of radiolabel was reached 0.1–2.0 h after dosing. Concentrations of radiolabel in tissues and organs at 48 h were very low (< 0.2%). Excretion was mainly urinary and was very rapid (essentially complete within 24 h). Faecal excretion accounted for 2–11% of the administered dose in rats. No parent compound was detected in the excreta. Only very minor metabolites (< 0.7% of the administered dose) were not identified. The main metabolic reaction was cleavage of the ester group, producing the enol that is subsequently metabolized to a range of metabolites.

In male rats given a high dose of spirotetramat at 1000 mg/kg bw, it was found that only 27% of the administered dose was excreted in the urine after 24 h. In addition, concentrations of radiolabel in the plasma were slightly higher than in the liver and kidney, and the decline in concentrations of radiolabel found in the tissues was minimal from 1 h to 8 h after dosing, with considerable quantities still remaining at 24 h (approximately 25%). These findings were consistent with saturation of cellular transport mechanisms, which may result in decreased excretion via urine and faeces and a potential for the accumulation of spirotetramat metabolites in the body after repeated high doses. The results of physiologically-based pharmacokinetic simulations supported this conclusion and suggested that repeated daily doses of spirotetramat at > 500 mg/kg bw lead to non-linear elimination kinetics, resulting in a higher than expected body burden in studies with repeated doses, despite some evidence of reduced absorption at such high doses.

In a comparative study of in vitro metabolism in hepatocytes from male rats, mice, and humans, differences in the proportions of several metabolites were observed; however, spirotetramat-enol was the first and most prominent metabolite detected and accounted for 66% and 100% of all metabolites in these studies, in mice and rats respectively. The relative efficiency of enol glucuronidation in isolated hepatocytes was: mouse > human > rat.

Toxicological data

Spirotetramat has low acute toxicity: oral and dermal LD_{50}s in rats were > 2000 mg/kg bw; the inhalation LC_{50} was > 4.18 mg/l of air. Spirotetramat is not a skin irritant in rabbits, although it is an irritant to rabbit eyes. Spirotetramat exhibited a skin sensitization potential in guinea-pigs (Magnussen & Kligman test) and mice (local lymph node assay).

In general, there were no target organs or effects that were common to all species. However, it should be noted that there were indications of immune-related effects in several species.

Mice appeared to be insensitive to toxicity caused by spirotetramat. In repeat-dose studies, mice given diets containing spirotetramat at the highest dose of 5000 ppm (equal to 1415 mg/kg bw per day), 7000 ppm (equal to 1305 mg/kg bw per day) or 7000 ppm (equal to 1022 mg/kg bw per day) for 4 weeks, 14 weeks or 18 months, respectively, showed no toxicological effects.

In a 14-week dietary study of toxicity in rats, the NOAEL was 2500 ppm (equal to 148 mg/kg bw) on the basis of decreased body-weight gain, an increased incidence of abnormal spermatozoa and hypospermia, an increased incidence of tubular degeneration, decreased absolute testicular weight, and accumulation of alveolar macrophages in the lungs of rats at 10 000 ppm (equal to 616 mg/kg bw per day). However these effects were reversible within 4 weeks in most rats after cessation of treatment. In the 1-year dietary study in rats, the NOAEL was 250 ppm (equal to 13.2 mg/kg bw per day) on the basis of an increased incidence of accumulation of alveolar macrophages in the lungs of males at 3500 ppm (equal to 189 mg/kg bw per day). Effects on body weight, and testes and sperm were observed at 7500 ppm.

The thymus and the thyroid were the main target organs in dogs. Reduced weight accompanied by histological evidence of involution and atrophy of the thymus was observed at 6400 ppm in the 4-week dose range-finding study, at 4000/2500 ppm in the 13-week study, and at 600 and 1800 ppm in the 1-year study. Although there was no clear dose–response relationship in the 1-year study, these findings were considered toxicologically significant because they occurred in all studies and because there were other indications that spirotetramat interferes with the immune system (skin sensitization, effect on lungs in rats, and allergic contact dermatitis in humans). Decreases in T4 and T3 concentrations were also observed, with an overall NOAEL of 600 ppm. Changes at this dose were inconsistent. Reduced body weight and haematological effects were observed at higher doses.

The occasional brain ventricular dilatation observed at 600 ppm (one male and one female) and at 1800 ppm (one male) in the 1-year study was not accompanied by any clear histopathological alterations. In addition, brain ventricular dilatation is occasionally reported to occur spontaneously in the strain of dogs used in the test. Consequently, the Meeting considered that this finding was of uncertain toxicological significance.

The Meeting concluded that the NOAEL in the 1-year study in dogs was 200 ppm, equal to 5 mg/kg bw per day, on the basis of effects on the thymus. This NOAEL is also protective for the equivocal findings of changes in thyroid hormones, and the brain ventricular dilatation of uncertain significance seen at 600 ppm.

Spirotetramat was tested in an extensive range of studies of genotoxicity. Negative results were found in studies in vivo and in vitro, except for one weakly and equivocally positive result in a study for chromosomal aberrations in vitro that was not reproduced in a second study with higher concentrations. The Meeting concluded that spirotetramat is unlikely to be genotoxic.

The carcinogenic potential of spirotetramat was studied in mice and rats. Spirotetramat was not found to be carcinogenic in either species. In rats, the NOAEL was 250 ppm, equal to 12.5 mg/kg bw per day, on the basis of structural changes in the kidney (renal tubular dilatation) at 3500 ppm. In this study, effects on the lungs were characterized by an increased incidence of accumulation of alveolar macrophages and of interstitial pneumonia at 7500 ppm and inconsistently at lower doses. These changes were of uncertain significance, possibly being indicative of effects of spirotetramat

on the immune system. Effects on body-weight gain, the testes, epididymis and bile duct were also observed at 7500 ppm.

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

Further support for findings of testicular effects in rats given spirotetramat at a high dose was provided by the results of a dose range-finding one-generation study and a two-generation study of reproductive toxicity. In the one-generation dietary study of reproductive toxicity in rats, severe toxicity was observed in sperm (motility and malformation) of parental males at 10 000 ppm (equal to 538 mg/kg bw per day), resulting in no pregnancies with a NOAEL of 6000 ppm (equal to 320 mg/kg bw per day). However, minimal effects on sperm parameters were observed in the F_1 generation at 6000 ppm (equivalent to 400 mg/kg bw per day in parents) with a NOAEL of 500 ppm (equal to 27.8 mg/kg bw per day in parents). At this dose, a significant (–14%) decline in pup-weight gain, possibly secondary to decreases in maternal body weight was observed. In the two-generation study of reproductive toxicity, abnormal sperm cells were reported in the F_1 generation, but not in parental male rats at 6000 ppm (equal to 487 mg/kg bw per day) and decreased reproductive performance was also observed in one of these males. Offspring toxicity also included decreased body weight in F_1 and F_2 pups in both sexes during lactation at 6000 ppm (equal to 419 mg/kg bw per day). Effects observed in the parental generation were reduction of body weight and/or body-weight gain, reduced terminal body weight, reduced food consumption (females) and increased multifocal tubular dilatation in the kidneys in rats at 6000 ppm. The NOAEL for parental toxicity was 1000 ppm (equal to 70.7 mg/kg bw per day) on the basis of decreases in body-weight gain in the parental generation. The NOAEL for reproductive toxicity was 1000 ppm (equal to 79.5 mg/kg bw in F_1 males) on the basis of abnormal sperm-cell morphology in the F_1 generation. The NOAEL for offspring toxicity was 1000 ppm on the basis of growth retardation at 6000 ppm.

Two studies of developmental toxicity in rats treated by gavage have been performed. Inconsistent and equivocal effects on the offspring, including retarded ossification and increased wavy ribs, were observed in one study at doses of 140 and 20 mg/kg bw per day. Maternal effects consisting mainly of reduced body-weight gain were observed at 1000 mg/kg bw per day and were associated with reduced offspring weight, reduced fetal weight, retarded ossification and a slight increase in the frequency of fetuses with any malformations. The overall NOAEL for maternal toxicity was 140 mg/kg bw per day and the overall NOAEL for developmental toxicity was 140 mg/kg bw per day.

In a study of developmental toxicity in rabbits treated by gavage, severe maternal toxicity was observed, including death and abortion, at 160 mg/kg bw per day. No effects were observed at 40 mg/kg bw per day, except one abortion, which was considered to be incidental. No significant effects were observed in the offspring and the NOAEL was 160 mg/kg bw per day, the highest dose tested. The NOAEL for maternal toxicity was 40 mg/kg bw per day.

The effects on sperm, testes and epididymis were studied in more detail in rats given spirotetramat at a dose of 1000 mg/kg bw per day. It was observed that the decreased epididymal sperm counts occurred after 21 days and not after 10 days of treatment. In another study in rats given the enol metabolite, testicular/sperm toxicity similar to that caused by spirotetramat was observed. Thus these effects are unlikely to be due to the presence of the acyl chain of this compound.

The Meeting concluded that spirotetramat causes toxicity in the testes and sperm that, at higher doses, affects reproductive performance in rats. The NOAEL for testes and sperm effects was 169 mg/kg bw per day, with a LOAEL of 370 mg/kg bw per day in a 2-year study in adult rats, and a NOAEL of 79.5 mg/kg bw per day and a marginal LOAEL of 400 mg/kg bw per day in young rats, respectively. The Meeting observed that these effects occurred at doses higher than those causing other types of systemic toxicity, on which the acceptable daily intake (ADI) and acute reference dose (ARfD) were based.

Two studies of acute oral neurotoxicity in rats have been conducted. The overall NOAEL was 100 mg/kg bw per day on the basis of urine staining and slight declines in motor and locomotor activity in male rats at 200 mg/kg bw per day.

Studies with four metabolites found in animals and plants – spirotetramat-*cis*-ketohydroxy, spirotetramat-desmethyl-ketohydroxy, spirotetramat-mono-hydroxy and spirotetramat-di-hydroxy – showed that these substances were of low acute oral toxicity in female rats (LD_{50} > 2000 mg/kg bw) and not mutagenic in an assay for gene mutation in strains of *S. typhimurium*. The plant-specific metabolite spirotetramat-enol-glucoside is rapidly absorbed from the gastrointestinal tract and extensively metabolized and excreted within 24 h. The metabolites formed from this compound in rats do not differ from those found in the metabolism study with spirotetramat in rats.

Spirotetramat caused two proven cases of allergic contact dermatitis in workers handling undiluted active ingredient. Neither a questionnaire survey among staff exposed to spirotetramat nor yearly surveillance of 12 workers exposed to spirotetramat revealed any further cases of sensitization.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.05 mg/kg bw per day based on a NOAEL of 200 ppm (equal to 5 mg/kg bw per day) identified on the basis of thymus involution in a 1-year study in dogs and with a safety factor of 100.

The Meeting established an ARfD of 1 mg/kg bw, based on a NOAEL of 100 mg/kg bw identified on the basis of altered motor and locomotor activity and FOB changes in a single-dose study in rats treated by gavage and with a safety factor of 100. This ARfD provides adequate protection from maternal toxicity and abortion observed at 160 mg/kg bw per day in the study of developmental toxicity in rabbit, even in the unlikely event that the observed effect could be attributed to a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of carcinogenicity [a]	Toxicity and carcinogenicity	7000 ppm, equal to 1022 mg/kg bw per day[c]	—
Rat	Two-year study of carcinogenicity [a]	Toxicity	250 ppm, equal to 12.5 mg/kg bw per day	—
		Carcinogenicity	7500 ppm, equal to 373 mg/kg bw per day [c]	3500 ppm, equal to 169 mg/kg bw per day
	Multigeneration reproductive toxicity [a d]	Parental	1000 ppm, equal to 70.7 mg/kg bw per day	6000 ppm, equal to 419 mg/kg bw per day
		Offspring	1000 ppm, equal to 79.5 mg/kg bw per day	6000 ppm equivalent to 400 mg/kg bw per day
		Reproductive	1000 ppm, equal to 79.5 mg/kg bw per day	6000 ppm, equal to 486.7 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	140 mg/kg bw per day	1000 mg/kg bw per day
		Embryo and fetal toxicity	140 mg/kg bw per day	1000 mg/kg bw per day
	Acute oral neurotoxicity[b,d]		100 mg/kg bw (overall)	200 mg/kg bw

Species	Study	Effect	NOAEL	LOAEL
Rabbit	Developmental toxicity [b]	Maternal toxicity	40 mg/kg bw per day	160 mg/kg bw per day
		Embryo and fetal toxicity	160 mg/kg bw per day[c]	—
Dog	1-year study of toxicity [a]	Toxicity	200 ppm, equal to 5 mg/kg bw per day	600 ppm, equal to 19 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

0–0.05 mg/kg bw

Estimate of acute reference dose

1 mg/kg bw

Information that would be useful for 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 spirotetramat

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid and nearly complete absorption,
Distribution	Extensive, highest in liver and kidney
Potential for accumulation	No evidence of significant accumulation at low doses
Rate and extent of excretion	Very fast and almost complete within 48 h.
Metabolism in animals	Extensive. Main metabolite spirotetramat-enol was formed by cleavage of ester bond. Other minor metabolites are formed by oxidative transformation or conjugation.
Toxicologically significant compounds (animals, plants and environment)	Spirotetramat and spirotetramat -enol

Acute toxicity

Rat, LD50, oral	> 2000 mg/kg bw
Rat, LD50, dermal	> 2000 mg/kg bw
Rat, LC50, inhalation	> 4.18 mg/l air (nose only)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Irritating
Skin sensitization	Skin sensitization potential (Magnussen & Kligman test) in guinea-pigs and local lymph node assay in mice

Short-term studies of toxicity

Target/critical effect	Thymus involution
Lowest relevant oral NOAEL	200 ppm (equal to 5 mg/kg bw per day) 1-year study in dogs
Lowest relevant dermal NOAEL	> 1000 mg/kg bw per day
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	No genotoxic potential

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Kidney (tubular dilatation), decreased absolute weight
Lowest relevant NOAEL	2-year, rat, 250 ppm (equal to 12.5 mg/kg bw per day)
Carcinogenicity	No carcinogenic potential in mice and rat

Reproductive toxicity

Reproduction target/critical effect	Abnormal sperm in F1 at parentally toxic dose
Lowest relevant reproductive NOAEL	Parental toxicity: 1000 ppm (equal to 70.7 mg/kg bw per day)
	Offspring toxicity: 1000 ppm (equal to 79.5 mg/kg bw per day)
	Reproductive toxicity: 1000 ppm (equal to 79.5 mg/kg bw per day)
Developmental target/critical effect	Increased incidence of retarded ossification in fetuses at maternally toxic doses in rats. None in rabbits.
Lowest relevant developmental NOAEL	Maternal toxicity: 40 mg/kg bw per day (rabbit)
	Developmental toxicity: 140 mg/kg bw per day (rat)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity	On the basis of behavioural effects, NOAEL was 100 mg/kg bw per day in rats

Medical data

	Two proven cases of allergic contact dermatitis in workers handling undiluted active ingredient. No other effects were observed.

Summary

	Value	Study	Safety factor
ADI	0–0.05 mg/kg bw	Dog, 1-year study of oral toxicity	100
ARfD	1 mg/kg bw	Rat, studies of acute oral neurotoxicity	100

References

Capt, A. (2006) [^{14}C]-BYI 08330 in SC240 formulation: comparative in vitro dermal absorption study using human and rat skin. Unpublished report No. SA 05255 from Bayer CropScience AG, Germany. Submitted to WHO by Bayer CropScience, Germany.

Brendler-Schwaab, S. (2003) BYI 08330 - Unscheduled DNA synthesis test with rat liver cells in vivo. Unpublished report No. AT00526, edition No. M-116087-01-2, dated 10 July 2003, from Bayer CropScience AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience, Germany.

Eigenberg, D.A. (2004a) An acute oral LD_{50} study in the rat with BYI 08330. Unpublished report No. 200398, edition No. M-069299-01-1, dated 6 May 2004, from Bayer CropScience LP, Research Triangle Park, North Carolina, USA. Submitted to WHO by Bayer CropScience, Germany.

Eigenberg, D.A. (2004b) An acute dermal LD_{50} study in the rat with BYI 08330. Unpublished report No. 200399, edition No. M-066937-01-2, dated 28 April 2004, from Bayer CropScience LP, Stilwell, Kansas, USA. Submitted to WHO by Bayer CropScience, Germany.

Eigenberg, D.A. (2004c) Technical grade BYI 08330: a subacute toxicity feeding study in the beagle dog. Unpublished report No. 201012, edition No. M-182239-01-1, dated 13 December 2004, from Bayer CropScience LP, Stilwell, Kansas, USA. Submitted to WHO by Bayer CropScience, Germany.

Eigenberg, D.A. (2005) Technical grade BYI 08330: a 90-day subchronic toxicity feeding study in the beagle dog. Unpublished report No. 201223, edition No. M-254183-01-1, dated 9 May 2005, from Bayer CropScience LP, Stilwell, Kansas, USA. Submitted to WHO by Bayer CropScience, Germany.

Eigenberg D.A. (2006a) A subacute dermal toxicity study in rats with BYI 08330. Unpublished report No. 201505, edition No. M-75227-01-1, dated 20 June 2006, from Bayer CropScience LP, Stilwell, Kansas, USA. Submitted to WHO by Bayer CropScience, Germany.

Eigenberg, D.A. (2006b) A chronic toxicity feeding study in the beagle dog with technical grade BYI 08330. Unpublished report No. 201486, edition No. M-274969-01-1, dated 6 July 2006, from Bayer Corporation LP, Stilwell, Kansas, USA. Submitted to WHO by Bayer CropScience, Germany.

Esdaile, D. (2004) BYI 08330: evaluation of potential dermal sensitization in the local lymph node assay. Unpublished report No. SA 04120, edition No. M-090707-01-2, dated 9 September 2004, from Bayer CropScience, Sophia Antipolis, France. Submitted to WHO by Bayer CropScience, Germany.

Gilmore, R.G. & Fickbohm, B.L. (2005) An acute oral neurotoxicity screening study with technical grade BYI 08330 in Wistar rats. Unpublished report No. 201283, edition No. M-254187-01-1, dated 13 April 2005, from Bayer CropScience LP, Stilwell, Kansas, USA. Submitted to WHO by Bayer CropScience, Germany.

Hass, M. & Diederich B. (2004) Metabolism of [azaspirodecenyl-3-^{14}C]BYI 08330 (spirotetramat) in lettuce. Unpublished report No. MEF-049/04 from BayerCropScience AG, Germany. Submitted to WHO by Bayer CropScience, Germany.

Herbold, B. (2002a) BYI 08330 - Salmonella/microsome test plate incorporation and preincubation method. Unpublished report No. AT00056, edition No. M-065358-01-2, dated 24 October 2002, from Bayer HealthCare, Wuppertal, Germany. Submitted to WHO by Bayer CropScience, Germany.

Herbold, B. (2002b) BYI 08330 - in vitro chromosome aberration test with Chinese hamster V79 cells. Unpublished report No. AT00055, edition No. M-065342-01-2, dated 24 October 2002, from Bayer HealthCare, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Herbold, B. (2002c) BYI 08330 - V79/HPRT-test in vitro for the detection of induced forward mutations. Unpublished report No. AT00055, edition No. M-065342-01-2, dated 24 October 2002 from Bayer HealthCare, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Herbold, B. (2002d) BYI 08330 - Micronucleus-test on the male mouse. Unpublished report No. AT00048, edition No. M-065314-01-2, dated 24 October 2002, from Bayer HealthCare, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Herbold, B. (2003) BYI 08330 - cytogenetic screening with Chinese hamster V79 cells. Unpublished report No. AT00194, edition No. M-075136-01-2, dated 13 January 2003, from Bayer HealthCare, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Herbold, B. (2005a) BYI 08330-*cis*-Ketohydroxy (project: BYI 08330) -Salmonella/microsome test – plate incorporation and preincubation method. Unpublished report No. AT02735, edition No. M-262850-01-3, dated 15 December 2005 from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Herbold, B. (2005b) BYI 08330-mono-hydroxy (project:BYI 08330) - Salmonella/microsome test - plate incorporation and preincubation method. Unpublished report No. AT02716, edition No. M-262976-01-2, dated 13 December 2005 from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Herbold, B. (2006a) BYI 08330 - Salmonella/microsome test - plate incorporation and preincubation method. Unpublished report No. AT03070, edition No. M-272000-01-2, dated 24 May 2006 from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Herbold, B. (2006b) BYI 08330-di-hydroxy (project: BYI 08330) - Salmonella/microsome test -plate incorporation and preincubation method. Unpublished report No. AT03069, edition No. M-271980-01-2, dated 24 May 2006, from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Holzum, B. (2001) BYI 08330 - Pilot developmental toxicity study in rabbits after oral administration. Unpublished report No. T3062735, edition No. M-084392-01-2, dated 14 November 2001, from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Honarvar, N. (2003) Chromosome aberration assay in bone marrow cells of the mouse with BYI 08330 RCC Unpublished report No. AR00070, edition No. M-084116-01-2, dated 24 March.2003, from Cytotest Cell Research, Rosdorf, Germany. ,Submitted to WHO by Bayer Crop Science, Germany.

Jensen, T. L. (2005) A revised homogeneity and stability study of BYI 08330 technical in rodent ration. Unpublished report No. 201363, edition No. M-258710-01-1, dated 4 October 2005, from Bayer CropScience, Kansas City, Missouri, USA. Submitted to WHO by Bayer Crop Science, Germany.

Kehrig, B & Steffens, W. (2006) Occupational medical experiences with spirotetramat/BYI 08330. Unpublished report No. M-277039-01-1, edition No. M-277039-01-1, dated 30 August.2006, from Bayer Industry Services, Dormagen, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Kennel, P. (2005) BYI 08330 - Evaluation of the potential reproductive toxicity in the male rat following daily oral administration by gavage. Unpublished report No. SA 04181, edition No. M-252001-01-2, dated 23 May 2005, from Bayer CropScience, Sophia Antipolis, France. Submitted to WHO by Bayer Crop Science, Germany.

Klaus, A.M. (2001) BYI 08330 - Pilot study on developmental toxicity in rats after oral administration. Unpublished report No. T3068559, edition No. M-021476-01-2, dated 8 May 2001, from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Klaus, A.M. (2004a) BYI 08330, synonym: FHN 08330: supplementary developmental toxicity study in rats after oral administration. Unpublished report No. AT01512, edition No. M-091750-01-2, dated 7 October 2004, from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Klaus, A.M. (2004b) BYI 08330, synonym: FHN 08330: developmental toxicity study in rats after oral administration. Unpublished report No. AT01413, edition No. M-086404-01-2, dated 23 August 2004, from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Klaus. A.M. (2004c) BYI 08330 - Developmental toxicity study in rabbits after oral administration. Unpublished report No. AT01003, edition No. M-122324-01-2, dated 17 February 2004, from Bayer HealthCare AG, Wuppertal, Germany, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Klempner, A. (2006a) [Azaspirodecenyl-3-14C]-BYI 08330:absorption, distribution, excretion and metabolism in the rat. Unpublished report No. MEF-048/04, edition No. M-268709-02-2, dated 15 February 2006, from Bayer CropScience AG, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Klempner, A. (2006b) [Azaspirodecenyl-3-^{14}C]-BYI 08330: depletion of residues and metabolites in plasma, urine, liver, kidney and testis of the male rat. Unpublished report No. MEF-06/328, edition No. M-275731 -01-2, dated 11 August 2006, from Bayer CropScience AG, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Klempner A. (2006c) [Azaspirodecenyl-3-^{14}C]-BYI 08330: distribution of the total radioactivity in male and female rats determined by quantitative whole body autoradiography (QWBA) including determination of the total radioactivity in excreta and exhaled $^{14}CO_2$. Unpublished report No. MEF-06/15, edition No. M-269337-01-2, dated 21 February 2006, from Bayer CropScience AG Bayer CropScience, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Klempner, A. (2006d) [Azaspirodecenyl-3-14C]-BYI 08330-enol-glucoside supplemental study: absorption, distribution, excretion and metabolism in the rat. Unpublished report No. MEF-06/006, edition No. M-268645-01-2, dated 9 February 2006, from Bayer CropScience AG, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Klempner, A. (2006e) [Azaspirodecane-3-14C]-BYI 08330-ketohydroxy: absorption,distribution, excretion and metabolism in the rat. Unpublished report No. MEF-06/007, edition No. M-268931-01-2, dated 20 February 2006, from Bayer CropScience AG, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Kroetlinger, F. & Mihail, F. (1998) Cyclic ketoenols BSN 3457, BSN 2342, FHN 7504, FHN 8330 – subacute exploratory toxicity studies in rat (application by feed over 4 weeks). Unpublished report No. MO-02-003395, dated 13 February 1998, from Bayer HealthCare AG, PH-GDD Toxicology, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Leuschner, J. (2002a) Acute skin irritation test (patch test) of BYI 8330 in rabbits. LPT Laboratory of Pharmacology & Toxicology KG, Hamburg, Germany. Unpublished report No. R8147, edition No. M-062870 -01-2, dated 25 April 2002, from Bayer CropScience AG, Submitted to WHO by Bayer Crop Science, Germany.

Leuschner, J. (2002b) Acute eye-irritation study of BYI 08330 by instillation into the conjunctival sac of rabbits LPT Laboratory of Pharmacology & Toxicology KG, Hamburg, Germany. Unpublished report No. R8146, edition No. M-062864-01-3, dated 25 April 2002, from Bayer CropScience AG, Submitted to WHO by Bayer Crop Science, Germany.

Mihail, F. & Kroetlinger, F. (1998) Cyclic ketoenols BSN 3457, BSN 2342, FHN 7504, FHN 8330 -subacute exploratory toxicity studies in rats (application by feed over 4 weeks). Unpublished report No. T0061869, edition No. M-040236-01-2, dated 13 February 1998, from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Pauluhn, J. (2002) BYI 08330 - Study on acute inhalation toxicity in rats according to OECD No. 403. Unpublished report No. 32020, edition No. M-064654-01-2, dated 15 May 2002, from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Schladt, L. (2001) BYI 08330 - subacute study with mice (keto-enol design). Unpublished report No. T2070951, edition No. M-035927-01-2, dated 13 September 2001, from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Schmitt, W. (2006a) Physiology-based pharmacokinetic simulation of BYI 08330 in male rats. Unpublished report No. BTS-WSM0602, edition No. M-274844-01-2, dated 21 July 2006, from Bayer Technology Services GmbH, Leverkusen, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Schmitt, W. (2006b) PBPK-Simulation of BYI 08330 in male rats at high doses. Unpublished report No. BTS-WSM0603-1, edition No. M-274847-02-2, dated 21 July 2006, amended 1 September 2006, from Bayer Technology Services GmbH, Leverkusen, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Schuengel, M. (2005a) BYI 08330-*cis*-ketohydroxy – acute toxicity in the rat after oral administration. Unpublished report No. AT02506, edition No. M-258306-01-2, dated 14 October 2005, from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Schuengel, M. (2005b) BYI 08330-mono-hydroxy – acute toxicity in the rat after oral administration. Unpublished report No. AT02687, edition No. M-262070-01-2, dated 8 December 2005, from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Schuengel, M. (2006a) BYI 08330-desmethyl-ketohydroxy - acute toxicity in the rat after oral administration. Unpublished report No. AT02927, edition No. M-269279-01-2, dated 6 April 2006, from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Schuengel, M. (2006b) BYI 08330-di-hydroxy – acute toxicity in the rat after oral administration. Unpublished report No. AT02995, edition No. M-270700-01-2, dated 5 May 2006, from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Society of Toxicology (1992).Recommendations for the conduct of acute inhalation limit tests. Prepared by the Technical Committee of the Inhalation Specialist Section, Society of Toxicology. *Fundam.Appl.Toxicol*., 18, 321–327.

Steffens, W. (2005) Assessment of potential skin sensitization incidence in workers in handling BYI 08330. Unpublished report No. M-257035-01-1, edition No. M-257035-01-1, dated 5 September 2005, from Bayer CropScience AG. Submitted to WHO by Bayer Crop Science, Germany.

Sur, R. & Spiegel, K. (2005) Metabolism of [azaspirodecenyl-3-^{14}C]BYI 08330 (spirotetramat) in confined rotational crop. Unpublished report No. MEF-05/288 from BayerCropScience, Germany.

Temerowski, M. (2006) BYI 08330 (spirotetramat) - Assessment of literature research in various databases. Unpublished report No. M-275046-01-1, edition No. M-275046-01-1, dated 27 July 2006, from Bayer CropScience AG. Submitted to WHO by Bayer Crop Science, Germany.

Temerowski, M. (2008) BYI 08330 (spirotetramat) – high-dose reproductive effects in male rats and their relevance to humans databases – position paper. Unpublished report No. M-297775-01-1, dated 20 February 2008, from Bayer CropScience AG. Submitted to WHO by Bayer CropScience AG, Germany.

Tinwell, H. (2006) BYI 08330-enol - Investigation of the testicular/sperm toxicity in the rat following 21 days of exposure by gavage. Unpublished report No. SA06011, edition No. M-273959-01-1, dated 30 June 2006, from Bayer CropScience SA, Sophia Antipolis, France. Submitted to WHO by Bayer Crop Science, Germany.

Totis, M. (2006) [Azaspirodecenyl-3-14C]-BYI 08330: comparison of the in vitro metabolism in liverbeads from male rat, mouse and human. Unpublished report No. M-274118-02-2, dated 6 July 2006, amended 9 August 2006, from Bayer CropScience SA, Sophia, Antipolis, France. Submitted to WHO by Bayer Crop Science, Germany.

Vohr, H-W. (2002) BYI 08330 - study for the skin sensitization effect in guinea-pigs (guinea-pig maximization test according to Magnusson and Kligman). Unpublished report No. 32273, edition No. M-076253-01-2, dated 29 July 2002, from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Vohr, H-W. (2004) BYI 08330 - study for the skin sensitization effect in guinea-pigs (Buehler patch test). Unpublished report No. AT01317, edition No. M-078494-01-2, dated 13 July 2004, from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Wahle, B.S. (2005a) Technical grade BYI 08330: a subchronic toxicity testing study in the rat. Unpublished report No. 201136, edition No. M-252787-01-1, dated 1 June 2005, from Bayer CropScience LP, Stilwell, Kansas, USA. Submitted to WHO by Bayer Crop Science, Germany.

Wahle, B.S. (2005b) Technical grade BYI 08330 (common name spirotetramat): a chronic toxicity testing study in the rat. Unpublished report No. 201285, edition No. M-260765-01-1, dated 15 November 2005 from Bayer CropScience, Stilwell, Kansas, USA. Submitted to WHO by Bayer Crop Science, Germany.

Wahle, B.S. (2006a) Technical grade BYI 08330 (common name spirotetramat): an oncogenicity testing study in the rat. Unpublished report No. 201358, edition No. M-273643-01-1, dated 8 March 2006, from Bayer CropScience, Stilwell, Kansas, USA. Submitted to WHO by Bayer Crop Science, Germany.

Wahle, B.S. (2006b) Technical grade BYI 08330 (common name spirotetramat): an oncogenicity testing study in the mouse. Unpublished report No. 201359-1, edition No. M-275506-02-1, dated 13 March 2006, from Bayer CropScience LP, Stilwell, Kansas USA. Submitted to WHO by Bayer Crop Science, Germany.

Wahle, M.S. (2005) Technical grade BYI 08330: a subchronic toxicity testing study in the mouse. Unpublished report No. 201284, edition No. M-255359-01-1, dated 14 July 2005, from Bayer CropScience, Stilwell, Kansas, USA. Submitted to WHO by Bayer Crop Science, Germany.

Wirnitzer, U. (2006) BYI 08330-desmethyl-ketohydroxy (project: BYI 08330) - Salmonella/microsome test – plate incorporation and preincubation method. Unpublished report No. AT03027, edition No. M-271090 -01-2, dated 9 May 2006, from Bayer HealthCare AG, Wuppertal, Germany. Submitted to WHO by Bayer Crop Science, Germany.

Young, A.D. (2006a) Technical grade BY1 08330 - A dose range-finding reproductive toxicity study in the Wistar rat (revised report). Unpublished report No. 201300-1, edition No. M-273578-02-1, date 30 May 2006, amended:12 July 2006, from Bayer CropScience LP, Stilwell, Kansas, USA. Submitted to WHO by Bayer Crop Science, Germany.

Young, A.D. (2006b) Technical grade BYI 08330 (common name spirotetramat): a two-generation reproductive toxicity study in the Wistar rat. Unpublished report No. 201426-1, edition No. M-274619-02, dated 30 May 2006, amended 13 July 2006, from Bayer CropScience LP, Stilwell, Kansas, USA. Submitted to WHO by Bayer Crop Science, Germany.