FLUTRIAFOL

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Explanation

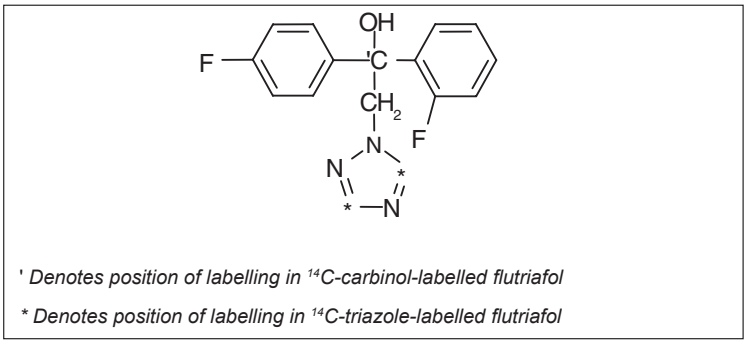
Flutriafol is the International Organization for Standardization (ISO)–approved name for (*RS*)-2,4′-difluoro-α-(1H-1,2,4-triazol-1-ylmethyl)benzhydryl alcohol (International Union of Pure and Applied Chemistry), which has the Chemical Abstracts Service No. 76674-21-0. Flutriafol is a contact and systemic fungicide belonging to the triazole class. It is used on a wide range of cereal crops and as a seed treatment. Its fungicidal mechanism of action is the inhibition of ergosterol biosynthesis and thus disruption of fungal cell wall synthesis.

Flutriafol was reviewed for the first time by the Joint FAO/WHO Meeting on Pesticide Residues at the request of the Codex Committee on Pesticide Residues. All critical studies contained statements of compliance with good laboratory practice.

Evaluation for acceptable daily intake

The structure of flutriafol showing the positions of the radioactive labels is given in Figure 1.

Figure 1. Structure of flutriafol showing position of carbinol and triazole labels



1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

Rats

Alderley Park (Wistar-derived) albino rats (five of each sex per group) were gavaged with a single dose of ^{14}C-carbinol-labelled flutriafol (specific activity 2.17 GBq/mmol / 373.03 kBq/mg; radiochemical purity > 98%) suspended in polyethylene glycol 600 (PEG600) at a dose level of 5 mg/kg body weight (bw). Excreta were collected up to termination at 7 days following administration. The levels of radioactivity in excreta and tissue samples were measured by liquid scintillation counting (LSC) following appropriate processing. In a whole-body autoradiography phase, two additional animals (one of each sex) were gavaged with a single dose of the test material (1.898 MBq/mg) and terminated at 48 hours after dosing. Longitudinal sagittal sections were cut and prepared for autoradiography. Radioactivity was also determined in urine, faeces and exhaled carbon dioxide.

In the main study phase, radioactivity was excreted rapidly (~95% within 48 hours) and approximately equally in urine and faeces, although faecal excretion was slightly greater in males (~52%) than in females (~45%). Little radioactivity was excreted after 72 hours. Negligible amounts of radioactivity were excreted as carbon dioxide, and total residual radioactivity in the carcass at 168 hours following dosing was low (< 1%). At 168 hours post-dosing, highest concentrations were in whole blood (0.1–0.2 µg equivalents [eq] per gram), with no other tissues having levels above 0.1 µg eq/g.

Whole-body autoradiography at 48 hours after dosing indicated that the bulk of the administered radioactivity was present in the contents of the gastrointestinal tract between the stomach and rectum, with lesser amounts present in the liver and kidney. Liver radioactivity appeared to be greater in males than in females, and the liver in males displayed a reticular appearance (interpreted as being indicative of preferential absorption at a particular area of the lobule), whereas hepatic distribution in females was more homogeneous. Renal radioactivity was observed at the cortico-medullary junction

Table 1. Metabolism study design

Radiolabel	Dose level (mg/kg bw)	No. of animals		Bile duct cannulation	Samples
		Males	Females		
^{14}C-carbinol	250	—	6	—	Urine, faeces
^{14}C-carbinol	250	2	2	✓	Bile, urine
^{14}C-carbinol	5	2	2	✓	Bile, urine, faeces
^{14}C-triazole	5	2	2	✓	Bile, urine, faeces

From Jones & Ladd (1986)

in both sexes. Traces of radioactivity were present in the adrenals in the female. No significant levels of radioactivity were noted in other organs (Jones et al., 1982).

Alpk:AP rats were gavaged with single doses of ^{14}C-carbinol- or ^{14}C-triazole-radiolabelled test material (^{14}C-carbinol label specific activity 2.2 GBq/mmol; ^{14}C-triazole label specific activity 2.2 GBq/mmol) at a dose level of 5 or 250 mg/kg bw in PEG600 (Table 1). Excreta were collected for 72 hours following dosing, and radioactivity was measured by LSC.

In the low-dose animals, excretion was rapid and primarily via the bile. Biliary excretion was extensive (~60–80% of the administered dose), with urinary excretion of approximately 10–25%; faecal excretion was low (< 10%) (Table 2). There were some differences in results between the sexes and radiolabel positions, but no obvious patterns. The excretion profile in bile indicates that the large majority of faecal radioactivity excreted by rats in the previous study (Jones et al., 1982) is likely to be biliary in origin.

At the high dose level, total recovery of radioactivity in all groups was incomplete (80–90%) at the 72-hour time point, indicating some saturation of absorption and/or excretion. Excretion of radioactivity in non-cannulated female rats administered the carbinol label was primarily via urine (Table 2), showing reasonable consistency with the non-cannulated females in the previous study (Jones et al., 1982). In bile duct–cannulated animals administered the triazole label, biliary excretion was more extensive in males (~71%) than in females (~47%). Urinary excretion was more extensive in females (~31%) than in males (~19%) (Table 2). The findings indicate that biliary excretion might be saturated in females administered a single high dose of 250 mg/kg bw.

An oral absorption value in the rat of greater than 90% can be estimated for flutriafol based on figures for the biliary and urinary excretion of radioactivity at the low dose level in this study (Jones & Ladd, 1986).

Groups of Sprague-Dawley rats (four of each sex) were gavaged with either a single dose of 250 mg/kg bw or 14 consecutive daily doses of ^{14}C-carbinol-labelled flutriafol (specific activity 1.835 GBq/mmol; suspended in PEG600) at 5 mg/kg bw. Urine and faeces were collected for up to 168 hours following the final dose; radioactivity was analysed by LSC. Following a single oral dose of flutriafol at 250 mg/kg bw, excretion was primarily urinary (~61% and ~68% in males and females, respectively), although faecal excretion was also a significant route (~33% and ~27% in males and females, respectively). The majority of the administered radioactivity was excreted within 72 hours of dosing.

The excretion of radioactivity within 24 hours of the administration of the first of the multiple low doses was proportionally greater than that seen within 24 hours of administration of the single high dose (Table 3), although the absolute proportions of the administered radiolabel excreted via each route were independent of dose level.

Residual levels in the carcass and tissue samples were low (< 1% of the administered dose), with highest concentrations in whole blood, 40- to 100-fold above levels in plasma, indicating concentration in and/or binding to blood cells (Millais, 2004).

Table 2. Excretion of radioactivity in rats administered flutriafol

Sampling time (h)	Radioactivity (% of administered dose)							
	5 mg/kg bw				250 mg/kg bw			
	^{14}C-carbinol		^{14}C-triazole		^{14}C-carbinol		^{14}C-triazole	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine								
0–24	7.14	11.06	9.06	13.98	—	16.69	4.21	6.12
24–48	4.31	13.66	11.17	9.68	—	26.97	12.99	11.13
48–72	0.46	0.27	1.75	0.43	—	17.21	1.58	14.14
Total	11.91	24.97	21.97	24.08	—	60.85	18.78	31.38
Faeces								
0–24	0.08	0.25	1.27	0.22	—	1.45	—	—
24–48	0.44	2.30	4.66	0.36	—	8.46	—	—
48–72	0.32	1.34	4.47	1.22	—	11.89	—	—
Total	0.84	3.89	10.40	1.79	—	21.79	—	—
Bile								
0–24	63.91	45.44	42.64	56.79	—	—	23.80	6.08
24–48	15.22	12.75	19.62	15.96	—	—	44.15	14.55
48–72	0.13	0.15	0.46	0.28	—	—	3.00	26.25
Total	79.26	58.34	62.72	72.01	—	—	70.95	46.87
Total								
0–24	71.13	56.75	52.96	70.98	—	18.12	28.01	12.20
24–48	19.97	28.69	35.45	25.99	—	35.41	57.14	25.68
48–72	0.91	1.76	6.68	1.92	—	29.11	4.58	40.38
Total	92.00	87.21	95.08	98.89	—	82.64	89.72	78.25

From Jones & Ladd (1986)

(b) Dermal route

In vitro

In an in vitro dermal absorption study of [^{14}C]flutriafol in a 125 g/l suspension concentrate, there was evidence of the extent of dermal absorption being limited by the applied concentration. Absorption of flutriafol from the undiluted product was low through both rat and human skin samples, but increased markedly on dilution. Dermal absorption values were 0.3%, 12% and 46% for human skin and 2.3%, 36% and 53% for rat skin for the concentrate, 0.79 g/l and 0.025 g/l dilutions, respectively, based on receptor fluid and application site after tape stripping (deLigt, 2004).

Rats

In an in vivo dermal absorption study of [^{14}C]flutriafol in a 125 g/l suspension concentrate, F344 rats were exposed to the undiluted product and 0.79 and 0.025 g/l dilutions for 8 hours. Urine and faeces were collected until sacrifice at 24, 96 or 216 hours following application (four rats per group per time point). Skin at the application site was shaved and tape-stripped (10 strips) at termination. Recovery in all groups was acceptable (means 93–99%). Absorption of radiolabel from both dilutions was essentially complete within 72 hours; with the concentrate, the rate of absorption was consistent over time. The amount of radioactivity excreted was relatively low in all groups, but increased with dilution. Faecal excretion of radioactivity was greater, up to 2-fold, than urinary excretion. The levels

Table 3. Excretion of radioactivity in rats receiving a single high or repeated low doses of flutriafol

Sampling time (h)	Radioactivity (% of administered dose)									
	Day 1		Day 1		Day 5		Day 10		Day 14	
	High dose		Repeated low dose							
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Urine										
0–6	1.89	4.37	20.4	21.4	15.9	27.5	22.4	29.6	11.9	27.6
6–24	17.8	17.7	29.8	32.6	34.0	27.5	28.4	27.5	36.7	33.1
24–48	29.8	34.1	—	—	—	—	—	—	9.67	4.59
48–72	7.64	9.38	—	—	—	—	—	—	3.37	1.26
72–96	2.22	1.19	—	—	—	—	—	—	1.26	0.71
96–120	0.74	0.42	—	—	—	—	—	—	0.56	0.40
120–144	0.38	0.25	—	—	—	—	—	—	0.43	0.35
144–168	0.22	0.12	—	—	—	—	—	—	0.28	0.24
Total	60.6	67.5	50.2	53.9	49.8	54.9	50.8	57.1	64.2	68.2
Faeces										
0–6	0.08	0.19	0.09	0.01	0.66	1.72	1.17	2.63	0.65	1.08
6–24	3.91	4.17	29.4	33.1	35.8	35.0	30.2	37.1	30.8	31.6
24–48	14.1	12.6	—	—	—	—	—	—	14.1	6.1
48–72	11.6	8.5	—	—	—	—	—	—	4.84	0.97
72–96	2.20	1.14	—	—	—	—	—	—	1.72	0.44
96–120	0.70	0.22	—	—	—	—	—	—	1.11	0.27
120–144	0.26	0.10	—	—	—	—	—	—	0.88	0.21
144–168	0.17	0.05	—	—	—	—	—	—	0.52	0.23
Total	33.1	26.9	29.5	33.1	36.4	36.7	31.4	39.8	54.7	40.8
Cage wash										
Total	2.79	4.29	2.01	2.25	1.74	2.07	2.73	2.25	3.41	2.92
Carcass	0.25	0.23	—	—	—	—	—	—	2.73	2.25
Recovery	96.8	99.0	81.6[a]	89.3[a]	88.0[a]	93.7[a]	84.9[a]	99.2[a]	125[b]	115[b]

From Millais (2004)

[a] Recovery of radioactivity during the 24 hours following dosing, relative to the administered dose.

[b] Recovery of radioactivity during the 168 hours following dosing, relative to a single administered dose.

of radioactivity in whole blood and plasma are consistent with binding to red blood cells, which is consistent with the results of oral dosing studies. Dermal absorption values of 1%, 10% and 12% were derived for the undiluted formulation, 0.79 g/l and 0.025 g/l dilutions, respectively, based on excretion, residues in carcass and residues in application site after tape stripping (deBie & Grossouw, 2004).

1.2 Biotransformation

Rats

Samples of urine, bile and faeces from the study by Jones & Ladd (1986) were analysed for metabolites of flutriafol by comparative thin-layer chromatography (TLC), high-performance liquid

chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR).

Analysis of methanol extracts of urine (containing > 96% of the urinary radioactivity) indicated extensive metabolism, with only trace amounts of unchanged parent compound detected. The metabolite profile and the relative proportions of metabolites were reported to be similar in urine collected from rats at the low and high dose levels; however, further details were not reported. The urinary metabolite profiles in bile duct–cannulated rats were stated to be similar to those in non-cannulated rats. The primary reactions were hydroxylation on the 2-fluorophenyl ring followed by conjugation. There was evidence of only limited cleavage to release the triazole moiety.

Chromatographic analysis of bile samples from rats administered either the low or high dose level showed that more than 95% of biliary radioactivity corresponded to polar metabolites believed to be conjugates. TLC of bile samples from rats administered either the ^{14}C-carbinol- or the ^{14}C-triazole-labelled test material did not show any evidence of cleavage of the triazole group. Acid hydrolysis resulted in a metabolic profile stated to be similar to that seen in urine. The profile of faecal metabolites was also reported to be similar to that in urine (Jones & Ladd, 1986).

Samples from the study of Millais (2004) were analysed using HPLC, NMR and liquid chromatography with mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS). Urinary metabolites were further characterized following treatment with β-glucuronidase/sulfatase. HPLC revealed a total of 19 metabolite regions (M1–19) in faecal extracts and urine samples; the major components were characterized.

The predominant urinary metabolite regions were M3, M5, M6, M8 and M15 (Tables 4 and 5), with no unchanged parent detected in the urine (Table 5). Metabolite profiles in the two sexes were broadly similar, although at 250 mg/kg bw, higher levels of unconjugated M15 were detected in the urine of males (15.5%) than in the urine of females (6.6%). Enzyme treatment increased the proportions of M4, M12, M14, M15 (markedly) and M18, indicating that these regions consisted largely of unconjugated metabolites (Table 5). Differences in metabolite patterns in high- and low-dose groups were mainly due to the level of conjugation. The metabolism was postulated to be initial oxidation and conjugation on the 2-fluorophenyl ring. The metabolite profile in faeces was broadly consistent with that in urine.

The proposed metabolic pathways for flutriafol in rats are shown in Figure 2.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Flutriafol is of moderate acute toxicity via the oral route, but of low toxicity via the dermal and inhalation routes (Table 6).

(b) Dermal and ocular irritation and dermal sensitization

Flutriafol (in 0.9% saline) was not irritating to rat or rabbit skin (Henderson & Parkinson, 1982; Sanders, 2005a). Flutriafol was a mild irritant to rabbit eyes; all effects had resolved within 72 hours (Henderson & Parkinson, 1982; Durando, 2007). Flutriafol did not produce any evidence of skin sensitization in a Magnusson and Kligman assay in guinea-pigs (Barber & Parkinson, 1982) or in a local lymph node assay in mice (Sanders, 2005b).

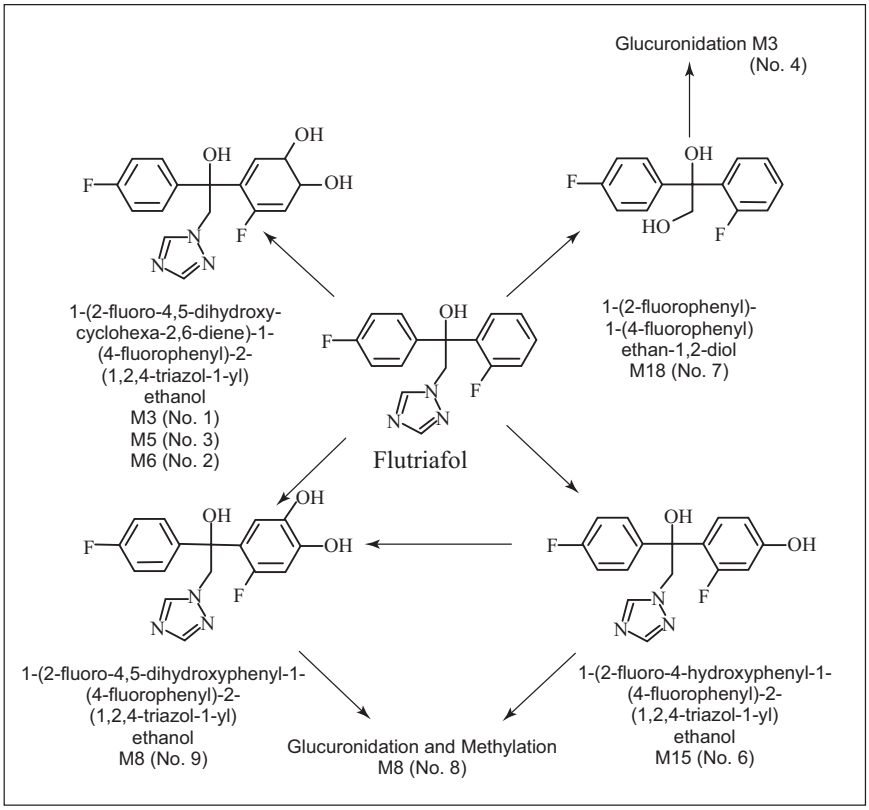
Table 4. Identity of major flutriafol metabolites

HPLC region	Chemical name
M3	1-(2-Fluoro-4,5-*cis*-dihydroxy-cyclohexa-2,6-diene)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol
	1-(2-Fluorophenyl)-1-(4-fluorophenyl) ethan-1,2-diol glucuronide
M5	(*S*,*S*)-1-(2-Fluoro-4,5-*trans*-dihydroxy-cyclohexa-2,6-diene)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol
M6	(*R*,*R*)-1-(2-Fluoro-4,5-*trans*-dihydroxy-cyclohexa-2,6-diene)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol
M8	1-(2-Fluoro-4-hydroxyphenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol glucuronide
	1-(2-Fluoro-4,5-dihydroxyphenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol glucuronide
M15	1-(2-Fluoro-4-hydroxy-5-methoxyphenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol
	1-(2-Fluoro-4-hydroxyphenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol
M18	1-(2-Fluorophenyl)-1-(4-fluorophenyl) ethan-1,2-diol

Table 5. Urinary metabolites in rats administered flutriafol

Metabolite region	Enzyme treated	Radioactivity (% of daily dose)									
		Repeated low dose								High dose	
		Males				Females				Males	Females
		Day 1	Day 5	Day 10	Day 14	Day 1	Day 5	Day 10	Day 14		
M3	−	4.8	4.9	5.2	5.0	5.1	6.0	6.3	7.8	6.6	8.7
	+	3.5	3.7	3.2	2.7	6.4	6.2	6.9	6.2	< 0.1	< 0.1
M4	−	0.3	0.2	0.5	0.6	0.5	0.3	0.3	0.3	< 0.1	< 0.1
	+	1.5	2.7	2.6	2.3	0.9	0.7	0.8	1.2	< 0.1	< 0.1
M5	−	6.9	5.9	5.8	6.8	12.0	12.2	13.4	11.9	6.4	7.5
	+	6.7	5.5	5.6	6.2	12.3	13.0	13.8	11.9	10.4	5.0
M6	−	5.6	6.2	6.5	6.5	8.6	9.7	9.1	9.9	6.6	9.0
	+	5.4	3.8	5.1	5.6	7.8	7.6	7.7	10.1	< 0.1	< 0.1
M8	−	10.4	9.7	8.6	7.6	9.5	10.5	10.5	12.7	4.6	8.6
	+	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	8.5	13.7
M14	−	0.1	0.1	0.4	0.5	0.1	0.2	0.1	0.1	1.7	3.2
	+	2.9	2.6	2.4	2.7	4.0	4.6	3.9	2.8	< 0.1	4.5
M15	−	3.5	5.3	4.3	4.5	1.2	1.4	1.3	1.3	15.2	6.6
	+	25.2	25.0	24.9	22.1	15.6	17.7	18.9	21.4	16.5	15.5
Flutriafol	−	0.2	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	+	0.1	0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	0.2	< 0.1	< 0.1
M18	−	0.1	0.2	0.4	0.4	0.1	< 0.1	0.1	< 0.1	2.5	1.6
	+	1.7	2.5	2.4	2.0	0.4	0.9	0.7	1.0	9.1	4.6

Figure 2. Proposed metabolism of flutriafol in rats



2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Alderley Park (C57BL/10J/Alpk) mice (five of each sex per group) received flutriafol (unspecified) in the diet at a concentration of 0, 50, 150, 500 or 1500 parts per million (ppm) for 29 days. Routine observations and investigations were performed. Gross necropsy was performed on all animals when weights of the brain, heart, liver, kidneys, spleen and testes were recorded. A comprehensive range of tissues from control, 500 and 1500 ppm animals were examined histopathologically; investigations in the other dose groups were limited to the liver, testes, epididymides, ovaries, cervix, uterus and adrenals. The level of reporting in this range-finding study is limited.

Dietary concentrations were not confirmed analytically, and doses of flutriafol were estimated to be 0, 7.5, 22.5, 75 and 225 mg/kg bw per day. Most top-dose animals were found dead during the study. Signs of toxicity at the top dose level included subdued behaviour, hunched posture, emaciation, shaking, piloerection and urinary staining. No deaths occurred and no signs of toxicity were observed at lower dose levels. Reduced body weight gain was evident at and above 500 ppm and to a lesser extent in males at 150 ppm (Table 7). Dose-related increases in absolute and relative liver weights were evident at 150 and 500 ppm (Table 7). Gross necropsy did not reveal any treatment-related findings in either sex at the top dose level; liver enlargement was observed in a number of animals at 500 ppm (further details not reported). Histopathology revealed hepatotoxicity in both sexes at all dose levels (Table 7).

A no-observed-adverse-effect level (NOAEL) cannot be determined for this range-finding study as a result of histopathology (lipid accumulation in the liver) of both sexes at 50 ppm (equivalent to 7.5 mg/kg bw per day), the lowest dose tested (Hodge & Robinson, 1985).

Table 6. Acute toxicity studies with flutriafol

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%); batch No.	Vehicle	Reference
Rat	AlPk	M F	Oral	1140 1480	—	93; P10	"Lissatan AC"	Henderson & Parkinson (1982)
Mouse	AlPk	M F	Oral	365 179	—	93; P10	"Lissatan AC"	Henderson & Parkinson (1982)
Rabbit	New Zealand White	F	Oral	300–400	—	93; P10	"Lissatan AC"	Henderson & Parkinson (1982)
Guinea-pig	Dunkin Hartley	M	Oral	300–400	—	93; P10	"Lissatan AC"	Henderson & Parkinson (1982)
Rat	AlPk	M F	Dermal	> 1000 (HDT)	—	93; P10	PEG300	Henderson & Parkinson (1982)
Rabbit	New Zealand White	M F	Dermal	> 2000 (HDT)	—	93; P10	PEG300	Henderson & Parkinson (1982)
Rat	AlPk	M F	Inhalation (nose only)	—	> 3.5 (MMAD 16 µm)	93; P10	None (aerosol)	Henderson & Parkinson (1982)
Rat	Sprague-Dawley	M F	Inhalation (nose only)	—	> 5.2 (MMAD 4 µm)	95.1; UPL BX1	None (aerosol)	Griffiths (2005)

F, female; HDT, highest dose tested; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male; MMAD, mass median aerodynamic diameter; PEG, polyethylene glycol

Rats

Alderley Park (Wistar derived) rats (eight of each sex per group) were administered flutriafol (purity 97%; batch No. P7) in the diet at a concentration of 0, 100, 300, 800, 2000 or 5000 ppm for 28 days. Standard observations and investigations were performed. A comprehensive range of tissues from control and top dose level animals were examined histopathologically, including oil red O staining of liver sections; investigations in intermediate-dose groups were limited to grossly abnormal tissues. Further liver samples from various groups were investigated by electron microscopy; peroxisome and smooth endoplasmic reticulum volumes were determined. Hepatic aminopyrine *N*-demethylase activity was also measured at termination (six rats of each sex per group).

Achieved doses were 0, 11, 34, 86, 215 and 517 mg/kg bw per day. One top-dose female was found dead on day 7. All top dose level animals were noted to be smaller and thinner than controls from day 4; other signs of toxicity in this group included ptosis, hunched posture, chromodacryorrhoea and piloerection. Signs of toxicity were not apparent at lower dose levels. Weight gain by both sexes at the top dose level was markedly reduced; feed consumption by these animals was lower during weeks 1–2 but was comparable to that of controls during weeks 3–4. Feed utilization efficiency was significantly lower (~50%) in both sexes at the top dose level and was also slightly (~10%) lower in 2000 ppm males. A range of haematology and clinical chemistry changes were noted at 2000 and 5000 ppm. Aminopyrine *N*-demethylase activity was increased in all groups in a dose-related manner (males, 19, 21, 27, 30, 36 and 26 µmol/h per gram; females 7, 8, 11, 18, 27 and 26 µmol/h per gram).

The weights of a number of organs at the top dose level were affected by the markedly lower body weights in this group. Mean absolute liver weights were significantly higher in males at and above

Table 7. Twenty-nine-day mouse study with flutriafol: summary of findings

Parameter	Sex	Dietary concentration (ppm)				
		0	50	150	500	1500
Weight gain (g)						
- weeks 0–1	Male	4.4	3.4	2.6	0.6*	—
	Female	3.0	2.6	2.0	1.0*	−1.3**
- weeks 0–4	Male	8.8	8.9	7.1	3.6**	—
	Female	5.0	5.3	5.2	2.3*	—
Histopathology in liver (n = 5)[a]						
- hepatocyte hypertrophy	Male	0	3 [3, 0, 0]	5 [0, 5, 0]	5 [2, 3, 0]	—
	Female	0	0	0	0	—
- cytoplasmic vacuolation	Male	0	0	3 [3, 0, 0]	5 [0, 3, 2]	—
	Female	0	0	0	2 [2, 0, 0]	—
- lipid accumulation	Male	0	2 [2, 0, 0]	4 [3, 1, 0]	5 [0, 5, 0]	—
	Female	0	3 [3, 0, 0]	4 [4, 0, 0]	5 [2, 3, 0]	2 [2, 0, 0]

From Hodge & Robinson (1985)
* $P < 0.05$; ** $P < 0.01$ (Student's t-test)
[a] Incidence [severity]. Laboratory severity scale: minimal, slight, moderate.

800 ppm and in females at and above 300 ppm (Table 8). Gross pathology revealed pale, enlarged, granular and/or yellow-brown livers with an accentuated lobular pattern at the top dose level; lower incidences of these findings were also noted at 2000 ppm. Microscopically, evidence of liver toxicity was seen at and above 2000 ppm and was characterized by centrilobular hepatocyte hypertrophy and vacuolation, fatty change and hydropic degeneration (Table 8). Hepatocyte hypertrophy was seen in males at 800 ppm. Electron microscopy revealed significantly increased hepatic smooth endoplasmic reticulum volume in 2000 ppm males, consistent with microsomal enzyme induction; hepatic peroxisome volume in 100 ppm females was comparable to that of controls. Reduced spermatogenesis, atrophy of the prostate and contracted tubules in the seminal vesicles were observed in top-dose males. Slight focal haemorrhage of the thymus was noted in some top-dose females. Oil red O staining revealed moderate to marked deposition of triglycerides in the liver of top dose level animals.

A NOAEL of 300 ppm (equal to 34 mg/kg bw per day) can be determined for this study, based on the marked increase (> 15%) in absolute and relative liver weights at 800 ppm (equal to 86 mg/kg bw per day). The statistically significantly higher relative liver weights in males at 100 ppm and in both sexes at 300 ppm are less than 10% in relative terms and are not considered to be adverse. The significantly elevated hepatic aminopyrine *N*-demethylase activity in both sexes at 300 ppm is considered to be adaptive rather than adverse (Milburn, 1982).

Alderley Park (Wistar-derived) rats (20 of each sex per group) received flutriafol (purity 93%; batch No. P10) in the diet at a concentration of 0, 20, 200 or 2000 ppm for 90 days. Routine observations and investigations were performed. All animals were subjected to gross necropsy, and main organs were weighed. A comprehensive range of tissues from control and top dose level animals were examined histopathologically; investigations in the intermediate-dose groups were limited to the liver, kidney, heart, lungs, gonads, uterus, epididymides, prostate, seminal vesicles and grossly abnormal tissues. Additional liver samples (from six rats of each sex per group) were investigated using electron microscopy; hepatic aminopyrine *N*-demethylase activity was measured in a further 10 samples from each sex per group.

Achieved doses were 0, 1.4, 13 and 148 mg/kg bw per day. Two low-dose males died during the study; neither death was considered to be treatment related. No signs of toxicity were observed during

Table 8. Twenty-eight-day rat study: liver weights and liver histopathology

Parameter	Sex	Dietary concentration (ppm)					
		0	100	300	800	2000	5000
Liver weights							
- absolute (g)	Male	14.24	15.80	15.96	17.09*	19.52**	16.81*
	Female	8.63	9.14	9.68**	10.10**	13.27**	15.43**
Relative (%)	Male	4.66	5.09**	5.07*	5.61**	7.21**	10.13**
	Female	4.28	4.49	4.66**	5.06**	6.60**	10.17**
Liver histopathology (n = 8)							
- hepatocyte hypertrophy	Male	0	0	1	6	8	8
	Female	0	0	0	0	5	7
- fatty change	Male	0	0	0	0	8	8
	Female	0	0	0	0	8	7
- hydropic degeneration	Male	0	0	0	0	3	2
	Female	0	0	0	0	0	5
- smooth endoplasmic reticulum volume (%)	Male	25.4	27.6	—	—	57.3*	—
	Female	20.9	22.5	—	—	—	—

From Milburn (1982)
* $P < 0.05$; ** $P < 0.01$ (Student's t-test)

the study period. Ophthalmoscopy and urinalysis did not reveal any treatment-related findings. Mean terminal body weights of both sexes at the top dose level were lower (~10%) than those of controls as a result of significantly lower weight gain from week 1 (Table 9). Overall feed consumption was slightly (but significantly) lower in all treated groups in a dose-related fashion.

Haematology revealed significant effects on erythrocyte parameters consistent with mild anaemia in both sexes at the top dose level at weeks 4 and 13. A range of clinical chemistry parameters consistent with hepatotoxicity were statistically significantly altered in top-dose animals, together with increases in blood urea nitrogen in top-dose females. Hepatic aminopyrine *N*-demethylase activity was significantly elevated in both sexes at and above 200 ppm.

The mean absolute and body weight–adjusted weights of a number of organs at the top dose level were influenced by the lower terminal body weights of both sexes. Marked increases in relative and absolute liver weights were seen at 2000 ppm (Table 9). The liver weight effects in 200 ppm females represent an increase of less than 10% and are therefore not considered to be of toxicological significance in the absence of any evidence of hepatotoxicity in this group. Gross necropsy revealed increased incidences of enlarged/swollen and pale livers with well-defined surface reticulation in top-dose males. Microscopically, liver effects were characterized by centrilobular hepatocyte hypertrophy and marked diffuse fatty vacuolation in both sexes at the top dose level, with a single incidence of focal necrosis in one top-dose male. Electron microscopy revealed significantly greater smooth endoplasmic reticulum volume in the hepatocytes of both sexes and an increase in lipid levels in centrilobular hepatocytes, usually in the form of pale droplets at the top dose level. Also in the 200 ppm males, this took the form of numerous small dense droplets additionally to a moderate proliferation of smooth endoplasmic reticulum (Table 9).

The NOAEL is 200 ppm (equal to 13 mg/kg bw per day), based on the reduced weight gain, haematological and clinical chemistry effects and liver histopathology at the top dose level. The significantly increased aminopyrine *N*-demethylase activity, slightly increased liver weights and increased smooth endoplasmic reticulum volume seen at 200 ppm are considered not to be adverse effects (Pigott et al., 1982).

Table 9. Ninety-day rat study with flutriafol: body weights, feed consumption and liver pathology and histopathology

Parameter	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Weight (g)								
- week 13	494.5	492.7	489.3	439.9	271.5	278.3	276.3	243.0
Weight gain (g)								
- week 1	54.4	54.7	54.9	20.6**	34.1	33.4	32.6	18.5**
- week 4	194.2	196.0	196.7	151.4**	101.8	98.8	93.9**	81.9**
- week 13	360.8	367.2	360.9	308.2**	156.8	161.6	157.9	127.8**
Feed consumption (g)								
- weeks 1–13	2327	2237*	2182**	2159**	1693	1583**	1567**	1373**
Liver weight (g)								
- absolute	17.3	18.0	17.7	23.6**	9.2	9.7	9.9*	11.0**
- adjusted for body weight	16.6	17.2	17.2	25.4**	9.1	9.4	9.6*	11.7**
Gross pathology of liver (n = 20)								
- liver pale/white	0	0	0	12	0	0	0	0
- liver surface reticulation	0	0	0	6	0	0	0	0
- liver swollen/enlarged	0	0	0	7	0	0	0	0
Histopathology of liver								
- hepatocyte hypertrophy	0	0	0	17	0	0	0	8
- fatty change	3	4	6	19	2	1	2	6
- focal necrosis	0	0	0	1	0	0	0	0
- smooth endoplasmic reticulum (%)	28.9	27.8	41.8**	50.6**	24.4	26.4	27.6	51.4**

From Pigott et al. (1982)
* $P < 0.05$; ** $P < 0.01$ (Student's t-test)

Dogs

Beagle dogs (four of each sex per group) were administered flutriafol (purity 93%; batch No. P10) in capsules at a dose level of 0, 1, 5 or 15 mg/kg bw per day for 90 days. Routine observations and investigations were performed pre-test and during dosing. All animals were subjected to gross necropsy; weights of the main organs were recorded. A comprehensive range of tissues from control and top dose level animals were examined histopathologically; a smaller selection of organs from intermediate-dose groups was investigated. In addition, samples of heart, liver and adrenals from control and top dose level animals were stained with oil red O; samples of liver and spleen from all animals were stained with Prussian blue. Liver samples were analysed for aminopyrine *N*-demethylase activity.

No deaths occurred and no signs of toxicity were observed during the study period. Feed consumption was unaffected by treatment. The mean terminal body weight of top-dose females was significantly lower than that of controls as a result of an initial slight weight loss and subsequently significantly reduced weight gain. Initial weight gain by top-dose males was also significantly reduced (Table 10). A tendency to higher white blood cell counts was seen in both sexes at the top dose level. Erythrocyte parameters were unaffected by treatment. Clinical chemistry investigations revealed significantly elevated serum alkaline phosphatase (ALP) activity in both sexes at the top dose level at all time points. Triglyceride concentrations were higher in top-dose females at all time points,

significantly at week 13. Hepatic aminopyrine *N*-demethylase activity was significantly elevated in both sexes at and above 5 mg/kg bw per day. Urinalysis did not reveal any clearly treatment-related effects (Table 10).

Mean absolute (males) and relative liver weights (both sexes) were significantly higher at the top dose level (Table 10). Relative liver weight was also significantly higher in females at 1 mg/kg bw per day; however, the Meeting concluded that this value does not form part of a dose–response relationship and is therefore not considered to be related to treatment. Mean absolute and relative ovary weights were significantly lower in all treated groups of females; however, a dose–response relationship is not apparent at 1 and 5 mg/kg bw per day. The effects on ovary weight at the top dose level are considered to be treatment related and might be secondary to reduced weight gain in this group. Additionally, one outlier with a high mean ovary weight (1.258 g) is noted in controls; the group mean value for absolute ovary weight excluding this individual is 0.927 g. Gross necropsy did not reveal any treatment-related findings. Microscopically, a slight increase in the number of haemosiderin-laden Kupffer cells was noted in the livers of both sexes at the top dose level; increased splenic haemosiderin content was noted in top-dose males.

A NOAEL of 5 mg/kg bw per day can be determined for this study, based on a range of findings at the top dose level of 15 mg/kg bw per day. The significantly elevated aminopyrine *N*-demethylase activity in both sexes at and above 5 mg/kg bw per day is considered to represent an adaptive rather than an adverse effect. The significantly lower ovary weights in females at 1 and 5 mg/kg bw per day are not considered to be of clear toxicological significance in the absence of a dose–response relationship or histopathological correlates (Kalinowski et al., 1982).

Beagle dogs (four of each sex per group) were administered flutriafol (purity 93%; batch No. P10) in capsules at a dose level of 0, 1, 5 or 20 mg/kg bw per day for 1 year. Dogs were observed at least daily for clinical signs; more detailed physical examinations were performed weekly, and full veterinary examinations (including ophthalmoscopy) were performed pre-test and during weeks 13, 26, 39 and 52. Body weights and feed consumption were recorded weekly throughout the study period. Blood samples were taken pre-test and during weeks 4, 13, 26 and 52 for the assessment of haematological and clinical chemistry parameters. All animals were subjected to gross necropsy; weights of the main organs were recorded. A comprehensive range of tissues from all animals were examined histopathologically; additional sections of liver were investigated following staining with oil red O and Perl's stain.

One top-dose female was killed for humane reasons during week 16 following observations of dehydration, emaciation, faecal blood and vomiting. No further deaths occurred, and no additional signs of toxicity were observed. Body weight gain over the initial 2 weeks of dosing was reduced significantly in top-dose animals. Ophthalmoscopy did not reveal any treatment-related findings. Initial weight gains were lower in both sexes at the top dose level (Table 11). With the exception of the decedent female, feed consumption was unaffected by treatment. Variations in erythrocyte parameters appeared to be secondary to pre-test values. Higher total leukocyte counts were noted in top-dose females; values were significantly higher at weeks 13 and 26, largely attributable to elevated neutrophil counts. Similar increases were seen in mid-dose, but not high-dose, males (Table 11). Plasma albumin concentrations were significantly lower in both sexes at the top dose level, more consistently in males. There were also increases in ALP and aspartate aminotransferase (AST) activities and serum triglyceride concentrations in the top-dose groups (Table 11). Serum iron and total iron-binding capacity were reduced in females in a dose-related fashion (Table 11).

Mean absolute weights of adrenals and liver were higher in both sexes at the top dose level. Absolute kidney weights were significantly higher in top-dose females only. Mean ovary weight was higher in top-dose females; however, the value did not attain statistical significance. Gross necropsy revealed granular or swollen livers in top-dose males and pale livers in top-dose females.

Table 10. Findings in dogs receiving flutriafol for 90 days

Parameter	Males				Females			
	Dose (mg/kg bw per day)							
	0	1	5	15	0	1	5	15
Weight, week 13 (kg)	12.30	12.63	12.55	12.58	11.83	11.87	11.93	10.98
Weight gain (kg)								
- week 1	0.28	0.13	0.13	0.03*	0.15	0.10	0.05*	−0.05**
- week 2	0.43	0.15	0.18	0.18	0.18	0.13	0.15	−0.03
- week 13	1.83	1.48	1.20	1.48	1.85	1.83	1.58	0.95**
Haematology								
White blood cells (10^3/µl)								
- pre-test	12.03	11.78	13.43	12.98	13.88	13.15	14.28	12.80
- week 4	15.61	14.22	13.27	16.00	12.34	14.82	12.65	14.30
- week 13	13.11	15.78	12.95	19.02**	12.96	13.57	13.28	14.54
Clinical chemistry								
ALP (U/l)								
- pre-test	185	130	172	192	191	214	181	193
- week 4	189	136	192	269*	175	207	186	250*
- week 13	143	95	165	217*	127	165	128	231**
Triglycerides (mg/dl)								
- pre-test	41.5	36.5	44.5	41.8	38.5	38.3	47.0	35.3
- week 4	46.0	41.5	62.6**	49.0	39.7	47.0	34.9	49.2
- week 13	40.6	33.3	43.3	43.7	30.8	38.0	32.8	50.8**
Aminopyrine *N*-demethylase (µmol/h per gram)	13.8	13.1	27.1**	35.3**	15.2	15.6	25.1**	37.9**
Organ weights								
Liver (g)								
- absolute	395	414	416	466*	333	379	380	401
- adjusted for body weight	403	410	415	463**	321	370*	363	438**
Spleen (g)								
- absolute	59.7	63.0	38.2	75.1	42.4	47.5	53.3	53.7
- adjusted for body weight	61.8	61.8	37.8	74.5	41.3	46.7	51.7	57.2
Testes/ovaries (g)								
- absolute	21.33	22.00	22.23	22.23	1.010	0.844*	0.860*	0.720**
- adjusted for body weight	21.48	21.92	22.20	22.18	1.016	0.848*	0.869*	0.700**
Histopathology (*n* = 4)								
Liver: haemosiderin	1	0	0	3	0	0	0	4
Spleen: haemosiderin	0	0	0	3	0	0	0	0

From Kalinowski et al. (1982)
U, units; *$P < 0.05$; ** $P < 0.01$ (Student's *t*-test)

Microscopically, hepatotoxicity was characterized by the increased deposition of lipid in centrilobular hepatocytes in top-dose females; however, similar findings were not apparent in males (Table 12). The severity of haemosiderin pigmentation in liver sinusoidal cells and spleen was increased in both sexes at the top dose level. Increased vacuolation of the adrenal cortex zona glomerulosa was noted in all animals of both sexes at the top dose level and in one animal of each sex at 5 mg/kg bw per day (Table 12).

A NOAEL of 5 mg/kg bw per day can be determined for this study based on the effects on body weight, haematology, clinical chemistry, organ weights and pathology in both sexes at 20 mg/kg bw per day. The increased vacuolation of the zona glomerulosa of the adrenal cortex seen in both sexes at 5 mg/kg bw per day is not considered to be of clear toxicological significance because of the low incidence and severity. Although reduced serum iron concentrations were seen in all groups, values are variable, do not attain statistical significance and do not form a dose–response relationship (Stonard, 1988).

Table 11. Findings in dogs receiving flutriafol for 1 year

Parameter	Males				Females			
	Dose (mg/kg bw per day)							
	0	1	5	20	0	1	5	20
Body weight gain (kg)								
- week 1	0.38	0.27	0.25	0.00*	0.13	0.22	0.17	−0.17*
- week 2	0.60	0.45	0.45	0.25*	0.20	0.38	0.27	−0.02*
- week 52	3.52	2.80	3.19	3.08	2.75	2.82	2.25	2.28
White blood cells (10^3/µl)								
- pre-test	13.9	11.9	11.8	14.9	10.6	12.7	12.8	11.8
- week 4	12.2	13.9	14.7*	12.7	12.3	11.8	12.3	13.0
- week 13	14.3	14.8	16.3	14.3	13.6	13.2	12.9	17.6*
- week 26	14.0	14.9	16.7	16.1	12.9	13.2	16.1*	16.3*
- week 52	15.9	17.2	18.4	14.1	13.2	12.6	13.5	17.1
Neutrophils (10^3/µl)								
- pre-test	5.95	5.55	5.80	7.72	4.30	4.80	5.40	5.15
- week 4	6.88	6.99	6.54	6.35	5.15	6.66	6.33	6.95
- week 13	8.98	7.75	8.67	8.30	6.96	7.04	7.28	11.13*
- week 26	7.61	8.76	10.71*	10.19	6.75	8.21	8.84	9.41
- week 52	9.76	10.04	11.08	7.32	6.31	6.28	6.50	9.02
Lymphocytes (10^3/µl)								
- pre-test	5.82	4.30	3.82	4.15	4.50	6.07	5.17	5.00
- week 4	4.05	4.04	4.52	5.53*	4.64	4.10	4.51	4.50
- week 13	3.93	4.45	5.17	4.80	4.44	4.74	4.50	4.29
- week 26	4.80	4.69	4.54	4.39	4.51	3.71	5.35	5.50
- week 52	4.79	5.09	4.35	4.79	4.71	5.25	5.20	6.10
Albumin (g/dl)								
- pre-test	3.57	3.50	3.57	3.38	3.60	3.52	3.63	3.45
- week 4	3.61	3.45	3.59	3.07**	3.59	3.58	3.64	3.36
- week 13	3.75	3.70	3.65	3.03**	3.63	3.71	3.67	3.29**

Table 11 (continued)

Parameter	Males				Females			
	Dose (mg/kg bw per day)							
	0	1	5	20	0	1	5	20
- week 26	3.56	3.45	3.54	2.90**	3.66	3.70	3.56	3.30*
- week 52	3.72	3.63	3.77	3.02**	3.62	3.70	3.59	3.36
ALP (U/l)								
- pre-test	205	195	209	209	178	165	209	220
- week 4	173	180	187	277**	167	181	196	349**
- week 13	153	170	152	299	149	174	127	426**
- week 26	115	107	123	318**	93	96	134	271**
- week 52	116	127	151	328**	94	106	158	346**
Triglycerides (mg/dl)								
- pre-test	39.8	35.5	43.8	39.0	39.0	37.5	38.3	42.3
- week 4	33.4	36.1	42.6	41.9	37.8	40.5	42.3	61.2**
- week 13	23.1	30.3	25.2	27.9	26.4	27.3	27.3	45.2**
- week 26	33.5	35.6	28.6	27.2	34.0	34.2	28.0	37.0
- week 52	26.0	26.4	28.1	34.0*	35.7	34.1	35.4	43.8
Iron (mmol/l)								
- week 26	17.6	12.9	10.9	13.8	17.3	18.6	12.2	8.8
- week 52	12.8	33.4*	14.7	18.4	23.2	26.6	16.0	11.3
Total iron-binding capacity (mmol/l)								
- week 26	63.3	52.9	53.9	55.0	65.5	58.3	56.0	53.4*
- week 52	62.6	69.0	53.7	56.8	68.9	63.4	61.4	50.9

From Stonard (1988)
ALP, alkaline phosphatase; U, units; *$P < 0.05$; ** $P < 0.01$ (Student's *t*-test)

(b) Dermal application

Groups of Sprague-Dawley rats (10 of each sex per group) received dermal applications of flutriafol (purity 95.1%; batch No. UPL BX1) in PEG400 (dose volume 1 ml/kg bw) at 0, 250, 500 or 1000 mg/kg bw per day for 28 days. Routine observations included mortality, clinical signs and feed consumption. A functional observational battery (FOB) was performed on days 8, 14, 22 and 29, with motor activity assessments performed on day 29. At necropsy, blood samples were taken for clinical chemistry and haematological examinations; all animals received a gross examination, and an extensive range of tissues from control and top-dose rats was examined histopathologically. Dose levels were confirmed analytically.

There were no deaths or signs of systemic toxicity. Erythema and scabbing were present at the application site at the top dose level and in females from the 500 mg/kg bw per day group. Gross pathological and histopathological findings were similar in control and treated groups. Statistically significant changes in relative thymus weight in males and serum succinate dehydrogenase activity and cholesterol level in females are not considered adverse, as the values in test groups were within the control ranges.

The NOAEL for systemic toxicity is 1000 mg/kg bw per day, the highest dose tested. The NOAEL for local effects is 250 mg/kg bw per day, based on erythema and scabbing at 500 mg/kg bw per day (Barnett, 2007a).

Table 12. Organ weight and pathology findings in dogs receiving flutriafol for 1 year

Observation	Sex	Dose (mg/kg bw per day)			
		0	1	5	20
Organ weight (g)					
Adrenal	Male	1.73	1.67	1.71	2.02
	Female	1.68	1.61	1.84	2.31**
Liver	Male	452	475	477	574**
	Female	368	385	401	513**
Kidney	Male	66.6	68.0	62.5	67.3
	Female	55.7	53.5	58.3	64.8*
Ovary	Female	1.26	1.50	1.27	1.79
Histopathology (n = 4)[a]					
Adrenal: increased cortical zona glomerulosa vacuolation	Male	0	0	1 [1, 0, 0, 0]	4 [0, 4, 0, 0]
	Female	0	0	1 [1, 0, 0, 0]	4 [2, 2, 0, 0]
Liver: vacuolation	Male	0	0	0	0
	Female	0	0	0	1 [1, 0, 0, 0]
Liver: increased perivascular connective tissue	Male	0	0	0	1 [1, 0, 0, 0]
	Female	0	0	0	0
Liver: increased centrilobular hepatocyte lipid	Male	0	0	0	0
	Female	0	0	0	3 [1, 2, 0, 0]
Liver: sinusoidal cell haemosiderin pigmentation	Male	4 [1, 2, 1, 0]	4 [1, 2, 0, 1]	4 [2, 2, 0, 0]	4 [0, 0, 0, 4]
	Female	4 [1, 2, 1, 0]	4 [2, 1, 0, 1]	4 [1, 1, 2, 0]	4 [0, 0, 0, 4]
Spleen: haemosiderin pigmentation	Male	4 [1, 2, 1, 0]	4 [1, 2, 1, 0]	4 [0, 2, 2, 0]	4 [0, 0, 0, 4]
	Female	4 [1, 1, 2, 0]	4 [2, 1, 1, 0]	4 [1, 1, 2, 0]	4 [0, 0, 3, 1]

From Stonard (1988)
* $P < 0.05$; ** $P < 0.01$ (Student's t-test)
[a] Incidence [severity]. Laboratory severity scale: minimal, slight, moderate, marked.

(c) Exposure by inhalation

No data were submitted.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Alderley Park (C57BL/10J/Alpk) mice (50 of each sex per group) were administered flutriafol (purity 93%; batch No. P10) in the diet at a level of 0 (two groups), 10, 50 or 200 ppm for 2 years. Routine observations and investigations were performed throughout the study. Blood smears (10 of each sex per group) were prepared following treatment for 12 and 18 months; a differential leukocyte count was performed, and erythrocyte morphology was investigated. Terminal blood samples were taken from all animals for the assessment of erythrocyte parameters. All animals were subjected to gross necropsy, and liver weights were recorded. A comprehensive list of tissues from all animals was investigated histopathologically.

Dietary analyses revealed the achieved concentrations, stability and homogeneity of the test material to be within acceptable limits. Achieved doses were 0, 1.2, 6.0 and 25 mg/kg bw per day in males and 0, 1.5, 7.4 and 30 mg/kg bw per day in females. A number of deaths occurred during the initial part of the study, apparently due to a failure to use the automatic water system effectively;

these animals were replaced. Overall survival was similar in all groups, was greater than 50% at 100 weeks and is considered to be acceptable for the assessment of carcinogenicity. The incidences of greying coat were increased in top-dose males; ocular discharge and thickened eyelids were observed with greater frequency in both sexes at the top dose level. Body weight and body weight gain were reduced at the top dose level (Table 13); a reduction at 50 ppm is not considered adverse, as it was transient in females and it was significant in males only at week 104, when mortality would have a marked impact on group means. Feed utilization efficiency was significantly reduced in top-dose males only (Table 13).

Examination of the blood smears prepared following treatment for 12 and 18 months did not reveal any effects of treatment. Investigation of terminal blood samples did not reveal any toxicologically significant effects on erythrocyte parameters. Significantly higher platelet and white blood cell counts were seen in top-dose males; white blood cell findings are attributable to significantly elevated neutrophil and lymphocyte counts. Similar findings were not apparent in females. There were no notable changes in blood parameters at 10 or 50 ppm.

Mean absolute and adjusted liver weights were significantly higher in both sexes at the top dose level; no other organs were weighed. Gross necropsy revealed slightly increased incidences of liver masses and discoloured and rough livers in top-dose males. Microscopically, liver toxicity was characterized by an increase in the incidence and severity of centrilobular fatty change and hepatocyte hypertrophy in both sexes at the top dose level (Table 13). The incidence and severity of centrilobular fatty change were also slightly increased in 50 ppm males. A marginally increased incidence of hepatocellular adenoma (4%) was observed in top-dose males; values are not statistically significant and are within the laboratory's historical control range (2, 0, 2, 0, 2, 0, 2, 0, 6, 0). Similar findings were not apparent in females, and the incidence of malignant hepatocellular tumours was unaffected by treatment in both sexes. The only non-hepatic finding of any note was a low incidence of vacuolation of white matter in the brain (Table 13); although this is a rare finding, it was not statistically significant, and there was no evidence of increased severity or a clear dose–response relationship.

No evidence of carcinogenicity was seen in this study; marginally increased incidences of benign hepatocellular tumours in males at the top dose level were within the historical control range. A NOAEL of 10 ppm (equal to 1.2 mg/kg bw per day) can be determined for this study, based on the increased incidence and severity of hepatic centrilobular fatty change in males at 50 ppm (equal to 6.0 mg/kg bw per day) (Hext, 1988).

Rats

Alpk:AP rats (64 of each sex per group) were administered flutriafol (purity 93%; batch No. P10) in the diet at a concentration of 0, 20, 200 or 2000 ppm for up to 104 weeks; 12 rats of each sex per group were sacrificed after treatment for 52 weeks. A standard range of observations and investigations was performed routinely during the study. All animals were subjected to gross necropsy; weights of the gonads, adrenals, kidneys, liver and brain were recorded. A comprehensive list of tissues from all animals was investigated histopathologically.

Dietary analyses showed the achieved concentrations, stability and homogeneity of the test material to be within acceptable limits. Achieved mean doses of flutriafol were 0, 1, 10 and 103 mg/kg bw per day and 0, 1.3, 13 and 130 mg/kg bw per day in males and females, respectively. A small number of accidental deaths occurred during blood sampling in the early part of the study. Mortality was comparable in all groups, and survival in all groups was above 50% at week 100. Survival in this study is considered to be adequate for the assessment of carcinogenicity. Clinical signs related to treatment were limited to increased incidences of rats appearing "thin" at the top dose level. Ophthalmoscopy revealed retinal pallor in both sexes at the top dose level, but was not investigated in the lower-dose groups (Table 14).

Cumulative weight gains by both sexes in top-dose animals were significantly lower throughout the study period. Reduced weight gain (~5%) in 200 ppm females at week 13 is not considered to

Table 13. Findings in mice receiving flutriafol for up to 2 years

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	0	10	50	200	0	0	10	50	200
Ocular discharge	60	25	52	69	172	63	101	96	93	240
Thickened eyelids	72	9	31	36	205	71	42	66	88	126
Survival, week 104 (%)	54	56	57	64	50	62	46	50	54	48
Body weight (g)										
- week 13	28.8	28.9	28.6	28.9	27.4**	23.7	23.6	23.7	23.2*	22.9**
- week 75	35.4	35.1	36.1	35.8	33.2**	28.7	28.3	29.6*	28.6	28.1
- week 104	34.0	33.2	33.2	32.2*	31.5**	28.1	27.7	27.8	28.0	26.4*
Weight gain (g)										
- week 12	8.7	9.1	8.4	9.2	7.3**	7.7	7.7	7.7	7.2*	7.2**
- week 52	13.8	14.2	13.7	13.7	12.0**	10.9	10.5	10.9	10.5	9.7**
Feed consumption, weeks 1–12 (g)	322.3	332.3	325.3	327.5	316.9*	360.3	361.3	356.3	350.7	348.6
Feed utilization, weeks 1–12 (g/100 g)	2.87	2.92	2.58	2.80	2.29**	2.14	2.13	2.30*	2.06	2.08
Liver weight (g)										
- absolute	1.67	1.65	1.63	1.68	2.19**	1.55	1.47	1.55	1.46	1.78**
- adjusted for body weight	1.64	1.64	1.61	1.70	2.24**	1.51	1.46	1.55	1.44	1.87**
Histopathology (n = 50)										
- liver fatty change	1	1	2	6	23	0	0	1	2	17
- liver centrilobular hypertrophy	1	0	1	1	14	0	0	0	1	3
- liver adenoma	1	0	1	1	2	0	1	0	0	0
- liver carcinoma	0	0	0	1	0	0	0	0	0	0
- brain, white matter vacuolation	0	0	0	1	2	0	0	0	1	1

From Hext (1988)
* $P < 0.05$; ** $P < 0.01$ (analysis of variance)

be of toxicological significance. Feed consumption and feed conversion efficiency were significantly lower throughout the study at the top dose level (Table 14). Haematology revealed statistically significant effects on erythrocyte parameters, consistent with mild microcytic anaemia, in both sexes at the top dose level. A tendency to higher total white blood cell and lymphocyte counts was seen in both sexes at the top dose level; findings were more marked in females (Table 14). Various clinical chemistry changes were seen at the top dose level: increases in total protein, cholesterol and total iron-binding capacity; and decreases in glucose, triglycerides and ALP (Table 14). Urinalysis revealed significantly lower urine volumes, higher specific gravity and lower pH in top-dose males.

At the interim sacrifice, mean adjusted kidney weights were significantly higher in females at and above 200 ppm, but only at the top dose level at terminal sacrifice; liver weights were significantly higher in both sexes at the top dose level. At terminal sacrifice, liver weights were significantly higher in both sexes at the top dose level and in males at 200 ppm (Table 15).

Gross necropsy of top-dose animals is reported to have found hepatic enlargement associated with discoloured foci. No further treatment-related findings were noted at gross necropsy. Histopathological investigation of the small number of animals dying prior to the interim sacrifice did not reveal any treatment-related findings. At the interim sacrifice, necropsy showed effects on the

Table 14. Findings in rats receiving flutriafol for 2 years

Parameter	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Body weight, week 104 (g)	625.4	621.6	613.4	549.0**	399.6	393.2	397.3	311.3**
Weight gain (g)								
- week 13	361.8	366.0	359.5	304.1**	161.4	158.8	153.5**	133.3**
- week 51	529.3	529.0	529.7	456.3**	240.6	233.3	232.3	175.9**
Feed consumption, weeks 1–13 (g)	2479	2503	2509	2270**	1841	1816	1825	1629**
Pale retina (n = 20)								
- week 52	0	nd	nd	3	0	nd	nd	3
- week 104	1	nd	nd	6	0	nd	nd	5
Haemoglobin (g/dl)								
- week 13	14.6	14.6	14.5	13.8**	14.6	14.2	14.5	13.8**
- week 26	15.8	15.8	15.9	15.1**	15.9	16.2*	16.0	14.9**
- week 52	16.4	16.5	16.4	15.6**	16.4	16.1	16.3	15.0**
Haematocrit (%)								
- week 13	46.3	45.9	46.3	44.1	47.3	46.5	47.4	45.1*
- week 26	47.9	47.9	48.1	46.3*	47.4	47.6	47.5	44.9**
- week 52	47.6	48.0	47.5	44.3**	46.3	45.1	45.8	42.3**
Mean corpuscular volume (fl)								
- week 13	50.3	49.7	49.3	49.0*	53.9	53.8	54.3	52.0**
- week 26	54.8	54.09	54.1	53.0**	58.8	58.8	59.4	55.3**
- week 52	55.3	56.0	54.7	52.5**	58.9	59.3	59.5	53.9**
Mean corpuscular haemoglobin (pg)								
- week 13	16.0	15.8	15.6	15.4	16.6	16.5	16.7	15.9*
- week 26	18.1	18.1	17.8	17.3**	19.7	20.0	20.1	18.4**
- week 52	19.1	19.3	18.8	18.5	20.9	21.1	21.2	19.1**
White blood cells (10^3/µl)								
- week 13	8.89	8.30	7.99	8.51	6.26	6.37	5.97	6.77
- week 26	7.38	6.89	7.53	8.02	4.87	4.66	4.50	5.71
- week 52	7.57	6.75	7.09	8.22	4.32	4.48	4.40	5.42*
Lymphocytes (10^3/µl)								
- week 13	7.12	6.78	6.54	6.90	5.02	5.41	4.87	5.57
- week 26	5.46	5.24	5.45	6.11	3.72	3.67	3.35	4.53*
- week 52	4.69	4.49	4.50	5.66*	2.97	3.20	2.79	4.02**
Total protein (g/dl)								
- week 13	7.13	7.18	7.22	7.52**	7.02	7.15	7.09	7.59**
- week 26	7.19	7.22	7.17	7.47*	7.32	7.28	7.38	7.89**
- week 52	7.41	7.36	7.24	7.42	7.63	7.79	7.72	8.17**
Glucose (mg/dl)								
- week 13	149	152	143	131**	143	140	145	137
- week 26	130	139*	133	130	134	129	136	126*
- week 52	131	127	123*	122*	132	128	131	128

Table 14 (continued)

Parameter	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Cholesterol (mg/dl)								
- week 13	74.3	76.3	74.0	67.8	72.4	77.8	75.6	95.8**
- week 26	85.3	86.3	85.3	79.2	82.1	83.4	82.2	106.3**
- week 52	116.3	135.1	126.3	108.6	98.2	104.2	99.8	137.2**
Triglycerides (mg/dl)								
- week 13	154	157	149	50**	90	98	106	97
- week 26	152	163	158	53**	126	108	136	114
- week 52	163	166	176	73**	171	186	181	152
ALP (U/l)								
- week 13	153	142	146	135*	95	90	87	82
- week 26	114	114	112	93**	60	58	60	51
- week 52	145	153	122*	100**	55	57	55	51
ALT (U/l)								
- week 13	48.3	51.9	50.1	74.3**	48.9	49.3	46.3	47.3
- week 26	58.6	58.3	54.3	64.0	53.1	58.0	60.8	54.6
- week 52	76.9	64..7	67.9	67.9	63.0	57.1	59.5	55.8
Serum iron (mmol/l), week 104	29.8	28.6	26.0	26.1	41.3	49.6	49.4	38.5
Total iron-binding capacity (mmol/l), week 104	86.4	67.5	73.0	92.8	76.3	88.8	88.4	107.0**

From Milburn et al. (1986)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; nd, not determined; U, units; * $P < 0.05$; ** $P < 0.01$ (analysis of variance)

liver (centrilobular hepatocyte hypertrophy and fatty change) in both sexes at the top dose level and in 200 ppm males. In animals dying during the study and killed at termination, the target organs were liver, spleen and adrenals (Table 16). An apparently dose-related increase in the incidence of peripheral neuropathy (described as foci of degenerate nerve fibres) was seen in all treated groups of males dying during the study. However, the pattern is less marked when the total incidence (intercurrent deaths plus terminal sacrifice) is considered (Table 16), and it is not mirrored in females, indicating that it might be merely a variation in the time of observation of a common finding.

The overall pattern of tumours was consistent with that expected for the strain. Treatment-related neoplastic changes were seen in the liver, with slightly increased incidences of adenoma in both sexes at the top dose level and increased incidences of carcinoma in males at and above 200 ppm. Historical control data (Pigott, 1994) from 11 studies initiated in the same laboratory between July 1980 and March 1987 showed that the incidences in the flutriafol-treated animals are within the historical control range (Table 16). The incidence of Leydig cell tumours was also increased in this group; however, the incidence (10.9%) is reported to be within the laboratory's historical control range (2.8–10.9%; results from three contemporary studies); the absence of this common tumour in the concurrent control group is considered to be unusual. Incidences of mammary gland tumours were markedly lower in top-dose females, possibly secondary to the lower body weight in this group.

No evidence of carcinogenicity was seen in this study; slight increases in the incidence of liver adenoma and Leydig cell tumours in top-dose males are within the historical control ranges. An overall NOAEL of 20 ppm (equal to 1.0 and 1.3 mg/kg bw per day in males and females, respectively) can be determined for this study, based on the histopathological findings in the liver seen at 200 ppm (equal to 10 and 13 mg/kg bw per day in males and females, respectively) (Milburn et al., 1986).

Table 15. Organ weights in rats receiving flutriafol for up to 2 years

Organ	Organ weights (g)							
	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Interim sacrifice								
Kidneys								
- absolute	3.65	3.61	3.72	3.46	2.20	2.18	2.39*	2.26
- adjusted[a]	3.51	3.55	3.65	3.79	2.13	2.16	2.31**	2.42**
Liver								
- absolute	21.8	21.0	23.9	26.6**	13.1	12.6	13.6	14.5*
- adjusted[a]	20.9	20.6	23.4	28.7**	12.5	12.5	12.9	16.0**
Terminal sacrifice								
Kidneys								
- absolute	4.21	4.43	4.58	4.61	2.84	2.80	2.71	2.77
- adjusted[a]	4.23	4.45	4.59	4.57	2.70	2.71	2.62	3.01*
Liver								
- absolute	19.8	20.9	22.0**	25.7**	14.5	13.9	13.7	15.6
- adjusted[a]	19.5	20.7	21.9**	26.2**	13.6	13.3	13.1	17.3**

From Milburn et al. (1986)
* $P < 0.05$; ** $P < 0.01$ (analysis of variance)
[a] Organ weights adjusted for terminal body weight by analysis of covariance.

2.4 Genotoxicity

Testing of the genotoxicity of flutriafol has been performed in a range of assays (Table 17). Equivocal results were seen at cytotoxic concentrations in two reverse mutation assays with mouse lymphoma L5178Y cells. The remaining in vitro and all the in vivo tests were negative. The overall database is considered adequate to conclude that flutriafol is unlikely to be genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Alpk:AP rats (F_0 generation; 15 males, 30 females per group) were administered flutriafol (purity 93%; batch No. P10) in the diet at a concentration of 0, 60, 240 or 1000 ppm for 12 weeks prior to mating to produce F_{1A} litters. Two females were housed with one male until mating or for a maximum of 10 days. If mating was unsuccessful, the male was removed and replaced with an animal of proven fertility. Litters were not standardized at day 4, and the F_{1A} pups were weaned at day 29. A week after weaning, F_0 parents were remated to produce F_{1B} litters; litters were weaned at day 29, but remained housed by litter until selection at day 36. Selected F_{1B} pups (15 males, 30 females per group) were administered the test material for 11 weeks prior to mating to produce the F_{2A} litters. Litters were not standardized, and the F_{2A} pups were weaned at day 29. A week after weaning, the F_1 parents were remated to produce F_{2B} litters.

All rats were observed daily for clinical and behavioural abnormalities; more detailed physical examinations were carried out weekly. F_0 and F_1 parents were subjected to gross necropsy; weights of the testes, ovaries and liver were recorded. Testes, epididymides, prostate, seminal vesicles, ovaries,

Table 16. Histopathology findings in rats receiving flutriafol for up to 2 years

Parameter	Incidence of finding							
	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Interim sacrifice								
Number of animals	*11*	*11*	*12*	*11*	*12*	*12*	*12*	*12*
Liver: centrilobular hypertrophy	0	0	1	10*	0	0	0	4*
Liver: fatty change	0	0	3	11*	0	0	0	1
Liver: haemosiderin accumulation	0	0	0	0	0	0	0	12*
Spleen: haemosiderin accumulation[a]	11 (1.1)	11 (1.5)	12 (1.7)	10 (1.7)	12 (1.8)	12 (2.0)	12 (2.0)	12 (3.0)
Intercurrent deaths								
Number of animals	*26*	*25*	*24*	*26*	*33*	*28*	*27*	*22*
Liver: centrilobular hypertrophy	0	0	0	2	0	0	0	1
Liver: fatty change	4	3	13*	24*	10	12	9	15
Liver: spongiosis	1	1	5	1	0	0	0	0
Liver: clear cell focus	1	1	2	3	0	1	0	0
Liver: haemosiderin accumulation	0	0	0	0	0	0	0	10*
Adrenal: macrophages	0	0	1	4	4	1	4	15*
Adrenal: vacuolation	4	3	9	12*	1	0	1	2
Thyroid: cystic foci	0	0	0	3	0	0	0	0
Spleen: increased haemosiderin	0	0	0	9*	9	3	7	18*
Peripheral neuropathy	2	4	10*	16*	10	5	8	7
Kidney: nephropathy	21	20	21	24	28	23	21	20
Seminal vesicle: atrophy	1	1	3	4	—	—	—	—
Terminal sacrifice								
Number of animals	*23*	*23*	*26*	*24*	*19*	*23*	*25*	*29*
Liver: centrilobular hypertrophy	0	0	0	1	0	0	0	3
Liver: fatty change	8	9	14	24*	2	3	6	18*
Liver: clear cell focus	8	12	23*	17*	2	1	1	0
Liver: haemosiderin accumulation	0	1	1	0	0	0	0	18*
Adrenal: macrophages	0	1	0	1	9	7	8	25*
Adrenal: vacuolation	17	14	21	12	4	1	3	2
Thyroid: cystic foci	0	0	1	2	1	1	0	1
Spleen: increased haemosiderin	0	0	1	2	1	0	2	25*
Peripheral neuropathy	17	18	18	13	15	16	9	17
Kidney: nephropathy	22	22	24	24	19	19	23	22
Total neoplastic								
Number of animals	*64*	*64*	*64*	*64*	*64*	*64*	*64*	*64*
Liver: adenoma (HC 0–6.7%)	0	0	0	1	0	0	0	2
Liver: carcinoma (HC 0–5.8%)	0	0	1	2	0	0	0	0
Leydig cell tumour (HC 2.8–10.9%)	0	4	3	7	—	—	—	—
Mammary: adenocarcinoma	—	—	—	—	6	4	5	2
Mammary: fibroadenoma	—	—	—	—	4	7	6	1
Total peripheral neuropathy (*n* = 52)	19	22	28	29*	25	21	17	24

HC, historical control range (11 studies 1980–1987); * $P < 0.05$

[a] Incidence (mean severity score).

Table 17. Results of studies of genotoxicity with flutriafol[a]

End-point	Test object	Concentration	Purity (%); batch	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2*uvrA*	3–5000 µg/plate, ±S9 (plate incorporation) 33–5000 µg/plate, ±S9 (pre-incubation)	95.1; UPL BX-1	Negative	Sokolowski (2006)
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	1.6–5000 µg/plate, ±S9	93; P10	Negative	Callander (1988)
Reverse mutation	Mouse lymphoma L5178Y cells	12.5–600 µg/plate, −S9 25–600 µg/plate, +S9	95.1; UPL BX-1	Equivocal ±S9 with cytotoxicity	Wollny (2006)
Reverse mutation	Mouse lymphoma L5178Y cells	50–800 µg/plate, −S9 150–1500 µg/plate, +S9	Not reported	Equivocal +S9 with cytotoxicity Negative –S9	McGregor & Riach (1984)
Chromosomal aberration	Human lymphocytes	91–1514 µg/ml, +S9 92–1250 µg/ml, −S9	95.1; UPL BX-1	Negative	Kunz (2007)
Chromosomal aberration	Human lymphocytes	25, 125, 250 µg/ml, ±S9	92.8; batch not reported	Negative	Howard (1989)
In vivo					
Chromosomal aberration	Alpk rats	0, 15, 75 or 150 mg/kg bw (gavage) single or 5 consecutive days	93; batch not reported	Negative	Richardson et al. (1982)
Bone marrow micronuclei	Alpk mice	0, 94 or 150 mg/kg bw (gavage) single	93; P10	Negative	Sheldon et al. (1986)
Liver unscheduled DNA synthesis	Alpk rats	0, 250, 500 or 1000 mg/kg bw (gavage) single	93; P10	Negative	Trueman (1987)

DNA; deoxyribonucleic acid; S9, 9000 × *g* supernatant from livers of rats

[a] Vehicle dimethyl sulfoxide for in vitro studies, corn oil for in vivo studies.

uterus and cervix of control and top-dose animals were investigated histopathologically; investigations in intermediate-dose groups were limited to the liver and grossly abnormal tissues. Clinically abnormal F_{1A} and F_{2A} pups were subject to gross necropsy at day 36 postpartum; the remainder were discarded. Non-selected F_{1B} (5 of each sex per group) and F_{2B} pups (10 of each sex per group) were subject to gross necropsy at day 36. Testes, epididymides, prostate, seminal vesicles, ovaries, uterus and cervix from control and top-dose animals were investigated histopathologically; investigations in intermediate-dose groups were limited to the liver and grossly abnormal tissues. Pups found dead at up to and including 18 days of age were subjected to gross necropsy. Those pups found dead at over 18 days were subjected to gross necropsy; the testes, epididymides, prostate, seminal vesicles, ovaries, uterus, cervix, liver and grossly abnormal tissues were examined histopathologically.

Some variation was evident between the numerical information in the main study report, the individual animal data and a supplementary analysis (Plassmann & Hofer, 2006). The variations were relatively minor and do not have an impact on the overall evaluation.

Dietary analyses revealed the stability, homogeneity and achieved concentrations of the test material to be within acceptable limits. Achieved doses were 0, 3.5, 14 and 56 mg/kg bw per day.

Parental animals. No treatment-related deaths occurred, and no signs of toxicity were observed. Body weight gains and feed consumption were consistently lower in the top-dose groups during most

phases of the study (Table 18). Liver weights of both sexes at the top dose level were significantly higher; histopathology revealed increased incidences of hepatic centrilobular hypertrophy in top-dose males and fatty change in both sexes at the top dose and in 240 ppm F_1 males. Mean testes weight (adjusted for body weight) was lower in top-dose F_1 males; mean ovary weight was increased in F_1 females (Table 18). Mating indices were comparable in all groups; however, the fertility index at the top dose level was lower for the F_{1A} litter; a similar finding was not apparent for the F_{1B} litter or F_2 litters (Table 19).

Offspring. The number of F_{1A} litters at the top dose level was lower as a result of the reduced fertility index in this group. Mean litter size was lower at the top dose level in all matings (Table 20). Pup birth weights, weight gains and the number of postnatal deaths were comparable in all groups. Necropsy of the F_{1B} and F_{2B} litters revealed evidence of hepatic fatty change at the top dose level.

A NOAEL for reproductive toxicity of 240 ppm (equal to 14 mg/kg bw per day) can be determined for this study, based on the consistently lower litter sizes in all generations at the top dose level and the lower fertility index in F_0 parents seen for the first generation.

A NOAEL for parental toxicity of 60 ppm (equal to 3.5 mg/kg bw per day) can be determined, based on the liver histopathology (fatty change) in F_1 males at 240 ppm.

A NOAEL for offspring toxicity of 240 ppm (equal to 14 mg/kg bw per day) can be determined, based on the liver histopathology (fatty change) at the top dose level (Tinston et al., 1986).

In a screening reproductive toxicity study, flutriafol (purity 95.1%; batch No. UPL BX 1) was administered to groups (five of each sex) of Han Wistar rats at a concentration of 0, 30, 60, 240 or 1000 ppm in the diet. The males were treated for 70 days and the females for 14 days prior to pairing (1:1). The resultant offspring were culled to four of each sex per litter at day 4, then reared to weaning, separated from the mother and reared for a further 2 weeks on their respective diets until necropsy. Regular assessments were made of clinical signs, body weights and feed consumption. Routine examinations were made of mating performance and gestation, parturition and lactation status. Age and body weight at vaginal opening and preputial separation were determined. Limited gross and microscopic examinations were performed, primarily of reproductive organs. Sperm analyses (morphology, motility and count) were performed on parental males from control and top-dose groups.

Table 18. Parental and offspring findings in rats receiving flutriafol

Parameter		Dietary concentration (ppm)							
		0	60	240	1000	0	60	240	1000
		Males				**Females**			
F_0	Pre-mating weight (g)	482.2	467.1	465.2	454.3	271.9	270.9	269.7	260.0
	Weight gain (g)	411.4	397.1	394.9	384.6	207.3	206.2	205.3	195.0
	Feed consumption (g)	2451	2381	2388	2326**	1669	1669	1664	1609**
	Feed utilization (g feed/g weight gain)	5.98	6.04	6.06	6.06	8.12	8.11	8.11	8.17
F_1	Pre-mating weight (g)	469.7	476.3	488.1	473.1	270.7	272.5	265.5	260.9
	Weight gain (g)	354.4	364.0	367.5	349.9	167.3	167.3	161.0	150.0
	Feed consumption (g)	2286	2351	2377	2355	1594	1631	1575	1566
	Feed utilization (g feed/g weight gain)	6.47	6.49	6.53	6.78	9.67	9.77	9.79	10.49**

Table 18 (continued)

Parameter		Dietary concentration (ppm)							
		0	60	240	1000	0	60	240	1000
		F_{1A}				**F_{1B}**			
F_0	Weight, day 0 (g)	274.1	272.5	277.6	260.3*	310.9	314.0	312.4	300.5*
	Weight gain, day 8 (g)	26.5	28.4	25.9	22.9	23.8	22.5	22.9	19.5*
	Weight gain, day 15 (g)	57.7	61.5	56.6	49.4**	52.5	50.8	50.9	42.8**
	Weight gain, day 22 (g)	134.9	138.1	134.3	121.9	121.4	127.5	120.1	106.8**
	Weight, day 22 (g)	409.0	410.6	411.9	382.2	432.3	441.5	432.5	407.3
		F_{2A}				**F_{2B}**			
F_1	Weight, day 0 (g)	287.0	284.1	282.4	276.5	312.8	319.4	309.0	304.4
	Weight gain, day 8 (g)	23.6	22.8	24.4	23.1	23.7	21.5	22.7	17.8**
	Weight gain, day 15 (g)	50.2	51.3	53.5	50.3	52.8	51.5	52.1	46.2**
	Weight gain, day 22 (g)	114.0	110.0	117.6	109.1	121.8	130.1	123.3	115.8
	Weight, day 22 (g)	401.0	394.1	399.7	385.6	434.6	449.5	432.3	420.2
Organ weights (g)		**Males**				**Females**			
F_0	Liver, absolute	21.3	20.8	21.9	25.7**	14.3	13.8	13.8	15.2
	Liver, adjusted	21.0	21.4	22.1	25.7**	13.6	13.8	13.8	16.0**
F_1	Liver, absolute	21.7	22.9	22.7	28.1**	12.6	13.1	12.9	14.0**
	Liver, adjusted	22.0	22.8	22.6	28.0**	12.6	12.8	12.9	14.4**
F_0	Testes/ovary, absolute	3.61	3.70	3.78	3.66	0.187	0.177	0.186	0.187
	Testes/ovary, adjusted	3.61	3.71	3.78	3.66	0.186	0.177	0.186	0.188
F_1	Testes/ovary, absolute	3.80	3.66	3.88	3.62	0.201	0.188	0.200	0.238
	Testes/ovary, adjusted	3.82	3.65	3.88	3.61*	0.201	0.190	0.200	0.235
Liver histopathology		**Males**				**Females**			
F_0	Centrilobular hypertrophy	0/15	0/15	0/15	2/15	0/30	0/30	0/30	0/30
F_1		0/15	0/15	0/15	4/15	0/30	0/30	0/30	0/30
F_0	Fatty change	0/15	1/15	0/15	8/15*	1/30	1/30	0/30	5/30
F_1		0/15	0/15	5/15*	13/15*	0/30	0/30	0/30	3/30

From Tinston et al. (1986)
* $P < 0.05$; ** $P < 0.01$

Table 19. Reproductive findings in rats receiving flutriafol

Parameter		First litter				Second litter			
		Dietary concentration (ppm)							
		0	60	240	1000	0	60	240	1000
		Males							
Mating index (%)	F_0	93	90	87	87	75	76	82	88
	F_1	87	87	93	87	86	76	80	85
Fertility index (%)	F_0	78	93	77	54	93	84	84	83
	F_1	73	85	86	77	87	90	96	97
		Females							
Mating index (%)	F_0	93	90	87	87	97	93	97	97
	F_1	87	87	93	87	93	93	93	97

Table 19 (continued)

Parameter		First litter				Second litter			
		Dietary concentration (ppm)							
		0	60	240	1000	0	60	240	1000
Fertility index (%)	F_0	78	93	77	54	100	93	90	93
	F_1	73	85	86	77	96	93	96	100
		F_{1A}				**F_{1B}**			
Litters (no.)	F_0	21	25	20	14	26	26	26	26
- with live pups		21	25	20	14	26	26	26	26
- all pups alive		16	22	15	14	24	24	21	22
- with dead pups		5	3	5	0	2	2	5	4
- all pups dead		0	0	0	0	0	0	0	0
		F_{2A}				**F_{2B}**			
Litters (no.)	F_1	18	22	24	20	26	26	27	29
- with live pups		18	22	24	20	26	26	26	28
- all pups alive		18	20	23	15	24	23	25	19
- with dead pups		0	0	0	0	0	0	1	1
- all pups dead		0	2	1	5	2	3	1	9

From Tinston et al. (1986)

Achieved mean doses were 0, 1.6, 3.3, 13 and 54 mg/kg bw per day in the male groups and 0, 2.3, 4.3, 18 and 73 mg/kg bw per day in the female groups. There were no deaths or treatment-related clinical signs and no effects on sperm parameters. At 1000 ppm, a number of effects were produced: reduced feed consumption and body weight gain in females during the pre-pairing, gestation and lactation periods; increased duration of gestation; decreased implantation numbers; increased implantation loss (partly due to increased number of pups born dead); decreased litter size; increased number of postnatal losses; increased liver weights; decreased pup weights from birth to weaning and thereafter; and delayed vaginal opening (possibly secondary to reduced delayed body weight). Hepatic fatty change and hepatocellular hypertrophy were seen in both sexes; ovarian stromal cell hyperplasia incidence and severity were slightly increased.

At 240 ppm (equal to 13 mg/kg bw per day), effects included increased post-implantation loss (to 13%); slightly decreased birth index; and hepatic fatty change and hepatocellular hypertrophy.

The NOAEL for parental, pup and reproductive effects was 60 ppm (approximately 4 mg/kg bw per day) (Gerspach, 2008a).

Groups of Han Wistar rats (24 of each sex) were exposed to flutriafol (purity 95.1%; batch No. UPL BX 1) at a concentration of 0, 30, 80, 150 or 300 ppm in the diet. Parental animals were exposed for 10 weeks before pairing and through gestation, lactation, weaning and selection of the F_2 parental generation through to weaning of the F_2 pups. Litters were culled to four of each sex on day 4. One pup of each sex per litter was given a gross and histopathological examination. All F_2 parental animals received a gross pathological and histopathological examination. Regular assessments were made of clinical signs, body weights and feed consumption. Routine examinations were made of mating performance and gestation, parturition and lactation status. Age and body weight at vaginal opening and preputial separation were determined. Sperm analyses (morphology, motility and count) were performed on parental males from control and top-dose groups.

Achieved doses were 0, 1.5, 4.0, 7.4 and 16 mg/kg bw per day in the male groups and 0, 2.0, 5.3, 9.7 and 21 mg/kg bw per day in the female groups. Findings seen in the screening study at

Table 20. Offspring findings for rats receiving flutriafol

Parameter	Time	Dietary concentration (ppm)			
		0	60	240	1000
F_{1A}					
Deaths	Days 1–29	9	10	18	6
Litter size (no.)	Day 1	11.7	11.6	12.2	11.2
	Day 29	11.2	11.2	11.3	10.8
Weight, M, F (g)	Day 1	6.1, 5.8	6.3, 5.9	6.1, 5.9	6.5, 6.2
Weight gain, M, F (g)	Day 5	3.2, 3.2	3.4, 3.5	2.9, 2.7	3.8, 3.6
	Day 29	71.8, 67.1	72.4, 68.5	69.0, 62.7	70.1, 64.6
F_{1B}					
Deaths	Days 1–29	23	15	25	21
Litter size (no.)	Day 1	11.3	11.9	10.8	9.7
	Day 29	10.4	11.3	9.9	8.9
Weight, M, F (g)	Day 1	6.2, 5.9	6.1, 5.7	6.2, 5.9	6.3, 5.9
Weight gain, M, F (g)	Day 5	3.2, 3.2	3.4, 3.3	3.4, 3.1	4.0*, 4.0**
	Day 29	73.8, 68.9	73.5, 67.8	74.1, 67.2	75.4, 70.4
F_{2A}					
Deaths	Days 1–29	10	8	7	4
Litter size (no.)	Day 1	12.0	11.2	10.8	9.5
	Day 29	11.4	10.8	10.5	9.3
Weight, M, F (g)	Day 1	6.4, 6.1	6.2, 5.8	6.6, 6.1	6.6, 6.2
Weight gain, M, F (g)	Day 5	3.5, 3.5	3.7, 3.7	3.7, 3.6	4.2, 4.0
	Day 29	70.6, 66.3	74.0, 69.9	74.9, 68.9	73.9, 68.6
F_{2B}					
Deaths	Days 1–29	18	12	4	11
Litter size (no.)	Day 1	10.7	12.0	9.9	9.4
	Day 29	10.0	11.6	9.7	9.3
Weight, M, F (g)	Day 1	6.3, 6.0	6.4, 6.0	6.6, 6.2	6.5, 6.1
Weight gain, M, F (g)	Day 5	3.6, 3.3	3.9, 3.8	3.8, 3.7	4.0, 3.8
	Day 29	74.9, 68.6	77.7, 71.3	75.8, 71.4	76.2, 69.7
Liver: fatty change, M, F					
F_{1A}		0/0, 0/1	0/2, 0/1	0/3, 0/1	0/2, 0/2
F_{1B}		0/6, 0/8	0/5, 0/6	0/5, 0/6	1/7, 1/6
F_{2B}		0/10, 0/10	0/10, 0/10	0/10, 0/11	5/10, 1/10

From Tinston et al. (1986)
F, female; M, male; * $P < 0.05$; ** $P < 0.01$

240 ppm were not repeated in this study at 300 ppm. There were no deaths or clinical signs of toxicity. Feed consumption and body weights were similar in all groups. There were no adverse effects on mating, estrous cycling, sperm parameters, gestation length, birth or litter parameters in the F_1 or F_2 generations (Table 21). The time to sexual maturation was slightly but statistically significantly increased in F_1 offspring of both sexes (Table 21), but the values were within the historical control range (26.7–28.3 days in males and 33.0–35.1 days in females), and this is not considered to represent an adverse effect. Post-implantation losses were slightly increased at the top dose level in both generations but had an insignificant effect on litter size (Table 21) and are not considered to be adverse.

Table 21. Reproductive findings in rats dosed with flutriafol

	Dietary concentration (ppm)				
	0	30	80	150	300
F_1 generation					
Post-implantation loss (%)	8.8	9.1	8.2	9.9	10.6
Litter size, day 1	12.2	12.3	11.6	12.0	12.1
Litter size, day 4 post-cull	8.0	8.0	7.9	7.7	7.7
Litter size, day 21	8.0	8.0	7.9	7.5	7.7
Body weight, day 21, M/F (g)	50/48	51/49	50/48	51/49	51/49
Preputial separation (day)	27.1 ± 0.8	27.2	27.5	27.6	27.9 ± 0.9*
Preputial separation (weight; g)	79 ± 6	—	—	—	84 ± 8
Vaginal patency (day)	33.7 ± 1.5	33.5	34.4	33.6	34.6 ± 1.8
Vaginal patency (weight; g)	108 ± 9	—	—	—	113 ± 11
F_2 generation					
Post-implantation loss (%)	8.3	7.5	8.3	9.3	10.7
Litter size, day 1	12.6	12.3	12.4	11.8	11.9
Litter size, day 4 post-cull	8.0	7.8	8.0	7.4	7.9
Litter size, day 21	8.0	7.8	8.0	7.4	7.9
Body weight, day 21, M/F (g)	52/51	52/51	52/51	52/51	52/51

From Gerspach, Flade & Chevalier (2009)
F, female; M, male; * $P < 0.05$

Liver weights were increased slightly (< 10%) at 300 ppm in parental animals of both sexes in both generations. The increased liver weight was associated with centrilobular hepatocyte hypertrophy. A parental NOAEL of 150 ppm (equal to 7.4 mg/kg bw per day) can be determined, based on the liver histopathology (fatty change) in F_1 males at 300 ppm.

There were no alterations in organ weights or histopathological findings in the reproductive organs examined.

The NOAEL for reproductive and pup toxicity is 300 ppm (equal to 16 mg/kg bw per day), the highest dose tested in this study (Gerspach, Flade & Chevalier, 2009). Some effects were observed at the highest dose level (e.g. the time to sexual maturation was slightly but statistically significantly increased in F_1 offspring of both sexes, and post-implantation losses were slightly increased at the top dose level in both generations), but these were marginal and considered to be of no biological significance.

(b) Developmental toxicity

Rats

Mated female Alderley Park rats (24 per group) were gavaged with flutriafol (purity 93%; batch No. P10) suspended in corn oil at 0, 10, 50 or 125 mg/kg bw per day on gestation days 6–15. Animals were observed daily for clinical signs; body weights and feed consumption were measured at intervals during the study. Dams were sacrificed and the uterine contents investigated on day 21. All fetuses were weighed and examined for external abnormalities. Approximately two thirds of the fetuses were investigated for internal abnormalities prior to evisceration and staining with alizarin red for the assessment of skeletal findings. The remaining fetuses were investigated by serial sectioning (heads) and dissection (viscera).

No deaths occurred during the study period. Signs of toxicity were limited to genital/ventral staining in 16 animals at the top dose level. Body weight gains and feed consumption at the top

dose level were significantly lower than those of controls over the dosing period and post-treatment. The numbers of corpora lutea and implantations were comparable in all groups; however, mean litter size at the top dose level was significantly lower than that of controls as a result of significantly higher post-implantation loss (Table 22). Mean gravid uterus weights, litter weights and fetal weights were also significantly lower at the top dose level; similar effects were not apparent in other treated groups.

The total incidences of external and visceral defects were unaffected by treatment; incidences of individual findings were comparable in all groups (Table 23). Three fetuses had major skeletal defects: at 50 mg/kg bw per day, a single fetus had multiple skeletal defects (missing vertebral arches); at 125 mg/kg bw per day, two fetuses had pronounced kinked ribs. The total incidence of minor skeletal defects was significantly increased (in a dose-related fashion) in all treated groups (Table 23). A number of individual findings were significantly increased in incidence at the top dose level, and a smaller number were significantly increased at 50 mg/kg bw per day. At 10 mg/kg bw per day, a small number of individual findings were increased in incidence, including partially ossified occipital, unossified odontoid, increased fontanelle size and unossified calcaneum (Table 23). Mean scores for the degree of manus and pes ossification were significantly higher in all treated groups (in a dose-related fashion), indicating delayed or reduced ossification. Although some of the findings at the lowest dose level were within the range of contemporary historical control data (Moxon, 2005a), the findings for odontoid and occipital ossification and manus and pes scores were outside the historical control range (Table 23).

No evidence of teratogenicity was seen in this study; litter size was reduced at the top dose level as a result of increased post-implantation loss associated with maternal toxicity. A maternal NOAEL of 50 mg/kg bw per day can be determined for this study, based on the clinical signs and body weight effects at the top dose level. Minor body weight effects at 50 mg/kg bw per day are not considered to be toxicologically significant. A developmental NOAEL cannot be determined for this study as a result of the increased incidences of a number of fetal skeletal parameters mainly indicative of reduced or retarded ossification at the lowest dose level (Killick et al., 1982).

In an initial range-finding developmental toxicity study, female Han Wistar rats (five per group) were administered flutriafol (purity 95.1%; batch No. UPL BX 1) by gavage at 0 (corn oil), 5, 10, 50 or 150 mg/kg bw per day from gestation days 6 to 20. The dams were sacrificed on gestation day 21, and the fetuses were removed by caesarean section. Fetuses were examined for external anomalies, and skeletons from all groups except 150 mg/kg bw per day were stained with alizarin red and alcian blue.

One top-dose animal was sacrificed on day 12. Treatment at 150 mg/kg bw per day resulted in the occurrence of behavioural changes or clinical signs and decreased feed consumption and strongly retarded body weight from the first day of dosing; this was considered to be mainly due to embryonic/fetal resorptions in all pregnant animals in this group. There were only two dams with only three live fetuses in which weights were also markedly low. Skeletal and cartilage examinations were not performed for this group. At 50 mg/kg bw per day, slightly reduced maternal feed consumption and retarded body weight development occurred during the treatment period.

The number of skeletal variations (rudimentary cervical ribs and supernumerary rudimentary thoracic ribs) was increased in fetuses from all treated groups. Increased incidences of branched xiphoid cartilage were noted at 50 mg/kg bw per day. One fetus at 150 mg/kg bw per day had multiple external abnormalities (a domed head, ablepharia, micrognathia and a suspected cleft palate).

This preliminary study failed to show a fetal NOAEL as a result of increases in thoracic and cervical ribs at 5 mg/kg bw per day and above. The maternal NOAEL was 10 mg/kg bw per day, based on reduced body weight gain. Within the limited study design, there was no indication of teratogenicity in the absence of severe maternal toxicity (Gerspach, 2008b).

Table 22. Maternal and litter findings in rats administered flutriafol during pregnancy

Parameter	Dose (mg/kg bw per day)			
	0	10	50	125
Maternal findings				
Body weight (g)				
- day 6	284.4	282.6	286.0	272.8
- day 15	326.0	326.2	327.4	303.6
- day 21	415.2	412.3	412.8	363.8
Weight gain (g) [% of control]				
- days 0–6	29.1	31.1	30.8	31.7
	[—]	[107%]	[106%]	[109%]
- days 6–9	6.4	10.4	6.0	3.0
	[—]	[163%]	[93.8%]	[46.9%]
- days 6–15	41.6	43.6	41.4	30.8*
	[—]	[105%]	[100%]	[74.0%]
- days 15–21	89.2	86.1	85.4	60.2*
	[—]	[96.5%]	[95.7%]	[67.5%]
Feed consumption (g/day) [% of control]				
- days 6–15	22.6	22.4	22.2	18.8**
	[—]	[99.1%]	[98.2%]	[83.2%]
- days 15–21	35.5	35.0	35.4	30.6**
	[—]	[98.6%]	[99.7%]	[86.2%]
Litter findings				
Mated (no.)	24	24	24	24
Litters (no.)	20	21	23	21
Corpora lutea (no.)	15	14.4	13.8	14.0
Implantations (no.)	12.9	12.6	11.8	12.4
Early intrauterine deaths [% of total]	15 [6.3%]	19 [7.2%]	16 [6.1%]	40 [14.8%]
- litters affected	6/20	13/21*	10/23	14/21*
Late intrauterine deaths [% of total]	0	2 [0.7%]	1 [0.4%]	46 [18.7%]
- litters affected	0/20	2/21	1/23	14/21**
Pre-implantation loss (%)	13.5	10.3	13.8	12.2
- litters affected	15/20	10/21	12/23	13/21
Post-implantation loss (%)	6.3	7.9	6.4	33.5
- litters affected	6/20	13/21*	10/23	17/21**
Litter size (no.)	12.2	11.7	11.1	8.4*
Gravid uterus weight (g)	84.4	81.7	79.2	61.5**
Litter weight (g)	63.9	59.9	57.8	38.7**
Fetal weight (g)	5.27	5.09	5.24	4.44**

From Killick et al. (1982)
* $P < 0.05$; ** $P < 0.01$ (*t*-test or Fisher's test)

Table 23. Fetal findings for rats administered flutriafol during pregnancy

Parameter	Historical control range[a] (%)	Dose (mg/kg bw per day)							
		0		10		50		125	
Total findings [fetal incidence]									
Minor external/visceral defects: total	—	6	[2.5%]	12	[5.0%]	9	[3.7%]	9	[5.3%]
Major external/visceral defects: total	—	—	—	1	[0.4%]	1	[0.4%]	1	[0.6%]
Minor skeletal defects: total	—	65	[40.6%]	92**	[55.4%]	109**	[64.9%]	101**	[88.6%]
Major skeletal defects: total	—	—	—	—	—	1	[0.6%]	2	[1.8%]
Skeletal findings [fetal incidence]									
Occipital: partially ossified	1–39	132	[82.5%]	159**	[95.8%]	166**	[98.8%]	114**	[100%]
Fontanelle: increased	0–3.6	—	—	2	[1.2%]	3	[1.8%]	16**	[14.0%]
Odontoid: not ossified	4–10	14	[8.8%]	29*	[17.5%]	42**	[25%]	64**	[56.1%]
Cervical arches 3–6 partially ossified	—	—	—	—	—	—	—	7**	[6.1%]
Cervical rib									
- unilateral	0–13	4	[2.5%]	6	[3.6%]	16**	[9.5%]	21**	[18.4%]
- bilateral	0–1	1	[0.6%]	3	[1.8%]	10**	[6.0%]	20**	[17.5%]
- unilateral/bilateral	0–14	5	[3.1%]	9	[5.4%]	26**	[15.5%]	41**	[36%]
14th rib									
- unilateral	4–16	16	[10%]	24	[14.5%]	28	[16.7%]	18	[15.8%]
- bilateral	4–12	19	[11.9%]	17	[10.2%]	63**	[37.5%]	81**	[71.1%]
- unilateral/bilateral	8–27	35	[21.9%]	41	[24.7%]	91**	[54.2%]	99**	[86.8%]
Kinked ribs	—	—	—	—	—	—	—	2	[1.8%]
1st sternebra partially ossified	—	3	[1.9%]	3	[1.8%]	4	[2.4%]	12**	[10.5%]
2nd sternebra partially ossified	—	7	[4.4%]	11	[6.6%]	10	[6.0%]	24**	[21.5%]
2nd sternebra misaligned	—	—	—	1	[0.6%]	1	[0.6%]	—	—
Calcaneum not ossified									
- unilateral	3–5	10	[6.3%]	7	[4.2%]	3	[1.8%]	—	—
- bilateral	79–85	112	[70%]	137**	[82.5%]	157**	[93.5%]	114**	[100%]
- unilateral/bilateral	83–99	122	[76.3%]	144*	[86.7%]	160**	[95.2%]	114**	[100%]
Mean manus score[b]	1.9–2.59	2.42		2.66*		2.65*		3.13**	
Mean pes score[b]	2.4–3.05	2.72		3.06**		3.21**		3.63**	

From Killick et al. (1982)

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

[a] From five contemporary studies using the same diet (Moxon, 2005a).

[b] Based on a laboratory scale of 1–4 for ossification (1 = good; 4 = poor).

A subsequent developmental range-finding study was intended to investigate the embryo and fetal effects seen during the first study. Female Han Wistar rats (five per group) were administered flutriafol (purity 95.1%; batch No. UPL BX 1) by gavage at 0 (corn oil), 2, 5 or 100 mg/kg bw per day from gestation days 6 to 20. The dams were sacrificed on gestation day 21, and the fetuses were removed by caesarean section. External and skeletal examinations were performed for all fetuses in all dose groups.

At 100 mg/kg bw per day, there were significant reductions (~15%) in maternal body weight gain during the dosing period. There were also increases in post-implantation losses and fetal resorptions, with an associated reduction in litter size (9.2 versus 11.6 in controls). Fetuses from this group had increased incidences of skeletal anomalies, including absence of hyoid body, interrupted hyoid body, bent hyoid bodies, zygomatic arch fusions, pelvic girdle displacements, extra ribs and variations in the cervical ribs. One fetus had a cleft palate.

There were no treatment-related findings at 2 or 5 mg/kg bw per day. A higher rate of post-implantation loss at 5 mg/kg bw per day is not considered biologically relevant, as the mean number of live fetuses per litter was greater than in controls.

The maternal and fetal NOAEL for this limited study is 5 mg/kg bw per day. Cleft palate is a rare finding in rats, and its presence at low incidences in both of the preliminary developmental studies indicates, together with other fetal anomalies, that flutriafol is considered teratogenic at maternally toxic doses (Gerspach, 2008c).

Presumed pregnant female Han Wistar rats (22 per group) were orally administered flutriafol (purity 95.1%; batch No. UPL BX 1) by gavage at 0 (corn oil), 2, 5, 10 or 75 mg/kg bw per day from gestation days 6 to 20. Regular assessments were performed for clinical signs, body weight and feed consumption. The dams were sacrificed on gestation day 21, and the fetuses were removed by caesarean section and examined externally. Approximately half of the fetuses were examined for visceral and 50% for skeletal (alizarin red and alcian blue staining) effects. Dams were subjected to a gross necropsy examination. An expert peer review was performed on the hyoid findings.

Achieved doses were confirmed by analysis as satisfactory. No mortality or clinical signs of toxicity were noted in the dams. Test item–related effects were apparent only at 75 mg/kg bw per day. There was a reduction in initial body weight gain and in net body weight gain (minus uterine contents) (Table 24). Post-implantation loss was increased in the top-dose group (Table 24). At external examination, one control fetus had micrognathia and one top-dose fetus had a cleft palate. There were two treatment-related effects evident at visceral examination of top-dose animals: misshapen nasopharynx (three fetuses in three litters) and displaced origin of the common carotid artery (four fetuses in four litters). Increased incidences of several abnormalities and variations in the skeleton (particularly the hyoid) occurred at the top dose level (Table 24).

No treatment-related skeletal findings were observed at 2, 5 or 10 mg/kg bw per day. The high incidence of rudimentary supernumerary ribs at 5 and 10 mg/kg bw per day is within the contemporary historical control range (23–44% of fetuses). Sporadic increases in skeletal findings such as zygomatic arch fusion and pelvic girdle displacement exhibited no dose–response relationship and are not considered to be treatment related (Table 24).

The pathology peer review changed some of the descriptions of the findings but did not alter the conclusion that at 75 mg/kg bw per day, flutriafol produces a high incidence of skeletal abnormalities, particularly of the hyoid. The presence of cleft palate, together with other anomalies (e.g. of the hyoid), indicates that flutriafol is considered teratogenic at maternally toxic doses.

The maternal and developmental NOAEL is 10 mg/kg bw per day, based on reduced body weight gain and extensive skeletal effects at 75 mg/kg bw per day (Gerspach, 2008d; T. Kvieholm, personal communication, 2011).

Table 24. Findings in pregnant rats dosed with flutriafol

Finding	Fetus or litter	Dose (mg/kg bw per day)				
		0	2	5	10	75
Body weight gain, days 6–8 (g)		5	5	5	5	3**
Body weight gain, days 6–16 (g)		22	21	21	22	18**
Net weight gain, days 0–21 (g)		26	28	27	27	18**
Mean litter size		13.1	11.6	12.1	13.5	11.7
Post-implantation loss (%)		4.3	3.6	4.7	1.7	12.8**
Fetal resorptions (%)		0.3	0.8	0	0	7.1**
External examination (fetuses)		*288*	*244*	*266*	*296*	*258*
Cleft palate		0	0	0	0	1
Micrognathia		1	0	0	0	0
Skeletal examination (fetuses)		*139*	*117*	*129*	*142*	*123*
Hyoid body interrupted	Fetus	0	0	0	0	9**
	Litter	0	0	0	0	4*
Hyoid body bent	Fetus	0	0	0	0	2
	Litter	0	0	0	0	2
Hyoid body accentuated curvature	Fetus	0	0	0	0	42**
	Litter	0	0	0	0	16**
Squamosal or zygomatic process of maxilla, additional ossification	Fetus	0	0	0	0	10**
	Litter	0	0	0	0	7**
Zygomatic arch fusion	Fetus	4	0	7	1	61**
	Litter	3	0	6	1	22**
Maxilla or mandible blue-stained foci	Fetus	0	0	0	0	59**
	Litter	0	0	0	0	18**
Cervical rib long	Fetus	0	0	0	0	9*
	Litter	0	0	0	0	4
Cervical rib rudimentary	Fetus	0	2	2	1	36**
	Litter	0	2	2	1	17**
Pelvic girdle caudal displacement	Fetus	2	1	12	3	16*
	Litter	2	1	5	1	11
Incompletely ossified sternebra 6	Fetus	0	2	0	2	14*
	Litter	0	2	0	2	8*
Supernumerary ribs, left	Fetus	1	1	5	3	15**
	Litter	1	1	2	1	10**
Supernumerary rudimentary ribs, left	Fetus	34	35	48**	50**	94**
	Litter	12	14	19**	17	22**
Supernumerary ribs, right	Fetus	2	1	4	3	13**
	Litter	2	1	3	2	9**
Supernumerary rudimentary ribs, right	Fetus	31	30	41	47**	96**
	Litter	14	15	18	17	22**
Supernumerary rudimentary ribs, total	Fetus (%)	34 (24)	35 (30)	48 (37)**	50 (35)**	96 (78)**
	Litter	16	16	20	18	22*
Non-ossified proximal phalanges of toes						
- toe 2 proximal phalanx, left	Fetus	4	3	6	0	23**
	Litter	3	2	5	0	13**

Table 24 (continued)

Finding	Fetus or litter	Dose (mg/kg bw per day)				
		0	2	5	10	75
- toe 3 proximal phalanx, left	Fetus	4	1	4	0	22**
	Litter	3	1	3	0	12**
- toe 4 proximal phalanx, left	Fetus	4	2	4	0	21**
	Litter	3	1	3	0	11**
- toe 2 proximal phalanx, right	Fetus	4	3	5	0	23**
	Litter	3	2	4	0	13**
- toe 3 proximal phalanx, right	Fetus	4	2	4	0	23**
	Litter	3	1	3	0	12**
- toe 4 proximal phalanx, right	Fetus	4	2	4	0	21**
	Litter	3	1	3	0	11**

From Gerspach (2008d); T. Kvieholm, personal communication, 2011
* $P < 0.05$; ** $P < 0.005$

Rabbits

Mated female Dutch White rabbits were dosed orally (by capsule) with flutriafol (purity 93%; batch No. P10) at a dose level of 0, 2.5, 7.5 or 15 mg/kg bw per day on days 6–18 of gestation. Dams were observed daily for clinical signs; body weights were measured daily throughout the dosing period and at termination. Animals were sacrificed on day 29, and the uterine contents were investigated. All fetuses were examined for external findings, visceral malformations by dissection and skeletal findings following alizarin red staining.

Two top-dose dams were sacrificed during the study, one as a result of the presence of a large scab on the chin and thorax and one owing to "considerable" weight loss following the refusal of food and water. Gross necropsy indicated that the deaths were not directly attributable to treatment. One 7.5 mg/kg bw per day dam was sacrificed after aborting part of its litter. Treatment-related clinical signs were limited to a slightly increased incidence of loose faeces at the top dose level. Weight gains at the top dose level were lower than those of controls, significantly during the treatment phase and the post-treatment phase. No significant effects were seen on body weights. Feed consumption at the top dose level was also significantly lower during the treatment and post-treatment phases.

Mean corpora lutea and implantation numbers were comparable in all groups. Post-implantation loss (both early and late embryonic deaths) was significantly increased at the top dose level only, resulting in a significantly lower number of viable fetuses in this group. Findings are attributable to the significantly increased incidence of total resorptions (seen in five dams) in this group. Gravid uterus weight and litter weights were unaffected by treatment; mean fetal weight was slightly (but not significantly) lower at the top dose level (Table 25).

The total incidences of major external/visceral and skeletal fetal defects were slightly higher at 7.5 and 15 mg/kg bw per day; these findings are due to single fetuses with multiple findings. At the top dose level, the multiple malformed fetus is present in a litter with a significant level of resorptions, and together these are considered to be evidence of fetotoxicity and possible teratogenicity. A number of minor external/visceral and skeletal findings were also noted in these pups. Slightly increased incidences of minor skeletal parameters indicative of reduced ossification were noted at the top dose level, although values did not attain statistical significance. The incidence of extra (bilateral normal/short) lumbar ribs was also higher in this group; however, the total incidence of extra lumbar ribs was comparable in all treated groups and within the normal range for this strain of rabbit (13–50%; Moxon, 2005b). Mean scores for manus and pes ossification were slightly (but not significantly) higher at the top dose level, indicating marginally delayed or reduced ossification (Table 26).

Table 25. Maternal findings and litter parameters in rabbits administered flutriafol

Parameter / time point	Dose (mg/kg bw per day)			
	0	2.5	7.5	15
Maternal findings				
Body weight (g) [% of control]				
- day 0	2109	2143 [102%]	2172 [103%]	2145 [102%]
- day 6	2133	2207 [103%]	2220 [104%]	2221 [104%]
- day 19	2181	2240 [103%]	2254 [103%]	2142 [98%]
- day 29	2339	2427 [104%]	2416 [103%]	2294 [98%]
Weight gain (g) [% of control]				
- days 0–6	29.1	31.1 [107%]	30.8 [106%]	31.7 [109%]
- days 6–9[a]	6.4	10.4 [163%]	6.0 [93.8%]	3.0 [46.9%]
- days 9–12[a]	14.0	14.4 [101.4%]	17.0 [121.4%]	13.3 [95.0%]
- days 12–15[a]	21.2	19.0 [89.6%[	18.4 [86.8%]	14.5 [68.4%]
- days 6–15	41.6	43.6 [105%]	41.4 [100%]	30.8* [74.0%]
- days 15–21	89.2	86.1 [96.5%]	85.4 [95.7%]	60.2* [67.5%]
- days 6–21[a]	130.8	129.7 [99.2%]	126.8 [96.9%]	91.0 [69.6%]
- days 0–21[a]	159.9	160.8 [101%]	157.6 [98.6%]	122.7 [76.7%]
Feed consumption (g) [% of control]				
- days 0–6	24.2	24.5 [101%]	24.6 [102%]	24.8 [102%]
- days 6–15	22.6	22.4 [99.1%]	22.2 [98.2%]	18.8** [83.2%]
- days 15–21	35.5	35.0 [98.6%]	35.4 [99.7%]	30.6** [86.2%]
Mated (no.)	18	18	18	19
Not pregnant (no.)	2	2	1	3
Deaths (no.)	1	0	1	2
Aborted (no.)	0	0	1	0
Total resorptions (no.)	0	1	0	5*
Litters with viable fetuses (no.)	15	15	15	9
Litter parameters				
Corpora lutea (no.)	9.0	8.5	7.9	9.9
Implantations (no.)	7.1	7.0	6.4	8.1
Pre-implantation loss (%)	20.9	13.4	17.5	8.7
- litters affected	11/15	12/15	8/15	5/11
Post-implantation loss (%)	13.1	13.8	5.8	45.5*
- litters affected	6/15	8/16	4/15	8/14
- excluding total resorptions	13.1	8.0	5.8	15.3
Early embryonic death (%)	10.4	7.1	5.0	31.0
- litters affected	5/15	6/16	4/15	6/14
Late embryonic death (%)	1.0	2.7	1.0	16.4
- litters affected	1/15	2/16	1/15	3/14
Viable fetuses (no.)	6.5	6.3	6.0	4.0*
- excluding total resorptions	6.3	6.5	6.0	6.7
Gravid uterus weight (g)	285	316	286	307

Table 25 (continued)

Parameter / time point	Dose (mg/kg bw per day)			
	0	2.5	7.5	15
Litter weight (g)	208	224	208	223
Fetal weight (g)	34.0	34.8	35.6	32.0

From Kilmartin & Godley (1982)
* $P < 0.05$; ** $P < 0.01$ (*t*-test)
[a] Statistical analysis not performed.

Table 26. Fetal findings and litter parameters in rabbits administered flutriafol

Parameter	Dose (mg/kg bw per day)			
	0	2.5	7.5	15
Fetal incidence (%)				
Minor external and visceral defects	5 (5.3%)	6 (5.9%)	4 (4.3%)	4 (6.6%)
Major external and visceral defects	0	0	1 (1.1%)[a]	1 (1.6%)[b]
Minor skeletal defects	27 (28.7%)	23 (22.5%)	30 (31.9%)	26 (42.6%)
Major skeletal defects	0	0	1 (1.1%)[a]	1 (1.6%)[b]
Skeletal findings (%)				
Frontals: partially ossified	2 (2.1%)	2 (2.0%)	6 (6.4%)	8 (13.1%)
Parietals: partially ossified	6 (6.4%)	4 (3.9%)	3 (3.2%)	4 (6.5%)
Interparietals				
- partially ossified	1 (1.1%)	2 (2.0%)	0	4 (6.5%)
- not ossified	0	0	0	2 (3.3%)
Occipitals: partially ossified	2 (2.1%)	0	0	2 (3.3%)
7th lumbar vertebra: not ossified	0	0	0	1 (1.6%)
Extra lumbar ribs				
- unilateral short	4 (4.2%)	9 (8.8%)	4 (4.2%)	6 (9.8%)
- unilateral normal	3 (3.2%)	4 (3.9%)	1 (1.1%)	1 (1.6%)
- bilateral short	1 (1.1%)	4 (3.9%)	4 (4.2%)	3 (4.9%)
- bilateral normal	3 (3.2%)	10 (9.8%)	14 (14.9%)	9 (14.7%)
- bilateral normal/short	2 (2.1%)	2 (2.0%)	4 (4.2%)	7 (11.5%)
- total	13 (13.8%)	29 (28.4%)	27 (28.7%)	26 (42.6%)
Mean manus score	1.88	1.88	1.77	1.95
Mean pes score	1.88	1.91	1.75	1.95

From Kilmartin & Godley (1982)
[a] Single fetus with encephalocoele, shortened jaw, cleft palate, microphthalmia, gastroschisis, clubbed foot and malformed skull; the remainder were normal.
[b] Single fetus with encephalocoele, shortened jaw, gastroschisis, clubbed foot and malformed skull; the remainder were totally resorbed.

A maternal NOAEL of 7.5 mg/kg bw per day can be determined, based on the clinical signs and body weight effects at the top dose level. A developmental NOAEL of 7.5 mg/kg bw per day can also be determined, based on the significantly increased post-implantation loss, significantly lower number of viable fetuses and slightly increased incidences of a number of fetal skeletal parameters indicative of reduced or retarded ossification at 15 mg/kg bw per day (Kilmartin & Godley, 1982).

2.6 Special studies

(a) Acute neurotoxicity

Crl:CD(SD) rats (10 of each sex per group) received flutriafol (purity 95.1%, batch No. BX-1) at a single oral gavage dose of 0, 125, 250 or 750 mg/kg bw using a corn oil vehicle. Dose levels were based on a range-finding study, which reported adverse clinical signs in the 1st hour after dosing at 500 mg/kg bw and above, with reduced body weight gain evident at 125 mg/kg bw and above. Regular, routine observations included clinical signs and appearance. An extensive FOB was performed pre-test and on days 1 (8 hours), 8 and 15; motor activity was monitored over a 90-minute session. At sacrifice on day 16, animals were perfused with 10% formalin. Brain and spinal cord were removed. Peripheral nerve samples were prepared from five rats of each sex per group. A wide range of brain, spinal cord, peripheral nerve and skeletal muscle sections were prepared and stained using haematoxylin and eosin (H&E), toluidine blue, luxol fast blue, crystal violet or Bielschovski's silver stain, singly or in combination.

Achieved doses were confirmed by analysis. At 750 mg/kg bw, four males and two females showed severe signs of toxicity and were sacrificed on day 2 or 3. There were no reports of clinical signs at 250 mg/kg bw. Body weight, particularly in males, was reduced over the first few days post-dosing at all dose levels, possibly secondary to reduced feed consumption (Table 27). At day 14, body weights were similar in all groups other than 750 mg/kg bw males. FOB and motor activity findings on day 1 were limited to the 750 mg/kg bw group, which had ataxia, ptosis, gait abnormalities and reductions in amount of movement and time spent moving. At days 7 and 14, there were alterations in urination pattern and slow breathing in males at 250 and 750 mg/kg bw; these findings are equivocal, as they were not evident on day 1 or in the repeated-dose neurotoxicity study (see below) at a dose of 172 mg/kg bw per day, but showed consistency and are considered potentially relevant to treatment with flutriafol. Variations in grip strength were within normal ranges. There were no significant findings at necropsy. A reduction in absolute brain weight at 750 mg/kg bw was considered not to be of biological relevance, as relative brain weights were unaltered. There were no indications of overt neurotoxicity or damage to the central or peripheral nervous system.

The NOAEL for neuropathy was 750 mg/kg bw, the highest dose tested. The NOAEL for neurotoxicity was 250 mg/kg bw, based on altered FOB and motor activity at 750 mg/kg bw. A NOAEL for general toxicity was not determined, as transient reductions in body weight and feed consumption were seen in all dose groups at the start of the study (Barnett, 2006a).

(b) Repeated-dose neurotoxicity

In a range-finding repeated-dose neurotoxicity study, Crl:CD(SD) rats (five of each sex per dose group) received flutriafol (purity 95.1%, batch No. BX-1) in the diet at a concentration of 0, 1000, 2000 or 4000 ppm ad libitum for 29 days. On day 29, a 1-minute open-field examination was performed. At 4000 ppm (~85 mg/kg bw per day), there was initial body weight loss and reduced feed consumption. At 2000 ppm (~170 mg/kg bw per day), there was a transient reduction in feed consumption. There were no treatment-related clinical signs or effects on the results of the open-field examination (Barnett, 2006b).

In the main study, Crl:CD(SD) rats (10 of each sex per dose group) received flutriafol (purity 95.1%, batch No. BX-1) in the diet at a concentration of 0, 500, 1500 or 3000 ppm ad libitum for 13 weeks. FOB and motor activity (1.5-hour session split into 5-minute segments) evaluations were conducted on all rats before the 1st day of exposure and during weeks 2, 4, 8 and 13 of the exposure period. At sacrifice, animals were perfused with 10% formalin. Brain and spinal cord were removed. Peripheral nerve samples were prepared from five rats of each sex per group. A range of brain (six regions), spinal cord (three levels), peripheral nerve and skeletal muscle sections were prepared and

Table 27. Body weight and feed consumption in rats receiving flutriafol

	Dose (mg/kg bw)							
	0		125		250		750	
	Males	Females	Males	Females	Males	Females	Males	Females
Body weight (g)								
- day 1	252	186	253	187	254	182	254	187
- day 2	256	187	248	187	242	177	225*	177
- day 3	266	194	261	196	257	190	221*	181
- day 7	295	210	292	210	291	204	257*	208
Body weight gain (g)								
- days 1–2	3.9	1.5	−5.0*	−0.1	−12*	−4.2*	−29*	−10*
Feed consumption (g/day)								
- days 1–2	19	12	11*	9	7*	7*	2*	5*
- days 1–8	25	19	24	19	24	18	15*	17

From Barnett (2006a)
* $P < 0.05$

stained using H&E, toluidine blue, luxol fast blue, crystal violet or Bielschovski's silver stain, singly or in combination.

Achieved doses and homogeneity were confirmed by analysis; mean doses were 0, 29, 84 and 172 mg/kg bw per day in males and 0, 33, 98 and 185 mg/kg bw per day in females. There were no deaths or clinical signs of toxicity or any abnormal findings at necropsy. Significant reductions in body weight gain and feed consumption were seen at 1500 and 3000 ppm, with transient body weight loss at 3000 ppm. FOB and motor activity results were similar across all groups. Slight reductions in hindlimb grip strength at 3000 ppm appeared to be related to the lower body weight. There were no indications of damage to the central or peripheral nervous system.

The NOAEL for neuropathy and neurotoxicity was 3000 ppm (equal to 172 mg/kg bw per day), the highest dose tested. The NOAEL for general toxicity was 500 ppm (equal to 29 mg/kg bw per day), based on reductions in body weight gain at the start of the study at 1500 ppm (equal to 84 mg/kg bw per day (Barnett, 2007b).

(c) Immunotoxicity

Female CD-1 mice (10 per group) received flutriafol (purity 95.1%; batch No. UPL BX1) in the diet for 28 days at 0, 50, 250, 500 or 1000 ppm. A positive control group received cyclophosphamide (50 mg/kg bw per day) intraperitoneally on days 25–28. Mice were observed routinely for clinical signs, feed consumption, body weight and mortality. Four days prior to sacrifice, mice received an intravenous injection (tail vein) of sheep red blood cells to investigate immunoglobulin M (IgM)–mediated response to antibody. At sacrifice, a blood sample was taken for haematological investigation; liver, spleen and thymus were removed from all animals and weighed. The spleen was examined for immunological response, and the livers from negative control and top-dose groups were examined histopathologically.

Achieved mean doses were 0, 9.8, 47, 94 and 208 mg/kg bw per day. There were no deaths, clinical signs or alterations in feed consumption. Transient body weight loss was seen at and above 500 ppm (Table 28). Absolute and relative liver weights were increased at and above 250 ppm, with periacinar hypertrophy and vacuolation present in all livers examined histopathologically (1000 ppm group only). Altered erythrocyte parameters were found at and above 250 ppm, progressing with

Table 28. Effects in female mice exposed to flutriafol in an immunotoxicity study

	Dietary concentration (ppm)					
	0	50	250	500	1000	CPA[a]
Body weight gain, days 1–4 (g)	0.6	0.4	0.5	−0.5	−1.7	—
Relative liver weight (%)	5.5	5.6	7.7*	10.8*	16.5*	5.4
Relative spleen weight (%)	0.46	0.45	0.41	0.46	0.49	0.24*
Relative thymus weight (%)	0.20	0.23	0.19	0.21	0.19	0.08*
Platelets (10^3/mm^3)	925	1250*	1130	1283*	1264*	—
White blood cells (10^3/mm^3)	6.8	7.3	8.9	11.0*	13.4*	—
IgM response (antibody-forming cells/10^6 spleen cells)	1626	1566	1309	1450	2000	0

From Barnett (2011)
CPA, cyclophosphamide; IgM, immunoglobulin M; * $P < 0.05$
[a] 50 mg/kg bw per day intraperitoneally.

administered dose. Increases in white blood cell counts were seen in all groups, primarily due to increases in lymphocyte and segmented neutrophils, achieving statistical significance at and above 500 ppm. Platelet counts were higher in all treated groups, but showed no dose–response relationship and were consistent with the control range ($407 \times 10^3 – 1491 \times 10^3$/mm^3). There were no effects on spleen or thymus weights or on the measured immunological response (Table 28). The positive control results confirmed the viability of the test system.

The NOAEL for general toxicity (within the limited investigations in the study) is 50 ppm (equal to 9.8 mg/kg bw per day), based on haematological changes and hepatotoxicity at 250 ppm (equal to 47 mg/kg bw per day). Flutriafol was not immunotoxic in this study, with a NOAEL of 1000 ppm (equal to 208 mg/kg bw per day) (Barnett, 2011).

3. Observations in humans

No reports of intentional or accidental poisoning with flutriafol have been found in the published literature. Health monitoring of production plant workers has not identified any cases of illness associated with flutriafol (Denman, 1994; McNie, 2002; I.M. Jensen, personal communication, 2011).

Comments

Biochemical aspects

Flutriafol is rapidly and extensively absorbed following oral administration to the rat at a dose level of 5 or 250 mg/kg bw in PEG600. Based on values for urinary and biliary excretion, oral absorption is greater than 90%. Flutriafol and/or its metabolites are widely distributed, with highest levels of radioactivity associated with red blood cells. Data from studies with repeated administration indicate that there is unlikely to be any bioaccumulation. Flutriafol is extensively metabolized in the rat, with only trace levels of unchanged parent detected in excreta following oral administration. The initial stage of metabolism is oxidation of the 2-fluorophenyl ring, followed by conjugation. Excretion was predominantly within 24 hours at 5 mg/kg bw, with similar amounts of radiolabel present in the urine and faeces. Biliary excretion was extensive (~80%), with evidence for enterohepatic circulation.

Toxicological data

Flutriafol has acute oral LD_{50} values of 1140–1480 mg/kg bw in the rat. Acute oral toxicity was higher in the mouse, rabbit and guinea-pig (LD_{50}s 179–400 mg/kg bw). Lower acute toxicity

was seen via the dermal (LD_{50} > 2000 mg/kg bw in rabbits) and inhalation (LC_{50} > 5.2 mg/l) routes. Flutriafol was not irritating to rat or rabbit skin but was a mild irritant to rabbit eyes; all ocular effects had resolved within 72 hours. Flutriafol did not produce any evidence of skin sensitization in a Magnusson and Kligman assay in guinea-pigs or in a local lymph node assay in mice.

The red blood cell and liver were identified as targets of flutriafol toxicity in short-term oral toxicity studies in the rat, mouse and dog. Increases in hepatocyte hypertrophy and liver weight with no other effects were considered to be adaptive and not treated as adverse effects; other histopathological findings in the liver, such as fatty vacuolation or necrosis, were treated as adverse. Reduced body weights, weight gains and/or feed consumption were also seen at high dose levels in all three species. Effects on haematological parameters consistent with mild microcytic anaemia were seen in the rat and mouse; such effects were less marked in the dog. In a 29-day study, the majority of mice exposed at 1500 ppm died. Increased liver weights and hepatocytic vacuolation were seen at intermediate doses. Hepatocytic lipid accumulation was seen at all dose levels; the lowest-observed-adverse-effect level (LOAEL) was 50 ppm (equivalent to 7.5 mg/kg bw per day). Hepatotoxicity in the rat at high dose levels was characterized by the disruption of lipid metabolism, increased liver weight, altered pigmentation and granulation, centrilobular hepatocyte hypertrophy and vacuolation, fatty change, focal necrosis and hydropic degeneration. At lower dose levels, findings of increased liver weight, centrilobular hepatocyte hypertrophy, elevated hepatic aminopyrine *N*-demethylase activity and proliferation of the smooth endoplasmic reticulum are consistent with an adaptive effect. The NOAEL in the 90-day rat study was 200 ppm (equal to 13 mg/kg bw per day). In the dog, hepatotoxicity was characterized by elevated serum alkaline phosphatase activity, perturbation of lipid metabolism, increased liver weight and (in the 1-year study) granular, swollen liver, hepatocytic vacuolation and lipid accumulation. Initial reductions in body weight and body weight gain with no associated reductions in feed consumption were seen at dose levels above 5 mg/kg bw per day. The NOAELs for the 90-day and 1-year dog studies were both 5 mg/kg bw per day.

In a 28-day dermal toxicity study in rats, there were no systemic effects; the NOAEL was 1000 mg/kg bw per day. The NOAEL for local effects was 250 mg/kg bw per day, based on erythema and flaking of the skin at the application site at 500 mg/kg bw per day.

The liver was identified as the target organ of flutriafol toxicity following chronic administration to the rat and mouse. Hepatotoxicity in the 2-year mouse study was characterized by increased liver weight and fatty change; the incidence of liver adenoma was marginally increased in males at the top dose level, but was within the historical control range. The NOAEL in the 2-year mouse study was 10 ppm (equal to 1.2 mg/kg bw per day), based on centrilobular fatty changes in the liver at 50 ppm (equal to 6 mg/kg bw per day). Flutriafol was not carcinogenic in mice.

Toxicity in the chronic rat study was characterized by clinical chemistry findings (reduced serum triglycerides and ALP activity and increased ALT activity in males; increased serum cholesterol in females) at 2000 ppm and increased liver weight and fatty change at 200 ppm and above. Incidences of liver adenoma and carcinoma were slightly increased at the top dose level, but were within historical control ranges. Evidence of anaemia was seen at the high dose level; effects on red blood cell parameters were accompanied in females by reduced serum iron concentration, elevated total iron binding capacity and haemosiderin accumulation in the spleen and liver. The NOAEL in the 2-year rat study was 20 ppm (equal to 1.0 mg/kg bw per day), based on increased fatty change and weight of the liver in males at 200 ppm (equal to 10 mg/kg bw per day). Flutriafol was not carcinogenic in rats.

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

Flutriafol has been tested for genotoxicity in an adequate range of in vitro and in vivo studies. Equivocal results were seen in two reverse mutation assays with mouse lymphoma L5178Y cells. The remaining in vitro and all the in vivo tests were negative.

The Meeting concluded that flutriafol is unlikely to be genotoxic.

Based on the absence of genotoxicity and absence of treatment-related carcinogenicity in mice and rats, the Meeting concluded that flutriafol is unlikely to be carcinogenic in humans.

Two reproductive toxicity studies in rats are available. Evidence of reproductive toxicity was seen in the first two-generation rat study; mean litter size was lower at the top dose level of 1000 ppm, and there was a reduced fertility index at the mating for the F_{1A} litter. The NOAEL for reproductive toxicity was 240 ppm (equal to 14 mg/kg bw per day). Minor maternal body weight effects and hepatotoxicity (fatty change) were seen at 240 ppm in both sexes. The NOAEL for parental toxicity was 60 ppm (equal to 3.5 mg/kg bw per day). Fatty changes in the liver were also seen in pups at the top dose level. The NOAEL for offspring toxicity was 240 ppm (equal to 14 mg/kg bw per day). Similar findings were seen in the range-finding component of the more recent rat reproductive toxicity study. Post-implantation losses (13%) were increased at doses causing mild to moderate maternal toxicity in the preliminary reproduction study at 240 ppm (equal to 13 mg/kg bw per day) and above. NOAELs for parental, pup and reproductive effects were 60 ppm (approximately 4 mg/kg bw per day). However, in the main study, there were no effects on reproduction, offspring or parents at the highest dose tested, 300 ppm (equal to 16 mg/kg bw per day). The Meeting concluded that the overall NOAELs from the two main reproductive toxicity studies for reproductive and offspring toxicity were 16 mg/kg bw per day.

There are four rat developmental toxicity studies on flutriafol. In the first guideline-compliant study, effects on the fetal skeleton mainly consistent with delayed ossification were seen at all dose levels; the LOAEL was 10 mg/kg bw per day. At the top dose level of 125 mg/kg bw per day, there were reductions in body weight gain (20–50%), feed consumption (~15%) and litter size (30%), the latter associated with increased post-implantation loss (33%). In both subsequent range-finding studies, there were marked increases in skeletal anomalies (especially of the hyoid) and reduced ossification, as well as single incidences of cleft palate at the top dose levels of each study (150 and 100 mg/kg bw per day). Marked maternal toxicity, clinical signs, body weight deficits and fetal resorptions were seen at the top dose levels. In the second guideline-compliant study, there was a single incidence of cleft palate at the top dose level of 75 mg/kg bw per day, a dose that also produced significantly reduced maternal body weight gain (~30%), increases in post-implantation loss (3-fold), skeletal anomalies (including the hyoid bone and supernumerary ribs) and delayed ossification. The NOAELs in this study for both developmental and maternal toxicity were 10 mg/kg bw per day.

Cleft palate is a very rare finding in rats that has been seen at high dose levels with a number of triazole compounds. The presence of single incidences of cleft palate in three of the rat developmental toxicity studies, although not statistically significant in isolation, cannot be discounted when the database is considered as a whole. Litter sizes at the top dose levels were significantly lower as a result of post-implantation loss, which could reduce the number of malformed fetuses observed at caesarean section. The NOAEL for teratogenicity in the rat was 10 mg/kg bw per day, based on the findings of cleft palate at and above 75 mg/kg bw per day.

In a rabbit developmental toxicity study, there was evidence of clinical signs, reduced maternal body weight gain (30%), reduced litter size (40%) and delayed ossification at the highest dose level of 15 mg/kg bw per day. One fetus at the top dose level and one at the intermediate dose level had multiple malformations that could not be unequivocally linked to flutriafol administration. The NOAELs for maternal and developmental toxicity were 7.5 mg/kg bw per day.

The Meeting concluded that flutriafol is teratogenic in rats.

In an acute neurotoxicity study in rats, there was no evidence of neuropathy at 750 mg/kg bw, the highest dose tested. The NOAEL for acute neurotoxicity was 250 mg/kg bw, based on altered FOB and motor activity findings on day 1 at 750 mg/kg bw. The NOAEL for general toxicity was less than 125 mg/kg bw, based on transient reductions in body weight gain in males at all doses.

In a repeated-dose neurotoxicity study, there were no signs of neuropathy or neurotoxicity at 3000 ppm (equal to 172 mg/kg bw per day), the highest dose tested. The NOAEL for general toxicity

was 500 ppm (equal to 29 mg/kg bw per day), based on reductions in body weight gain at the start of the study at 1500 ppm (equal to 84 mg/kg bw per day) and above.

In a 28-day immunotoxicity study in female mice, there was no reduction in the IgM response to challenge with sheep red blood cells or changes in spleen or thymus weights. The NOAEL for immunotoxicity was 1000 ppm (equal to 208 mg/kg bw per day), the highest dose tested. The NOAEL for general toxicity was 50 ppm (equal to 9.8 mg/kg bw per day), based on reduced erythrocyte mean cell volume and hepatotoxicity at 250 ppm (equal to 47 mg/kg bw per day).

Medical monitoring of production plant workers has not identified any cases of occupational illness related to flutriafol.

The Meeting concluded that the available database on flutriafol is adequate to characterize the potential risk to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.01 mg/kg bw on the basis of the NOAEL of 1.0 mg/kg bw per day in the 2-year rat study, based on increases in fatty changes and increased weights of the liver in males at 10 mg/kg bw per day. A safety factor of 100 was applied. The ADI is supported by the NOAEL in the carcinogenicity study in mice of 1.2 mg/kg bw per day, based on the increased incidence and severity of hepatic centrilobular fatty change in males at 6 mg/kg bw per day.

The Meeting established an acute reference dose (ARfD) of 0.05 mg/kg bw on the basis of the NOAEL of 5 mg/kg bw per day in the 90-day and 1-year toxicity studies in dogs based on reduced body weight gain (males) or body weight loss (females) after 1 week (the first time of measurement) and subsequently reduced body weight gain during the early part of the study, although feed consumption was unaffected by treatment. A safety factor of 100 was applied. This provides a margin of greater than 1000 between the ARfD and the LOAEL for cleft palate in rats (75 mg/kg bw per day).

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	10 ppm, equal to 1.2 mg/kg bw per day	50 ppm, equal to 6 mg/kg bw per day
		Carcinogenicity	200 ppm, equal to 25 mg/kg bw per day[b]	—
Rat	Ninety-day study of toxicity[a]	Toxicity	200 ppm, equal to 13 mg/kg bw per day	2000 ppm, equal to 148 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	20 ppm, equal to 1.0 mg/kg bw per day	200 ppm, equal to 10 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 103 mg/kg bw per day[b]	—
	Multigeneration studies of reproductive toxicity[a,c]	Reproductive toxicity	300 ppm, equal to 16 mg/kg bw per day	1000 ppm, equal to 56 mg/kg bw per day
		Parental toxicity	60 ppm, equal to 3.5 mg/kg bw per day	240 ppm, equal to 14 mg/kg bw per day
		Offspring toxicity	300 ppm, equal to 16 mg/kg bw per day	1000 ppm, equal to 56 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	10 mg/kg bw per day	75 mg/kg bw per day
		Embryo and fetal toxicity	10 mg/kg bw per day	75 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
Rabbit	Developmental toxicity study[d]	Maternal toxicity	7.5 mg/kg bw per day	15 mg/kg bw per day
		Embryo and fetal toxicity	7.5 mg/kg bw per day	15 mg/kg bw per day
Dog	Ninety-day and 1-year studies of toxicity[c,e]	Toxicity	5 mg/kg bw per day	15 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

0.05 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and extensive absorption: > 90% based on urinary and biliary excretion
Dermal absorption, rat (125 g/l suspension concentrate formulation)	~1% concentrate; ~10% in use dilutions; 8 h exposure and 9-day monitoring
Distribution	Widely distributed; highest levels in red blood cells
Potential for accumulation	No evidence for accumulation
Rate and extent of excretion	Rapid, equally in urine and faeces; extensive biliary excretion (~80%) with enterohepatic circulation
Metabolism in animals	Extensive metabolism; only trace amount of unchanged parent detected
Toxicologically significant compounds (animals, plants and the environment)	Flutriafol

Acute toxicity	
Rat, LD_{50}, oral	1140–1480 mg/kg bw
Rabbit, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.2 mg/l
Rabbit, dermal irritation	Non-irritant
Rabbit, ocular irritation	Mild irritant
Guinea-pig, dermal sensitization (Magnusson and Kligman)	No evidence

Mouse, dermal sensitization (local lymph node assay)	No evidence

Short-term studies of toxicity

Target/critical effect	Body weights (from start of dosing); red blood cells (anaemia) and liver (weight, clinical chemistry, fatty change)
Lowest relevant oral NOAEL	5 mg/kg bw per day (90-day and 1-year dog studies)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (systemic; rats)
Lowest relevant inhalation NOAEC	No data

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Liver (increased weight and fatty change)
Lowest relevant NOAEL	1.0 mg/kg bw per day (rat); 1.2 mg/kg bw per day (mouse)
Carcinogenicity	Not carcinogenic

Genotoxicity

	Some equivocal in vitro results; negative in vivo; considered unlikely to be genotoxic

Reproductive toxicity

Reproduction target/critical effect	Reduced litter size
Lowest relevant reproductive NOAEL	16 mg/kg bw per day
Developmental target/critical effect	Reduced litter size, delayed ossification (rat and rabbit); hyoid abnormalities (rat); cleft palate (rat)
Lowest relevant developmental NOAEL	7.5 mg/kg bw per day (rabbit); 10 mg/kg bw per day (rat)

Neurotoxicity/delayed neurotoxicity

Acute study	Altered FOB and motor activity; neurotoxicity NOAEL 250 mg/kg bw (rat)
Ninety-day study	Not neurotoxic

Other toxicological studies

Immunotoxicity	Not immunotoxic (female mice)

Medical data

	No adverse effects reported in production plant operators

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Two-year rat study (supported by 2-year mouse study)	100
ARfD	0.05 mg/kg bw	Ninety-day and 1-year studies in dogs	100

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