ETHIPROLE

First draft prepared by
Marloes Busschers[1] and Jürg Zarn[2]

[1] Charles River Laboratories, 's-Hertogenbosch, the Netherlands
[2] Federal Food Safety and Veterinary Office (FSVO), Bern, Switzerland

Explanation....11
Evaluation for acceptable intake....12
1. Biochemical aspects....12
1.1 Absorption, distribution and excretion....12
(a) Main experiment....12
(b) Pharmacokinetics....13
(c) Tissue kinetics....14
(d) Biliary excretion....14
1.2 Biotransformation....15
2. Toxicological studies....15
2.1 Acute toxicity....15
2.2 Short-term studies of toxicity....15
(a) Oral administration....15
(b) Dermal application....25
(c) Exposure by inhalation....25
2.3 Long-term studies of toxicity and carcinogenicity....26
2.4 Genotoxicity....34
2.5 Reproductive and developmental toxicity....34
(a) Multigeneration studies....34
(b) Developmental toxicity....36
2.6 Special studies....39
(a) Acute neurotoxicity....39
(b) Subchronic neurotoxicity....42
(c) Mechanistic studies....43
(d) Immunotoxic effects....44
(e) Studies on metabolites....44
3. Observations in humans....46
Comments....46
Toxicological evaluation....50
References....53

Explanation

Ethiprole (Fig. 1) is the common name approved by the International Organization for Standardization for 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-ethylsulfinylpyrazole-3-carbonitrile (International Union of Pure and Applied Chemistry), with the Chemical Abstracts Service number 181587-01-9.

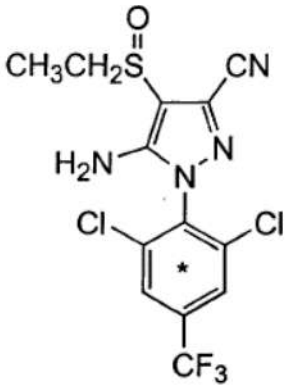
Ethiprole is a non-systemic insecticide of the fiproles (phenylpyrazoles) group. It acts by interfering with the flow of chloride ions through the γ-aminobutyric acid–regulated chloride channel, thereby disrupting the central nervous system activity of insects.

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

All critical studies contained statements of compliance with good laboratory practice (GLP) and were conducted in accordance with relevant national or international test guidelines, unless

otherwise specified. No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Fig. 1. Structure of ethiprole (company codes: RPA 107382 and AE 0316423)



Note: Asterisk (*) represents position of the ^{14}C label.

Evaluation for acceptable intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption, distribution and excretion of ethiprole were studied in rats following the administration of [phenyl-U-^{14}C]ethiprole in a single oral low dose or a single oral high dose, the administration of a single oral low daily dose of non-radiolabelled ethiprole repeated for 14 days followed by administration of a radioactive dose, and in a biliary excretion study. In these studies, a single radiolabelled position was used because there was evidence to show that the molecule cleaved only minimally during the biotransformation process (McCorquodale & Anderson, 1999).

(a) Main experiment

The absorption, distribution and excretion of total radioactivity were determined following a single oral administration of [phenyl-U-^{14}C]ethiprole (batch no. 2CFS94, purity 99.7% [non-radiolabelled material]; and batch no. GRX 485A, purity 99.3% [radiolabelled material]) to two groups of five male and five female Sprague Dawley rats at 5 or 1000 mg/kg body weight (bw). A third group (five males and five females) received a single oral administration of [phenyl-U-^{14}C]ethiprole at 5 mg/kg bw following 14 daily oral administrations of non-radiolabelled ethiprole (5 mg/kg bw). Urine and faeces were collected quantitatively up to 168 hours post-dosing from all animals. The animals were humanely killed at 7 days post-dosing, and selected tissues were retained in order to investigate the tissue residues of [phenyl-U-^{14}C]ethiprole.

At the low dose, results for the cumulative recovery of total radioactivity were consistent with the findings from a pilot experiment. The majority of the dose was recovered within the first 48 hours post-dosing, with a mean of about 86% observed for both males and females. The predominant route of elimination was via faeces, with means of 67% (males) and 55% (females) of the administered dose excreted by this route by 168 hours post-dosing. Urinary excretion accounted for means of 24% and 36% of the administered dose in males and females, respectively. By the end of the 168-hour collection period, the overall mean recoveries in males and females were 92% and 94%, respectively.

Following administration of [phenyl-U-^{14}C]ethiprole to male and female rats at the high dose, excretion of total radioactivity was slower than that observed following administration at the low dose, with mean recoveries of about 77% and 64% observed by 48 hours post-dosing. The major route of elimination was again via faeces, with a mean of about 88% of the dose observed for males and females

after 168 hours. Urinary excretion accounted for means of about 3% and 5% in males and females, respectively. By the end of the 168-hour collection period, the overall mean recoveries in male and female rats were about 92% and 93%, respectively.

Results for the recovery of total radioactivity following the administration of [phenyl-U-^{14}C]ethiprole to male and female rats at the low dose after the repeated oral administration of non-radiolabelled ethiprole were similar to those obtained following the single administration of [phenyl-U-^{14}C]ethiprole at the low dose. The predominant route of elimination was via faeces, with means of about 71% (males) and 56% (females) of the dose excreted by this route. Urinary excretion accounted for means of about 23% and 35% of the dose for males and females, respectively. Excretion of the dose was rapid, with means of about 82% (males) and 86% (females) of the radioactivity recovered within the first 48 hours post-dosing. Mean recoveries of about 94% were observed in both males and females by the end of the 168-hour collection period.

Following the administration of [phenyl-U-^{14}C]ethiprole at both doses, low concentrations of radioactivity were observed in most tissues. The highest concentrations were found in the tissues associated with metabolism (liver and kidney). Concentrations of total radioactivity in whole blood were higher in females than in males both for the single low dose and following repeated administration of non-radiolabelled ethiprole. However, this difference was not evident at the high dose.

(b) Pharmacokinetics

The pharmacokinetics of [phenyl-U-^{14}C]ethiprole was investigated following oral administration. Two groups of five male and five female Sprague Dawley rats received a single oral dose of [phenyl-U-^{14}C]ethiprole at 5 or 1000 mg/kg bw. The levels of total radioactivity were measured in whole blood samples collected at intervals up to 168 hours post-dosing.

At the low dose, concentrations of total radioactivity in whole blood increased rapidly to mean peak concentrations of 2.11 and 1.63 µg equivalents (equiv)/g in males and females, respectively, at 8 hours post-dosing. Mean concentrations declined in a biphasic manner, with concentrations decreasing rapidly to 1.47 µg equiv/g (males) and 1.03 µg equiv/g (females) at 24 hours post-dosing. Thereafter, the rate of decline slowed, with low concentrations of radioactivity in whole blood from males and females (0.09 and 0.17 µg equiv/g, respectively) observed at 168 hours post-dosing.

At the high dose, concentrations of radioactivity in whole blood increased gradually to mean peak concentrations of 41.7 µg equiv/g in males at 24–48 (mean 33.6) hours post-dosing and 29.8 µg equiv/g in females at 48 hours post-dosing. Following each peak, concentrations decreased slowly to means of 2.9 µg equiv/g (males) and 3.0 µg equiv/g (females) at 168 hours post-dosing.

The mean peak concentrations in whole blood, C_{max}, were observed at 8 and 24–48 hours post-dosing (= T_{max}) in both sexes at the low and high doses, respectively. Within dose groups, T_{max} estimates demonstrated minimal variability. Whole blood concentrations of [phenyl-U-^{14}C]ethiprole had mean elimination half-lives ($t_{½\ el}$) of between 44.33 and 49.18 hours, except in low-dose females (113.98 hours), for which interanimal variation was more pronounced. This parameter was not, however, considered to change significantly as a function of dose.

Estimates for C_{max} and both area under the concentration–time curve (AUC) parameters increased with increasing dose of [phenyl-U-^{14}C]ethiprole. The mean $AUC_{(0–t)}$ was 94.88 and 79.38 µg equiv·h/mL for males and females, respectively, at the low dose and 2620 and 2240 µg equiv·h/mL for males and females, respectively, at the high dose. $AUC_{(0–\infty)}$ and C_{max} demonstrated similar trends. This increase was not dose proportional, as a 200-fold increase in dose resulted in fold increases (males and females, respectively) of 28 and 22 for $AUC_{(0–\infty)}$, 28 and 28 for $AUC_{(0–t)}$ and 20 and 18 for C_{max}. There was no consistent effect of dose on $t_{½\ el}$ in this study.

(c) Tissue kinetics

The tissue kinetics of [phenyl-U-^{14}C]ethiprole was investigated in male and female Sprague Dawley rats following a single oral administration of [phenyl-U-^{14}C]ethiprole at 5 or 1000 mg/kg bw. Four male and four female rats were killed at each of 8, 24 and 48 hours post-dosing at the low dose and 48, 72 and 96 hours post-dosing at the high dose, and selected tissues, organs and biological fluids were retained for the determination of total radioactivity.

At the low dose of 5 mg/kg bw, the highest tissue concentrations were observed in glandular tissues (adrenal, pancreas and thyroid) and those associated with metabolism (kidney and liver). High concentrations of radioactivity were also observed in the renal fat. Peak concentrations were observed for all tissues at 8 hours post-dosing (the first sampling time). The mean percentages of dose associated with the liver and kidneys at this time were approximately 12% and 1% (males) and 11% and 1% (females), respectively. Following each peak, concentrations of radioactivity decreased in all tissues.

At the high dose of 1000 mg/kg bw, peak concentrations of radioactivity were observed at 48 hours post-dosing (the first sampling time). The pattern of tissue distribution was similar to that observed at the low dose, with the highest concentrations observed in glandular tissues (adrenal, pancreas and thyroid) and those associated with metabolism (kidney and liver). High concentrations of radioactivity were also observed in the renal fat. The mean percentages of dose associated with the liver and kidneys at this time were approximately 1% and less than 1% for both sexes, respectively.

(d) Biliary excretion

During the bile duct cannulation experiments, two groups of five male and five female Sprague Dawley rats fitted with bile duct cannulae received a single oral administration of [phenyl-U-^{14}C]ethiprole at either 5 or 1000 mg/kg bw. Samples of urine, faeces and bile were collected from each animal at intervals up to 96 hours post-dosing.

At the low dose of 5 mg/kg bw, the major route of excretion was via bile, with means of about 67% (males) and 52% (females) of the dose recovered by 96 hours post-dosing. Excretion via urine and faeces over the same period accounted for, respectively, about 11% and 11% (males) and about 30% and 11% (females) of the administered dose. Absorption of the administered dose was rapid. The low level of excretion via faeces, approximately 11% (both sexes) of the dose, indicated that at least about 89% of an oral dose of [phenyl-U-^{14}C]ethiprole at the low dose was absorbed, with the bulk of this absorbed within 24 hours post-dosing.

By the end of the study period, at 96 hours post-dosing, the mean recovery of total radioactivity from bile, urine, faeces and cage wash accounted for about 92% and about 96% of the dose in males and females, respectively. Recovery of total radioactivity from the remaining carcasses was low, accounting for means of 1.49% and 3.49% of the dose in males and females, respectively, suggesting that low residues remained in the tissues. Recovery in all samples by the end of the study period represented means of 93.9% and 99.1% of the administered dose in males and females, respectively.

At 1000 mg/kg bw, the major route of excretion was via faeces, with means of 86.0% (males) and 79.3% (females) of the dose recovered by 96 hours post-dosing. Excretion via urine and bile over the same period accounted for, respectively, about 1.1% and 8.9% (males) and about 1.5% and 7.0% (females) of the administered dose. Absorption of the administered dose was low. Excretion via faeces of 86.0% (males) and 79.3% (females) of the dose indicated that it was likely that only about 14% (males) and 20% (females) of an oral dose of [phenyl-U-^{14}C]ethiprole at the high dose were absorbed, with the bulk of this absorbed within 48 hours post-dosing. By the end of the study period, at 96 hours post-dosing, the mean recovery of total radioactivity from bile, urine, faeces and cage wash accounted for about 97% of the administered dose for males and about 88% for females. Recovery of total radioactivity from the remaining carcasses was low, accounting for means of 0.36% and 5.78% of the dose in males and females, respectively, suggesting that low residues remained in the tissues. Recovery in all samples by the end of the study period represented means of about 97% and 94% of the administered dose in males and females, respectively (McCorquodale & Anderson, 1999).

1.2 Biotransformation

The biotransformation of ethiprole in rats was investigated in the same study (McCorquodale & Anderson, 1999).

Analysis of the excreted radioactivity indicated that orally administered [phenyl-U-^{14}C]ethiprole was extensively metabolized. The pattern of metabolites in urine was similar in male and female rats following administration of the low dose. The major components were the polar glucuronide conjugate of hydroxy-MB 45897 and the sulfinic acid RPA 104615, as well as the less polar (non-conjugated) MB 45897 and, in female urine, the carboxylic acid RPA 112705. A similar pattern of metabolites was observed after repeated low doses or a single high dose. The excreted radioactive components could be identified to a proportion of approximately 65% or 80% of the administered radioactivity at the low or high dose.

Three primary parallel metabolic pathways could be derived from the metabolites observed, followed by further reactions, including conjugate formation:

1) Hydrolysis of the nitrile group of ethiprole to form the amide RPA 112916.
2) Reduction of the sulfoxide group of ethiprole to form the sulfide RPA 107566. A subsequent alkyl oxidation results in the carboxylic acid RPA 112716.
3) Oxidation of the sulfoxide group of ethiprole to form the major metabolite (i.e. the sulfone RPA 097973), followed by further metabolic reactions:
    - The sulfone RPA 097973 can be alkyl hydroxylated to generate RPA 114345, which is subsequently metabolized by alkyl oxidation to form RPA 112705 and by conjugation with sulfuric acid. Further metabolic steps can follow.
    - An oxidative dealkylation of the sulfone RPA 097973 forms the sulfuric acid RPA 104615. Replacement of the sulfuric acid group by a hydroxy group generates the intermediate hydroxy-MB 45897, which subsequently can be stabilized as the sulfate or glucuronide conjugate.
    - The nitrile group of the sulfone RPA 097973 can be further hydrolysed to the amide (step 1), resulting in RPA 112917.

The proposed metabolic pathway of ethiprole in the rat is shown in Fig. 2.

2. Toxicological studies

2.1 Acute toxicity

The result of an acute toxicity study with ethiprole is summarized in Table 1. No studies were submitted on the acute dermal or inhalation toxicity of ethiprole or on its potential for ocular or dermal irritation or dermal sensitization.

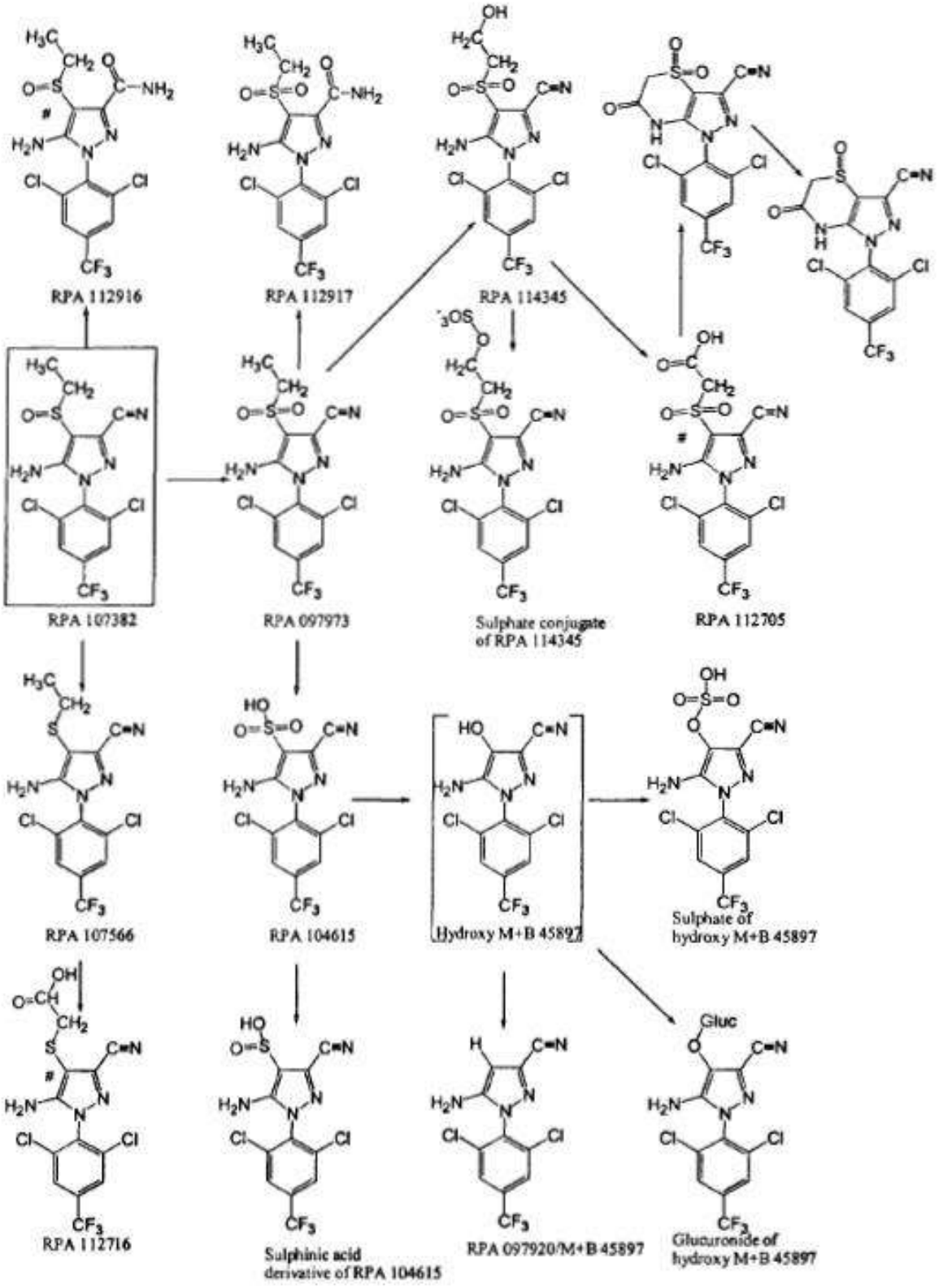
2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Ethiprole (batch no. CDR9706; purity 93.0%) was administered in the diet to groups of 15 male and 15 female C57B1/6N mice for at least 28 days at a dietary concentration of 0, 50, 250, 1000 or 2500 parts per million (ppm) (equal to 0, 9.3, 47.4, 186.2 and 458.0 mg/kg bw per day for males and 0, 11.8, 57.9, 234.4 and 513.0 mg/kg bw per day for females, respectively) to establish suitable doses for an 80-week carcinogenicity study. Mortality and clinical signs were checked daily. Body weight and feed consumption were measured weekly throughout the study. Clinical chemistry was performed towards the end of the dosing phase. All animals were subjected to necropsy, selected organs were weighed and designated tissues were sampled and examined microscopically. In addition, total cytochrome P450 content in the liver was determined.

Fig. 2. Proposed metabolic pathway of ethiprole in rats



[#] Tentatively identified by co-chromatography with a reference standard.
[ ] Postulated intermediate.
Source: McCorquodale & Anderson (1999)

Table 1. Study of acute toxicity of ethiprole

Species	Strain	Sex	Route	Purity (%)	Result	Reference
Rat	Wistar	M + F	Oral	93.0%[a]	LD_{50} > 7 080 mg/kg bw	Steiblen (1997)

bw: body weight; F: females; LD_{50}: median lethal dose; M: male
[a] Batch no. CDR9706.

The highest dietary concentration of 2500 ppm caused the death or required the premature termination of almost all animals and was considered to have exceeded the maximum tolerated dose. Ethiprole occasionally induced increased motor activity in males and females at 2500 ppm. The body weight gains of animals at 2500 ppm were comparable to those of controls. At 2500 ppm, statistically significantly lower feed consumption was noted in males and females during the first week of treatment. Decreases in mean total bilirubin concentrations were seen in males from 50 ppm onwards and in females from 250 ppm onwards. Higher mean aminotransferase activities were seen in males at 1000 and 2500 ppm. Ethiprole induced a dose-related increase in total liver cytochrome P450 content in males at 1000 and 2500 ppm (123% and 142% of controls, respectively). In the liver, dose-related increases in absolute and relative weights were observed at 250, 1000 and 2500 ppm in males (115%, 140% and 170% of control values for absolute, respectively; $P < 0.01$) and in females (115%, 143% and 210% of control values for absolute, respectively; $P < 0.01$). The 10% increased absolute liver weight in females at 50 ppm was not statistically significant. Liver enlargement and/or pale colour were observed in most animals at 1000 and 2500 ppm at gross necropsy. At 250 and 1000 ppm, histopathological examination revealed centrilobular to panlobular hepatocellular hypertrophy in both sexes and hepatocyte microvacuolation (characterized by the cytoplasmic accumulation of small vacuoles in the hepatocytes) and necrosis in males.

In the absence of other corroborative changes, the minimally higher liver weight without histopathological changes in females at 50 ppm and the lower total bilirubin in males at 50 ppm were not considered to be toxicologically relevant. The no-observed-adverse-effect level (NOAEL) was 50 ppm (equal to 9.3 mg/kg bw per day), based on increased liver weights and histopathological changes in the liver at 250 ppm (equal to 47.4 mg/kg bw per day) (Dange, 1999).

Rats

In a 28-day toxicity study, ethiprole (batch no. CDR9706; purity 93%) was administered to groups of 10 male and 10 female Wistar (RJ: WI (IOPS AF)) rats in the diet at a concentration of 0, 20, 100, 500 or 2500 ppm (equal to 0, 1.8, 9.2, 46.1 and 219.3 mg/kg bw per day for males and 0, 2.0, 9.6, 46.3 and 220.2 mg/kg bw per day for females, respectively). Mortality and clinical signs were checked daily. Body weight and feed consumption were measured weekly throughout the study. Neurotoxicity assessment and ophthalmic examinations were performed on all animals during the acclimatization period and during week 3 of dosing. Clinical chemistry, haematology and urine analysis were performed towards the end of the dosing phase. In addition, a blood sample was collected during week 4 for triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) analysis. All animals were subjected to necropsy, selected organs were weighed and designated tissues were sampled and examined microscopically.

At the top dose of 2500 ppm, there were signs of overt toxicity, with the premature deaths of two males; however, ethiprole induced no treatment-related clinical signs. The body weight gains at 2500 ppm were reduced compared with control values throughout the study in males (final body weight 92% of control value) and only during the first week of treatment in females (final body weight 100% of control value). At 2500 ppm, statistically significantly lower feed consumption was noted in males during the first 2 weeks and in females during the first week. Increases in prothrombin time were noted in males at 500 and 2500 ppm (120% and 140% of control values, respectively; $P < 0.001$). Decreased prothrombin times observed in females at 20, 100 and 500 ppm were considered to be incidental and unrelated to treatment, as they were not dose related and/or not biologically relevant. A tendency towards higher platelet counts was noted in males and females at 2500 ppm (124% and 117% in males

and females, respectively). Higher mean alanine aminotransferase activity was observed in males at 2500 ppm (464% of control value; $P < 0.001$). Mean total protein concentrations were higher in females at 500 and 2500 ppm (116% and 113% of control values; $P < 0.001$). Slight, but statistically significant, increases were also observed in males at 2500 ppm (107% of control value; $P < 0.01$) and in females at 100 ppm (107% of control value; $P < 0.05$). Lower mean albumin concentration was noted in males at 2500 ppm (91% of control value; $P < 0.001$). Mean total cholesterol concentrations were higher in males at 2500 ppm (212% of control value; $P < 0.001$) and in females at 500 and 2500 ppm (169% and 247% of control values; $P < 0.001$). In females, these changes were associated with higher mean triglyceride concentrations at 500 and 2500 ppm (184% and 349% of control values, respectively; $P < 0.001$).

Ethiprole induced a statistically significant imbalance of thyroid hormones in animals treated at 500 and 2500 ppm, resulting in higher levels of TSH and T_3 and lower levels of T_4 in both sexes (Table 2). Although statistically not significant, the groups begin to separate into sensitive and less sensitive subpopulations at 100 ppm.

Table 2. Twenty-eight-day study in the rat: mean T_3, T_4 and TSH plasma levels (week 4)

	0 ppm	20 ppm	100 ppm	500 ppm	2 500 ppm
Males					
No. of animals examined	9–10	9	9–10	10	9
T_3 (mmol/L)	0.439	0.410	0.447	0.447	0.608*
T_4 (mmol/L)	31.7	29.2	26.3	22.5***	15.2***
TSH (ng/mL)	2.75	2.14	3.64	6.93**	9.88***
Females					
No. of animals examined	9–10	7–9	8–10	9–10	9–10
T_3 (mmol/L)	0.407	0.430	0.438	0.355	0.605**
T_4 (mmol/L)	17.9	21.9	21.6	15.1	15.9
TSH (ng/mL)	1.18	1.23	2.72	5.18***	4.51**

ppm: parts per million; T_3: triiodothyronine; T_4: thyroxine; TSH: thyroid stimulating hormone; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ (Mann-Whitney U test [TSH] or Dunnett's test [T_3, T_4])
Source: Dange (2001a)

In the liver, dose-related increases in both absolute and relative weights were observed at 500 and 2500 ppm in males (135% and 198% of control values for absolute weight, respectively; $P < 0.001$) and in females at 100, 500 and 2500 ppm (approximately 120%, 196% and 262% of control values for absolute weight, respectively; $P < 0.001$). In the thyroid, dose-related increases in both absolute and relative weights were observed at 500 and 2500 ppm in males (141% and 153% of control values for absolute weight, respectively; $P < 0.001$ and $P < 0.01$, respectively) and in females (140% and 160% of control values for absolute weight, respectively; $P < 0.01$ and $P < 0.001$, respectively). In the adrenals, higher absolute and relative weights were observed in males at 500 and 2500 ppm (116% and 123% of control values for absolute weight, respectively; $P < 0.01$ and $P < 0.001$, respectively) and in females at 100, 500 and 2500 ppm (123%, 115% and 135% of control values for absolute weight, respectively; $P < 0.01$, not statistically significant and $P < 0.001$, respectively) (Table 3).

At the histopathological examination, the liver with centrilobular to panlobular hepatocellular hypertrophy, the thyroid with follicular cell hypertrophy and the adrenals with vacuolation of the cortex were found to be the target organs in animals at 500 and/or 2500 ppm. The kidney was also affected at 2500 ppm, with both sexes showing tubular golden brown pigment. At 100 ppm, one female was observed with vacuolation of the adrenal cortex; the incidence was low, but, based on the dose–effect relationship, 100 ppm should be considered the minimal effect dose for this finding.

Table 3. Twenty-eight-day study in the rat: effects of ethiprole on liver, thyroid and adrenal weights

		0 ppm	20 ppm	100 ppm	500 ppm	2 500 ppm
Males						
Number of animals		10	9	10	10	8
Terminal body weight (g)		338.6	339.9	332.8	334.2	310.4*
Liver weight	Absolute (g)	10.3	10.1	10.3	13.9***	20.4***
	Relative to body (%)	3.03	2.96	3.10	4.16***	6.58***
	Relative to brain (%)	529	515	529	714***	1 080***
Thyroid weight	Absolute (g)	0.017	0.018	0.019	0.024***	0.02***
	Relative to body (%)	0.004 9	0.005 3	0.005 8	0.007 3***	0.008 4**
	Relative to brain (%)	0.861	0.914	0.985	1.25**	1.39**
Adrenal weight	Absolute (g)	0.057	0.052	0.054	0.066**	0.070***
	Relative to body (%)	0.017	0.015	0.016	0.020**	0.022***
	Relative to brain (%)	2.92	2.65	2.76	3.39*	3.69***
Females						
Number of animals		10	9	10	7	10
Terminal body weight (g)		201.4	212.3	215.2	221.1	210.3
Liver weight	Absolute (g)	5.98	6.27	7.20***	11.7***	15.6***
	Relative to body (%)	2.85	2.96	3.35***	5.31***	7.43***
	Relative to brain (%)	323	345	387**	631***	851***
Thyroid weight	Absolute (g)	0.015	0.016	0.014	0.021**	0.024***
	Relative to body (%)	0.007 2	0.007 6	0.006 7	0.009 5*	0.011 3***
	Relative to brain (%)	0.807	0.889	0.771	1.13**	1.30***
Adrenal weight	Absolute (g)	0.060	0.066	0.074**	0.069	0.081***
	Relative to body (%)	0.029	0.031	0.035*	0.031	0.039***
	Relative to brain (%)	3.26	3.64	3.96*	3.68	4.42***

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$
Source: Dange (2001a)

The NOAEL was 20 ppm (equal to 1.8 mg/kg bw per day), based on adrenal effects (increased weight with slight increase in vacuolation) and slight, non-statistically significant effects on thyroid hormones at 100 ppm (equal to 9.2 mg/kg bw per day) (Dange, 2001a).

It should be noted that no adrenal vacuolation was reported in the 90-day toxicity study in the rat, which used doses of 0, 5, 20, 500 and 2500 ppm (see below).

In a 90-day toxicity study, ethiprole (batch no. CDR9706; purity 93%) was administered continuously via the diet to groups of 10 male and 10 female Wistar (RJ: WI (IOPS AF)) rats at a dietary concentration of 0, 5, 20, 500 or 2500 ppm (equal to 0, 0.296, 1.17, 30.5 and 155 mg/kg bw per day for males and 0, 0.373, 1.50, 37.6 and 188 mg/kg bw per day for females, respectively). Mortality and clinical signs were checked daily. Body weight was measured weekly, and feed consumption was measured daily throughout the study. Ophthalmic examinations were performed on all animals during the acclimatization period and during week 4 of dosing. Clinical chemistry, haematology and urine analysis were performed towards the end of the dosing phase. In addition, a blood sample was collected

during weeks 2, 6 and 13 for T_3, T_4 and TSH analysis. All animals were subjected to necropsy, selected organs were weighed and designated tissues were sampled and examined microscopically.

One female at 500 ppm and seven males at 2500 ppm were found dead. All these mortalities occurred between days 19 and 90 and were considered treatment related. One male and one female at 500 ppm and one male at 2500 ppm were moribund and were terminated. Clinical signs were observed at 2500 ppm and consisted of piloerection, increased and/or reduced motor activity and irritability to touch. These signs were observed on only very few occasions during the study. In addition, most of the males that died at 2500 ppm exhibited a general pallor before death. Body weights were significantly lower than those of controls at 2500 ppm during the first 5 weeks of dosing for males and only during the first week of treatment for females. At 2500 ppm, significantly lower feed consumption was noted in males during the first 3 weeks and in females during the first week. Prothrombin times were higher in males at 500 and 2500 ppm. In addition, a tendency towards higher platelet counts was noted at 2500 ppm in males (128% of control value; not statistically analysed) and in females (122% of control value; $P < 0.05$).

Higher mean total protein concentrations were noted in males at 500 and 2500 ppm (108% and 121% of control values, respectively; $P < 0.01$ and not statistically analysed, respectively) and in females at the same doses (119% and 125% of control values, respectively; $P < 0.01$). Higher mean total cholesterol concentrations were observed in females at 500 and 2500 ppm (214% and 333% of control values, respectively; $P < 0.01$). These changes were associated with higher mean triglyceride concentrations (137% and 141% of control values, respectively; not statistically significant and $P < 0.05$, respectively). In addition, a tendency towards slightly higher calcium and potassium concentrations and slightly lower chloride concentrations was noted at 500 and 2500 ppm.

Ethiprole induced an imbalance of thyroid hormones in animals treated at 500 and 2500 ppm, resulting in higher levels of TSH and T_3 and lower levels of T_4 (Table 4).

No treatment-related urine analysis changes were observed at any dose in either sex.

Statistically significant increases in liver and thyroid weights were observed at 500 and 2500 ppm. In the liver, dose-related increases in both absolute and relative weights were observed at 500 and 2500 ppm in males (157% and 257% of control values for absolute weight, respectively; $P < 0.01$ and not statistically analysed, respectively) and in females (196% and 292% of control values for absolute weight, respectively; $P < 0.01$). In the thyroid, dose-related increases in both absolute and relative weights were observed at 500 and 2500 ppm in males (148% and 162% of control values for absolute weight, respectively; $P < 0.01$) and females (144% and 156% of control values for absolute weight, respectively; $P < 0.01$).

At termination, histopathological examination revealed treatment-related microscopic findings in the liver, thyroid and kidney at 500 and/or 2500 ppm. In the liver, centrilobular hepatocyte hypertrophy attributable to treatment was noted in both sexes at 500 and 2500 ppm. Changes were generally more pronounced in females. Mild to moderate amounts of golden brown intracytoplasmic pigment were also observed in the majority of females at 2500 ppm.

In the thyroid, follicular cell hypertrophy/hyperplasia was noted in almost all examined animals of both sexes administered 500 or 2500 ppm. In addition, slight follicular epithelial hypertrophy/hyperplasia was seen in a single male at both 20 and 5 ppm, and mild follicular epithelial hypertrophy/hyperplasia was observed in two males at 20 ppm. However, these findings at these lower doses were not considered to be toxicologically relevant in view of the low incidence involved, the slight/mild nature of the change and the absence of corroborative changes in thyroid weights or thyroid hormone levels. In the kidney, an increased degree of cortical tubular brown pigment was reported in all surviving males and females at 2500 ppm.

The NOAEL was 20 ppm (equal to 1.17 mg/kg bw per day), based on mortality, changes in thyroid hormone levels, changes in clinical chemistry parameters and increased liver and thyroid weights associated with microscopic changes in these target organs at 500 ppm (equal to 30.5 mg/kg bw per day) (Dange & Foulon, 2002a).

Table 4. Ninety-day study in the rat: effects of ethiprole on mean T_3, T_4 and TSH plasma levels

Interval	Parameter	0 ppm	5 ppm	20 ppm	500 ppm	2 500 ppm
Males						
Week 2	No. of animals examined	10	10	10	10	10
	T_3 (mmol/L)	0.78	0.72	0.80	0.77	0.89
	T_4 (mmol/L)	36.2	36.5	37.7	28.6**	20.3**
	TSH (ng/mL)	0.57	0.65	0.62	1.13**	1.17**
Week 6	No. of animals examined	10	10	10	10	7
	T_3 (mmol/L)	0.74	0.63	0.71	0.85	0.71
	T_4 (mmol/L)	39.3	37.7	37.6	30.5**	21.3**
	TSH (ng/mL)	0.71	0.72	0.86	1.56**	2.13**
Week 13	No. of animals examined	8	7	8	8	4
	T_3 (mmol/L)	0.66	0.75	0.66	0.83	1.09*
	T_4 (mmol/L)	36.8	36.3	37.5	29.8*	22.2**
	TSH (ng/mL)	0.83	0.80	0.74	1.50**	1.63*
Females						
Week 2	No. of animals examined	10	10	10	10	10
	T_3 (mmol/L)	0.81	0.81	0.89	0.81	0.79
	T_4 (mmol/L)	28.3	29.1	28.1	25.5	22.0
	TSH (ng/mL)	0.48	0.42	0.48	0.76**	0.97**
Week 6	No. of animals examined	10	10	10	9	10
	T_3 (mmol/L)	0.77	0.72	0.85	0.96	1.16**
	T_4 (mmol/L)	31.4	34.6	32.9	24.1**	23.6**
	TSH (ng/mL)	0.52	0.52	0.48	0.82**	1.35**
Week 13	No. of animals examined	5	8	7	5	10
	T_3 (mmol/L)	0.73	0.76	0.91	1.08	1.26**
	T_4 (mmol/L)	23.5	26.3	29.8	22.1	22.9
	TSH (ng/mL)	0.64	0.64	0.62	0.81	1.44**

ppm: parts per million; T_3: triiodothyronine; T_4: thyroxine; TSH: thyroid stimulating hormone; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Source: Dange & Foulon (2002a)

Dogs

In a 90-day toxicity study, ethiprole (batch no. CDR9706; purity 93%) was administered continuously via the diet to groups of four male and four female beagle dogs at a dietary concentration of 0, 30, 90 or 200 ppm (equal to 0, 1.0, 3.2 and 7.6 mg/kg bw per day for males and 0, 1.1, 3.6 and 8.5 mg/kg bw per day for females, respectively). Mortality and clinical signs were checked daily. Body weight and feed consumption were measured weekly throughout the study. Ophthalmic examinations were performed on all animals during the acclimatization period and during week 12 of dosing. Clinical chemistry, haematology and urine analysis were performed in weeks 6 and 13. In addition, a blood sample was collected during weeks 2 and 13 for T_3, T_4 and TSH analysis. All animals were subjected to necropsy, selected organs were weighed and designated tissues were sampled and examined microscopically.

At 200 ppm, one moribund female was prematurely terminated on day 24. Clinical signs were limited to this female and consisted of bloody faeces, diarrhoea, soiled fur, prostration, tremors and increased salivation. This female had the highest ethiprole intake over weeks 1–3 (10.8 mg/kg bw per day, compared with 5, 7 and 8.4 mg/kg bw per day for the other three females in that group).

Treatment-related decreases in body weight and body weight gain were observed at 90 and 200 ppm in males. In males, differences were statistically significant only at 200 ppm and included lower mean body weight from day 36 onwards and decreases in mean body weight gain at weeks 2, 4 and 5 and overall for weeks 1–12. At 90 ppm, mean body weight was 88% of control values by day 85, and overall body weight gain (weeks 1–12) was 57% of that of controls. In females, although no apparent effect was observed on mean body weight at 200 ppm, a lower body weight gain was observed frequently (statistically significant at weeks 1 and 4) (Table 5). At 200 ppm, statistically significantly lower feed consumption was noted in males during the first 5 weeks.

Table 5. Ninety-day study in the dog: effects of ethiprole on body weight and body weight gain

	0 ppm	30 ppm	90 ppm	200 ppm
Males				
Body weight (g)				
Day 01	7 385	7 227	7 079	7 015
Day 36	8 396	8 209	7 703	6 903*
Day 43	8 526	8 362	7 779	6 895*
Day 50	8 728	8 567	7 903	6 992*
Day 58	8 813	8 686	7 903	6 952**
Day 64	8 966	8 823	7 997	6 951**
Day 71	9 200	9 050	8 175	7 050**
Day 78	9 279	9 095	8 183	6 946**
Day 85	9 464	9 207	8 279	6 971**
Body weight gain (g/day)				
Overall (weeks 1–12)	24.4	23.8	13.8	−0.6**
Females				
Body weight (g)				
Day 01	5 493	5 614	5 657	5 708
Day 08	5 548	5 671	5 632	5 510
Day 43	6 109	6 276	6 186	6 162 (*n* = 3)
Day 50	6 190	6 338	6 275	6 198 (*n* = 3)
Day 58	6 325	6 494	6 341	6 146 (*n* = 3)
Day 64	6 431	6 584	6 485	6 408 (*n* = 3)
Day 71	6 650	6 800	6 625	6 400 (*n* = 3)
Day 78	6 681	6 878	6 710	6 555 (*n* = 3)
Day 85	6 785	7 100	6 825	6 716 (*n* = 3)
Body weight gain (g/day)				
Overall (weeks 1–12)	15.3	17.1	13.8	8.2 (*n* = 3)

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$
Source: Bigot (2002)

Statistically significant increases were observed in alkaline phosphatase activities at 200 ppm in females in weeks 7 and 13. The same tendency was noted at 200 ppm in males in week 13 only, but it did not reach statistical significance. Ethiprole did not induce any changes in T_3 or T_4 at any dose in either sex; TSH could not be measured, probably because of the frozen storage period of the plasma samples (7–10.5 months).

In the thymus, lower absolute and relative weights were noted in males at 90 and 200 ppm (80% and 32% of control values, respectively; not statistically significant and $P < 0.05$, respectively). These effects were considered to be related to lower terminal body weights. In male sexual organs, lower absolute and relative weights were observed at 90 and 200 ppm (65% and 50% of control values in the epididymis, 59% and 44% of control values in the testis and 28% and 17% of control values in the prostate, respectively) (Table 6).

At 30 ppm, lower absolute organ weights were also noted in the prostate (41% of control value; $P < 0.01$), in the epididymis (66% of control value) and in the testis (69% of control value) (Table 6). Taking into account that these effects were not associated with any untoward histological changes and that in the subsequent 1-year study (Chevalier, 2001) in beagle dogs, no similar changes were observed up to 90 ppm, the lower sexual organ weights noted at 30 ppm were considered not to be toxicologically relevant.

In the brain, a higher relative weight was noted at 200 ppm in males (130% of control value; $P < 0.05$) (Table 6). This effect was considered to be related to lower terminal body weights. At termination, small testes and small prostate were seen in one male at 200 ppm. No treatment-related macroscopic changes were observed at 30 or 90 ppm in males or at any dose in females.

Table 6. Ninety-day study in the dog (males): effects of ethiprole on brain, thymus, epididymis, testis and prostate weights

		0 ppm	30 ppm	90 ppm	200 ppm
Number of animals examined		4	4	4	4
Terminal body weight (kg)		9.5	9.2	8.3	6.9**
Brain weight	Absolute (g)	76.2	78.2	77.5	75.2
	Relative to body (%)	0.825	0.850*	0.950	1.075*
	Relative to brain (%)	–	–	–	–
Thymus weight	Absolute (g)	8.95	11.524	7.10	2.90*
	Relative to body (%)	0.095	0.123	0.080	0.040
	Relative to brain (%)	11.8	14.8	9.15	3.70
Epididymis weight	Absolute (g)	2.87	1.90	1.85	1.45
	Relative to body (%)	0.027	0.017	0.020	0.017
	Relative to brain (%)	3.75	2.45	2.33	1.93
Testes weight	Absolute (g)	13.32	9.22	7.82*	5.85**
	Relative to body (%)	0.145	0.100	0.092	0.082
	Relative to brain (%)	17.4	11.8*	10.1*	7.73**
Prostate weight	Absolute (g)	6.10	2.52**	1.67**	1.05**
	Relative to body (%)	0.065	0.027**	0.022*	0.015**
	Relative to brain (%)	7.98	3.28**	2.15**	1.40**

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$
Source: Bigot (2002)

In the liver, slight to mild centrilobular hepatocyte hypertrophy attributable to treatment was noted in two males at 90 ppm and in one male at 200 ppm. Glycogen depletion was noted in one male and one female treated at 200 ppm. In the thymus, atrophy was noted in two males at 90 ppm and in four males and two females treated at 200 ppm. In the sexual organs of males, immaturity was reported in all males, including controls, but the severity of this finding was more pronounced at 90 and 200 ppm (Table 7).

Table 7. Ninety-day study in the dog: selected microscopic findings at termination

	Males				Females			
	0 ppm	**30 ppm**	**90 ppm**	**200 ppm**	**0 ppm**	**30 ppm**	**90 ppm**	**200 ppm**
Liver								
Number examined	4	4	4	4	4	4	4	3
Hepatocyte hypertrophy – centrilobular becoming generalized	0	0	2	1	0	0	0	0
Glycogen depletion	0	0	0	1	0	0	0	1
Thymus								
Number examined	4	4	4	4	4	4	4	3
Atrophy	1	0	2	4	0	0	0	2
Epididymis								
Number examined	4	4	4	4	–	–	–	–
No sperm	0	0	1	2	–	–	–	–
Few sperm	1	2	2	1	–	–	–	–
Testis								
Number examined	4	4	4	4	–	–	–	–
Immaturity	4	4	4	4	–	–	–	–
Prostate								
Number examined	4	4	4	4	–	–	–	–
Immaturity	0	1	3	4	–	–	–	–

ppm: parts per million
Source: Bigot (2002)

The NOAEL was 30 ppm (equal to 1.0 mg/kg bw per day), based on reduced body weight gain and decreased thymus weight with atrophy at 90 ppm (equal to 3.2 mg/kg bw per day) (Bigot, 2002).

In a 1-year toxicity study, ethiprole (batch no. CDR 9706; purity 93%) was administered to groups of five male and five female beagle dogs in the diet at a concentration of 0, 9, 30 or 90 ppm (equal to 0, 0.27, 0.70 and 2.73 mg/kg bw per day for males and 0, 0.22, 0.76 and 2.51 mg/kg bw per day for females, respectively) for at least 52 weeks. Mortality and clinical signs were checked daily. Body weight was measured during the acclimatization period and weekly thereafter. Feed consumption was measured daily throughout the study. Ophthalmic examinations were performed on all animals during the acclimatization period and during week 12 of dosing. Clinical chemistry, haematology and urine analysis were performed towards the end of the dosing phase. In addition, a blood sample was

collected during weeks 2 and 12 for T_3, T_4 and TSH analysis. All animals were subjected to necropsy, selected organs were weighed and designated tissues were sampled and examined microscopically.

The only treatment-related findings in this study were slight decreases in the overall body weight gain in both males and females at 90 ppm (−19% and −23% in males and females, respectively) (Table 8). At 9 ppm, one male presented with emaciation from week 15, with body weight loss noted from week 12 (−200 g compared with week 11) until week 16. Thereafter, the body weight of this dog was stable (around 7.5 kg), reaching 7.9 kg at the end of the treatment period. No variation in feed consumption was noted. In the absence of signs of emaciation in the 30 ppm group, the relationship to treatment was doubtful, and the body weight loss in this male was probably unrelated to the test substance.

Table 8. One-year study in the dog: effects of ethiprole on body weight and body weight gain

	0 ppm	**9 ppm**	**30 ppm**	**90 ppm**
Males				
Body weight (kg)				
Week 1	7.4 ± 0.53	7.3 ± 0.54	7.6 ± 0.29	7.3 ± 0.55
Week 13	8.7 ± 1.16	8.7 ± 0.89	10.0 ± 0.39	9.0 ± 0.57
Week 52	11.0 ± 1.71	10.1 ± 2.11	12.2 ± 1.06	10.0 ± 1.28
Body weight gain (g/day)				
Overall (weeks 1–52)	3.7	2.9	4.8	3.0
Females				
Body weight (kg)				
Week 1	6.7 ± 0.60	6.6 ± 0.51	6.6 ± 0.61	6.6 ± 0.66
Week 13	8.6 ± 0.80	8.8 ± 0.55	8.5 ± 0.93	7.8 ± 0.54
Week 52	9.6 ± 1.11	10.3 ± 1.84	9.9 ± 1.26	8.9 ± 1.00
Body weight gain (g/day)				
Overall (weeks 1–53)	3.1	3.9	3.4	2.4

ppm: parts per million
Source: Chevalier (2001)

The NOAEL was 30 ppm (equal to 0.70 mg/kg bw per day), based on overall reduced body weight gain at 90 ppm (equal to 2.51 mg/kg bw per day) (Chevalier, 2001).

(b) Dermal application

No studies were submitted.

(c) Exposure by inhalation

No studies were submitted.

2.3 Long-term studies of toxicity and carcinogenicity

The long-term toxicity and carcinogenic potential of ethiprole were evaluated in a 78-week study in the mouse and a 104-week study in the rat. Both studies included a 52-week long-term toxicity phase.

Mice

In a chronic toxicity and carcinogenicity study, ethiprole (batch no. CDR 9706; purity 93%) was administered to groups of 50 male and 50 female C57BL/6J mice for at least 78 weeks at a dietary concentration of 0, 10, 50, 150 or 300 ppm (equal to 0, 1.7, 8.6, 25.6 and 50.8 mg/kg bw per day for males and 0, 1.7, 12.5, 36.3 and 73.5 mg/kg bw per day for females, respectively). For evaluation of toxic effects, 10 satellite males and 10 satellite females per group were terminated after a 1-year treatment. Throughout the study, clinical signs and mortality were checked daily, and careful examination was carried out weekly to assess possible neurotoxic effects. Palpation of possible masses was carried out every 4 weeks from weeks 4 to 52 and every 2 weeks thereafter. Body weight and feed consumption were measured at weekly intervals during the first 13 weeks of the study and every 4 weeks thereafter. Haematological investigations (differential white cell count) were carried out in week 52 and at the end of the study (principal animals). Before the termination of satellite animals in week 52, blood was taken for the determination of liver enzyme activities (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase). At the end of the appropriate scheduled treatment period (week 52 or 78), all animals were terminated and submitted to a macroscopic postmortem examination. Designated organs were weighed. A microscopic examination was performed on all samples of tissues from satellite animals, from principal animals of the control and high-dose groups, and from principal animals that died or were killed prematurely during the study.

Mortality was slightly higher in females of the 300 ppm group (11/50) compared with the control group (4/50). There were no treatment-related clinical signs noted at any dose during either phase of the study. No signs of neurotoxic effects were observed, and there was no evidence that treatment produced an increase in the incidence or reduction in the onset time of palpable tumours. The body weight gain was similar in the controls and the treated groups for the males, but was slightly lower for the females given 50, 150 or 300 ppm during the first 4 weeks of the treatment period, without correlation with variations in feed consumption. As the lower gain was slight, transient, of low magnitude, not dose related, recorded only in females and not observed in the 28-day study in mice at much higher doses, it was not considered to be of toxicological relevance. After week 4, the body weight changes were similar to those of the controls. No effects on feed consumption or differential white cell count were observed.

Increases of greater than 115% (117–122%) of control values were noted in absolute and relative liver weights in males dosed at 300 ppm after 52 and 78 weeks. These increases were, however, without correlative histopathological changes, and they were therefore considered to be of doubtful toxicological importance.

There were no treatment-related non-neoplastic findings at any dose after treatment for either 52 or 78 weeks. The non-neoplastic findings observed in all organs and tissues were recognized as those that are commonly recorded in ageing mice of this strain and age and were regarded as being of no toxicological importance. After 78 weeks of treatment, the only neoplastic change was an increase (P = 0.0133) in the incidence of hepatocellular adenomas in females at 300 ppm (6/50 versus 0/50 in controls). The incidence and time of onset of the liver neoplasms were similar in all groups. All neoplasms were recorded in animals at termination, suggesting that treatment with the test substance did not shorten the time of onset of this lesion. In addition, the incidence and time of onset of the hepatocellular carcinomas were similar in all groups (Table 9).

Other (without any dose–response relationship) observed neoplastic lesions found in the haemolymphoreticular system (as assessed from the microscopic examination of the liver and lymphoid organs) were diagnosed as malignant lymphoma (lymphocytic, pleiomorphic or heterogenous [mixed cell] types) and histiocytic sarcoma.

Table 9. Mouse carcinogenicity study: effect of ethiprole on incidence of hepatic neoplastic lesions

	0 ppm	10 ppm	50 ppm	150 ppm	300 ppm
Males					
Hepatocellular adenoma	5/49	5/50	4/50	1/50	1/50
Hepatocellular carcinoma	0/49	3/50	1/50	0/50	1/50
Total hepatocellular adenoma + carcinoma	5/49	8/50	5/50	1/50	2/50
Females					
Hepatocellular adenoma	0/50	2/50	1/50	2/50	6/50
Hepatocellular carcinoma	0/50	0/50	0/50	0/50	0/50
Total hepatocellular adenoma + carcinoma	0/50	2/50	1/50	2/50	6/50

ppm: parts per million
Source: Richard (2002)

The NOAEL for toxicity was 150 ppm (equal to 36.3 mg/kg bw per day), based on a decrease in survival rate in females at 300 ppm (equal to 73.5 mg/kg bw per day). The NOAEL for carcinogenicity was 150 ppm (equal to 36.3 mg/kg bw per day), based on an increase in the incidence of hepatocellular adenomas in females at 300 ppm (equal to 73.5 mg/kg bw per day) (Richard, 2002).

Rats

In a chronic toxicity and carcinogenicity study, ethiprole (batch no. CDR9706; purity 93%) was administered in the diet to groups of 60 male and 60 female Wistar (RJ: WI (IOPS AF)) rats at a dietary concentration of 0, 5, 20, 75 or 250 ppm (equal to 0, 0.22, 0.85, 3.21 and 10.8 mg/kg bw per day for males and 0, 0.29, 1.17, 4.40 and 14.7 mg/kg bw per day for females, respectively). Additional groups were similarly dosed: groups of 10 males and 10 females were used for the interim kill at 52 weeks (all dose groups), and groups of 15 males and 15 females were used for a 90-day recovery phase after 52 weeks of exposure (control and high dose only). Mortality and clinical signs were checked daily. Body weight and feed consumption were measured weekly for the first 13 weeks and every 4 weeks thereafter. Ophthalmic examinations were performed on all animals during the acclimatization period and at 1 and 2 years. Clinical chemistry, haematology determinations and urine analysis were performed during months 6, 12, 15, 18 and 24 on selected animals. In addition, a blood sample was collected during weeks 2, 9, 24 and 52 and during weeks 2, 6 and 13 of the recovery period for T_3, T_4 and TSH analysis. All remaining animals of the chronic, recovery and carcinogenicity phases were subjected to necropsy, selected organs were weighed and designated tissues were sampled and examined microscopically.

No treatment-related changes in the mortality rate or clinical signs were observed. The body weight changes in males and females at 75 and 250 ppm were slightly increased compared with controls, especially during the first year of treatment. The overall feed consumption was generally higher in males and lower in females, but without a dose–response relationship.

Prothrombin times were higher in males and lower in females at 75 and 250 ppm. However, the effect in males was only greater than a 10% increase compared with controls at 6 months in the 250 ppm group (126%); in females, the effects were slight (87–92% of control values), with no consistent dose–response relationship. In addition, at 250 ppm in females, the mean platelet counts were statistically significantly higher on months 12 (125% of control value) and 24 (135% of control value). After the recovery period, no relevant differences were noted between control and high-dose animals, indicating that these effects were reversible.

In females at 250 ppm, mean total cholesterol concentration was higher at months 6 (144% of control value; $P < 0.001$), 12 (155% of control value; $P < 0.001$), 18 (184% of control value; $P < 0.01$) and 24 (133% of control value; not statistically significant).

Mean total bilirubin concentrations were lower at 75 and 250 ppm in both sexes (63–76% and 50–61% of control values in males, respectively, and 56–67% and 40–53% of control values in females, respectively; dose related) and at 20 ppm in males only at 18 months (69% of control value). After the 3-month recovery period, no relevant changes were noted between controls and the high-dose animals, indicating that these effects were reversible.

Ethiprole induced an imbalance of thyroid hormones, resulting in higher levels of TSH and/or lower levels of T_4 in males and females at 75 and 250 ppm (Table 10). During the recovery period, TSH and T_4 returned to control levels within 2 weeks of the withdrawal of treatment at 250 ppm. The imbalance of TSH and T_4 was most likely secondary to a treatment-induced increase in the metabolic activity of the liver, causing an increased biliary clearance of T_4 and, consequently, an overstimulation of the thyroid due to increased levels of TSH.

At 52 weeks, treatment-related organ weight changes were noted in the liver and thyroid. There were statistically significantly increased liver weights at 250 ppm in both sexes (130% and 119% of control values for absolute and relative liver weights in males, respectively; and 145% and 146% of control values for absolute and relative liver weights in females, respectively). The increased liver weights at 75 ppm in females were slight (112% and 114% of control values for absolute and relative weights, respectively). The thyroid weights were increased in males at 75 ppm (136% and 121% of control values for absolute and relative [not statistically significant] weights) and at 250 ppm in both sexes (168% and 150% of control values for absolute and relative weights in males, respectively; and 120% and 124% of control values for absolute and relative weights in females, respectively). No treatment-related changes in organ weights were noted in the reversibility groups.

At the end of the carcinogenicity phase, treatment-related organ weight changes were noted in the liver and thyroid. There was a statistically significantly increased liver weight at 250 ppm in both sexes (123% and 125% of control values for absolute and relative weights in males, respectively; and 138% and 137% of control values for absolute and relative weights in females, respectively). The increased liver weight at 75 ppm in females was slight (113% and 114% of control values for absolute and relative weights, respectively). The thyroid weight was increased at 75 ppm in both sexes (138% and 136% of control values for absolute and relative weights in males, respectively; and 128% and 133% of control values for absolute and relative weights in females, respectively) and at 250 ppm in both sexes (121% of control values for males and 135–136% of control values for females, for both absolute and relative weights), reaching statistical significance in females only. No treatment-related changes in organ weights were noted in the reversibility groups.

At 52 weeks, treatment-related changes were noted in the liver and thyroid. In the liver, treatment-related centrilobular hepatocyte hypertrophy was noted in all females at 250 ppm. This change, characterized by an appreciable increase in the size of hepatocytes in the area of the central vein, was generally graded slight to mild and is considered to represent a minor adaptive change. In the thyroid, follicular cell hypertrophy was noted in animals of both sexes dosed at 20 ppm and above. This change, which was characterized by an increase in height of the follicular epithelial cells accompanied by a decrease in the follicular size and in colloid content, was graded slight to moderate. There was evidence of a dose–effect relationship in both sexes, more prominently in females than in males. In addition, colloid mineralization was observed in a small proportion of males (2/9) and females (4/10) at 250 ppm (Table 11).

At the end of the reversibility phase after 52 weeks of treatment, the centrilobular hepatocyte hypertrophy was fully reversible. In the thyroid gland, the colloid mineralization and slight follicular cell hypertrophy were still present, with some indications that reversibility was in progress.

Histopathological examination at the termination of the carcinogenicity phase revealed a number of treatment-related non-neoplastic findings in the liver: bile duct hyperplasia and sclerosis and focal sinusoidal dilatation. These changes were noted in many animals, with the incidence and severity

Table 10. Rat chronic toxicity and carcinogenicity study: effects of ethiprole on mean T_3, T_4 and TSH plasma levels

		0 ppm	5 ppm	20 ppm	75 ppm	250 ppm
Males						
No. of animals examined		23–25	10	10	10	24–25
Week 2	TSH (ng/mL)	6.28	6.30	6.58	6.06	9.01***
	T_4 (ng/mL)	35.2	34.1	36.5	34.1	32.5*
	T_3 (ng/mL)	0.675	0.661	0.770	0.691	0.699
Week 9	TSH (ng/mL)	6.48	6.98	6.79	7.07	7.32
	T_4 (ng/mL)	27.7	27.6	28.9	27.2	24.1**
	T_3 (ng/mL)	0.352	0.412	0.421	0.391	0.372
Week 24	TSH (ng/mL)	6.51	6.50	7.15	5.72	8.87***
	T_4 (ng/mL)	23.7	24.5	26.7	25.6	25.6
	T_3 (ng/mL)	0.260	0.287	0.372**	0.339	0.402***
Week 52	TSH (ng/mL)	6.66	5.96	6.76	5.56	7.12
	T_4 (ng/mL)	24.0	22.5	23.0	23.0	22.3
	T_3 (ng/mL)	0.420	0.389	0.397	0.376	0.469
Females						
No. of animals examined		24–25	10	9–10	10	23–25
Week 2	TSH (ng/mL)	5.05	5.45	5.09	5.49	8.07***
	T_4 (ng/mL)	29.1	28. 9	26.6	27.3	25.5
	T_3 (ng/mL)	0.656	0.759	0.660	0.692	0.646
Week 9	TSH (ng/mL)	4.75	4.91	5.22	5.69*	7.09***
	T_4 (ng/mL)	24.6	25.6	23.0	22.3	21.1*
	T_3 (ng/mL)	0.503	0.531	0.478	0.473	0.483
Week 24	TSH (ng/mL)	4.64	5.50	5.21	4.95	7.21***
	T_4 (ng/mL)	22.7	23.1	19.3	19.1	19.5
	T_3 (ng/mL)	0.410	0.415	0.397	0.392	0.472
Week 52	TSH (ng/mL)	5.09	4.70	5.49	5.91	7.30**
	T_4 (ng/mL)	21.9	19.9	23.4	16.8***	18.9*
	T_3 (ng/mL)	0.548	0.527	0.600	0.472	0.538

ppm: parts per million; T_3: triiodothyronine; T_4: thyroxine; TSH: thyroid stimulating hormone; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Source: Dange & Foulon (2002b)

being slightly higher in females at 250 ppm. Also in the liver, eosinophilic and basophilic "tigroid" foci of cellular alteration were noted in many animals from all groups. The incidence of both types (and the severity of "tigroid" foci) was slightly higher in males dosed at 250 ppm in comparison with controls. In females, the incidence of "tigroid" altered hepatocyte foci was lower in animals dosed at 250 ppm, with no effect on the incidence of eosinophilic foci of cellular alteration. In the thyroid, colloid mineralization was the predominant finding in rats of both sexes at 250 ppm and in females at 75 ppm. Females at 250 ppm also showed significant diffuse follicular hypertrophy. These changes are consistent with minor, chronic stimulation. In the kidneys, chronic progressive nephropathy was noted

in most animals from all groups. The severity, but not the incidence, was slightly higher in rats of both sexes at 250 ppm. In addition, the incidence of arteritis/periarteritis in the kidney was marginally higher than in controls in females at 250 ppm. This change may be related to the increased severity of chronic progressive nephropathy.

Table 11. Rat chronic toxicity and carcinogenicity study: non-neoplastic microscopic findings at week 52 (chronic phase) and week 104 (carcinogenicity phase)

			Dietary concentration (ppm)									
			Males					Females				
Organ	**Finding**		**0**	**5**	**20**	**75**	**250**	**0**	**5**	**20**	**75**	**250**
52 weeks												
Liver	Number examined		10	10	10	10	9	10	10	10	10	10
	Centrilobular hypertrophy		0	1	0	0	0	0	0	1	0	10
Thyroid	Number examined		10	10	10	10	9	10	10	10	10	10
	Follicular cell hypertrophy		0	0	1	3	5	0	0	2	3	9
	Mineralization, colloid		0	0	0	0	2	0	0	0	0	4
Ovaries	Number examined		–	–	–	–	–	10	0	0	2	10
	Mineralization, unilateral		–	–	–	–	–	1	0	0	0	0
	Sertoliform hyperplasia, unilateral		–	–	–	–	–	1	0	0	0	0
	Sertoliform hyperplasia, bilateral		–	–	–	–	–	0	0	0	0	0
	Cyst(s), unilateral		–	–	–	–	–	2	0	0	0	3
	Cyst(s), bilateral		–	–	–	–	–	3	0	0	2	1
104 weeks												
Liver	Number examined		60	60	60	60	60	60	60	60	60	60
	Hyperplasia, bile ducts	Slight	23	33	29	26	30	16	22	15	24	18
		Mild	14	1	4	14	8	3	1	2	5	14
		Moderate	3	0	1	0	1	0	0	0	0	1
		Total	40	34	34	40	39	19	23	17	29	33
	Sclerosis, bile ducts	Slight	18	29	26	28	33	9	13	6	17	22
		Mild	11	9	10	11	9	1	0	0	0	7
		Moderate	0	0	0	0	0	0	0	1	0	0
		Total	29	38	36	39	42	10	13	7	17	29
	Dilatation, sinusoidal, focal	Slight	4	2	9	8	5	14	13	14	17	28
		Mild	0	0	0	0	0	1	0	0	0	2
		Total	4	2	9	8	5	15	13	14	17	30
	Cellular alteration, basophilic, “tigroid”, focal	Slight	30	33	38	33	37	30	30	27	32	17
		Mild	2	2	3	6	10	10	13	8	4	4
		Moderate	0	0	0	0	3	2	0	0	2	0
		Marked	0	0	0	0	1	0	0	0	1	0
		Total	32	35	41	39	51	42	43	35	39	21

			Dietary concentration (ppm)									
			Males					Females				
Organ	**Finding**		**0**	**5**	**20**	**75**	**250**	**0**	**5**	**20**	**75**	**250**
	Cellular alteration, eosinophilic type, focal	Slight	23	20	16	16	32	22	32	24	17	29
		Mild	3	3	3	4	8	5	3	6	6	2
		Moderate	0	0	1	0	0	0	0	2	0	0
		Total	26	23	20	20	40	27	35	32	23	31
Thyroid	Number examined		60	60	59	60	59	59	59	60	60	60
	Mineralization, colloid	Slight	4	4	4	9	12	0	1	3	5	16
		Mild	3	2	0	2	6	0	0	0	2	16
		Moderate	1	1	1	3	11	0	0	0	2	10
		Marked	1	0	0	0	0	0	0	0	0	0
		Total	9	7	5	14	29	0	1	3	9	42
	Hypertrophy follicular, diffuse	Slight	1	0	2	3	3	0	0	0	3	14
		Mild	1	1	2	0	0	0	0	0	1	6
		Total	2	1	4	3	3	0	0	0	4	20
Kidneys	Number examined		60	60	60	60	60	60	60	60	60	60
	Nephropathy, chronic, progressive	Slight	33	32	33	36	28	37	36	30	28	26
		Mild	10	8	9	6	16	1	2	2	9	11
		Moderate	6	3	5	7	10	3	1	2	1	5
		Marked	0	0	1	1	3	0	0	0	3	2
		Severe	0	1	0	0	0	0	0	0	1	3
		Total	49	44	48	50	57	41	39	34	42	47
	Arteritis/ periarteritis	Slight	0	0	1	2	0	0	1	0	2	2
		Mild	0	0	0	0	1	0	0	0	1	2
		Moderate	0	0	0	0	0	0	1	0	0	2
		Total	0	0	1	2	1	0	2	0	3	6
Lungs	Number examined		60	60	60	60	60	60	59	60	60	59
	Alveolar macrophages, focal	Slight	22	22	24	18	21	17	18	19	24	29
		Mild	3	4	1	1	3	6	3	4	3	6
		Moderate	2	0	0	0	0	0	0	0	0	1
		Total	21	26	25	19	30	23	21	23	27	36
Ovaries	Number examined		–	–	–	–	–	60	60	60	60	60
	Atrophy		–	–	–	–	–	9	10	17	16	7
	Hyperplasia, tubular, focal		–	–	–	–	–	12	15	17	10	13
	Hyperplasia, sertoliform, focal		–	–	–	–	–	28	29	28	24	29
	Cyst(s), unilateral		–	–	–	–	–	25	29	25	29	16
	Cyst(s), bilateral		–	–	–	–	–	28	29	31	29	32

ppm: parts per million
Source: Dange & Foulon (2002b)

At 52 weeks, there was no evidence of a treatment-related increased incidence of neoplastic changes, nor were there any treatment-related neoplastic changes in the recovery groups.

In Table 12, the numbers of neoplastic findings in animals that died prematurely and animals that survived until scheduled termination in the carcinogenicity phase are combined. Neoplastic findings in the target organs (liver and thyroid) in the animals that died prematurely were as follows: Thyroid follicular cell adenoma was seen in four males dosed at 250 ppm, with no similar lesion in controls. Focal follicular cell hyperplasia was seen in one control male, one 5 ppm male, four 250 ppm males and one 250 ppm female. In the liver, hepatocellular adenoma was seen in one male dosed at 250 ppm. In the subcutis, adipose tissue lipoma was observed in three males at 250 ppm. In the ovaries, sex cord stromal tumours were found in females dosed at 5, 75 and 250 ppm (0, 1, 0, 4 and 3 in the 0, 5, 20, 75 and 250 ppm groups, respectively).

Table 12. Rat chronic toxicity and carcinogenicity study: microscopic neoplastic findings in animals that died prematurely and at scheduled termination

		Dietary concentration (ppm)									
		Males					**Females**				
Organ	**Finding**	**0**	**5**	**20**	**75**	**250**	**0**	**5**	**20**	**75**	**250**
Liver	Number examined	60	60	60	60	60	60	60	60	60	60
	Hepatocellular adenoma	0	0	0	0	3	0	0	0	1	0
Thyroid	Number examined	60	60	59	60	59	59	59	60	60	60
	Hyperplasia, follicular cell, focal	2	1	0	1	5	0	1	0	1	2
	Adenoma, follicular cell	0	0	0	0	4	0	0	0	0	2
	Carcinoma, follicular cell	0	0	0	0	0	0	0	1	1	0
	Total with focal proliferative lesions	2	1	0	1	8	0	1	1	2	4
Subcutis	Number examined	60	60	60	60	60	60	60	60	60	60
	Lipoma	0	0	1	1	5	0	0	0	1	1
Ovaries	Number examined	–	–	–	–	–	60	60	60	60	60
	One benign granulosa cell tumour	–	–	–	–	–	0	0	1	1	1
	One benign thecoma	–	–	–	–	–	2	0	0	0	0
	One sertoliform adenoma	–	–	–	–	–	2	4	1	4	7
	Two sertoliform adenomas	–	–	–	–	–	0	1	0	2	2
	One benign sex cord stromal tumour	–	–	–	–	–	0	0	1	0	0
	Total sex cord stromal tumours	–	–	–	–	–	4	5	3	7	10

ppm: parts per million
Source: Dange & Foulon (2002b)

At the scheduled termination, thyroid follicular cell adenoma was noted in two females in the 250 ppm group. In the liver, hepatocellular adenoma was noted in two males at 250 ppm and in one female at 75 ppm. In the subcutis, adipose tissue lipoma was observed in one male at 20 ppm, one male and one female at 75 ppm, and two males and one female at 250 ppm. In the ovaries, sex cord stromal tumours were found in all groups, but with a dose-related increase (4, 4, 3, 3 and 7 in the 0, 5, 20, 75 and 250 ppm groups, respectively).

A variety of spontaneous tumours were noted in animals from all groups, with no evidence of an effect of treatment. Tumours were commonly encountered (10 or more in a group) in the pituitary gland, thyroid gland (C-cells) and mammary gland (females). The spectrum was consistent with the range of spontaneous lesions encountered in ageing rats of this strain.

Overall, there was an increase in several tumours at 250 ppm: hepatocellular adenoma in males, subcutaneous lipomas in males and thyroid adenomas in males and females, which were in general outside the incidence in concurrent controls and the laboratory historical control data (Table 13) and therefore were considered treatment related. The increase in ovary sex cord stromal tumours was considered equivocal.

Table 13. Summary of background data for the selected tumours, compiled from the original study and the testing laboratory's historical control data

Tumour type	Study control group data	Testing laboratory's historical control data (2000–2003)
Subcutaneous lipoma		
Males		
Mean % incidence (no. of tumours / no. of rats examined / no. of studies)	n/a	1.8 (7 / 400 / 7)
Study range: maximum % (no. of tumours / no. of rats examined in that study)	0 (0 / 60)	3.3 (2 / 60)
Females		
Mean % incidence (no. of tumours / no. of rats examined / no. of studies)	n/a	1.8 (4 / 401 / 7)
Study range: maximum % (no. of tumours / no. of rats examined in that study)	0 (0 / 60)	2.0 (1 / 49)
Hepatocellular adenoma		
Males		
Mean % incidence (no. of tumours / no. of rats examined / no. of studies)	n/a	2.0 (8 / 405 / 7)
Study range: maximum % (no. of tumours / no. of rats examined in that study)	0 (0 / 60)	3.3 (2 / 60)
Females		
Mean % incidence (no. of tumours / no. of rats examined / no. of studies)	n/a	1.5 (6 / 405 / 7)
Study range: maximum % (no. of tumours / no. of rats examined in that study)	0 (0 / 60)	5.0 (3 / 60)
Ovary sex cord stromal tumours (total)		
Females		
Mean % incidence (no. of tumours / no. of rats examined / no. of studies)	n/a	1.0 (4 / 405 / 7)
Study range: maximum % (no. of tumours / no. of rats examined in that study)	2.4 (4 / 60)	3.7 (2 / 55)

F: female; M: male; n/a: not applicable

The slightly increased incidence of follicular cell hypertrophy observed at 12 months in males and females was not observed at 24 months and was therefore considered to be within biological variation because of the low control incidence.

The NOAEL for toxicity was 20 ppm (equal to 0.85 mg/kg bw per day), based on observed effects in the thyroid and/or liver (histopathological changes, increased organ weights and/or altered thyroid hormone or bilirubin levels) at 75 ppm (equal to 3.21 mg/kg bw per day). Increased incidences of tumours (subcutaneous lipoma in males, hepatocellular adenoma in males and thyroid adenomas in males and females) were noted at 250 ppm (equal to 10.8 mg/kg bw per day). The slight increase in ovary sex cord tumours at 250 ppm (equal to 14.7 mg/kg bw per day) was considered equivocal. The NOAEL for carcinogenicity was 75 ppm (equal to 3.21 mg/kg bw per day) (Dange & Foulon, 2002b).

2.4 Genotoxicity

Ethiprole is not mutagenic to bacterial or mammalian cells in vitro and is not clastogenic in human lymphocytes in vitro. It is not clastogenic and does not induce DNA repair in vivo. Ethiprole shows no genotoxic potential either in vitro or in vivo (Table 14).

Table 14. Overview of genotoxicity with ethiprole[a]

End-point	Test object	Concentration	Batch no., purity	Results	Reference
In vitro					
Bacterial gene mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2 *uvr*A	1st experiment: 8, 40, 200, 1 000 and 5 000 µg/plate in DMSO (± S9) 2nd: 156.25 (with S9 only), 312.5, 625, 1 250, 2 500 and 5 000 µg/plate in DMSO (± S9)	CDR9706, 93%	Negative	Ballantyne (1998)
Mammalian cytogenetic test	Human lymphocytes	Up to 800 µg/mL in DMSO (± S9)	OP9750149, 93%	Negative	Marshall (1998)
Mammalian cell gene mutation	Mouse lymphoma L5178Y *TK*$^{+/-}$	15.625–500 µg/mL in DMSO (± S9)	CDR9706, 93%	Negative	Fellows (1999)
In vivo					
Micronucleus test in mice	CD-1 mice	500, 1 000 and 2 000 mg/kg bw in 0.5% methylcellulose	CDR9706, 93%	Negative	Burman (1999)
Unscheduled DNA synthesis	Han Wistar rat (liver)	2 000 mg/kg bw in 0.5% methylcellulose	CDR9706, 91.5%	Negative	Howe (2001)

bw: body weight; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *TK*: thymidine kinase

[a] Positive and negative (solvent) controls were included in all studies.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a two-generation study of reproductive toxicity, four groups of 30 male and 30 female CD (Sprague Dawley) rats were given diets containing ethiprole (batch no. CDR9706; purity 92.7%) at a concentration of 0, 10, 75 or 500 ppm (equal to 0, 0.66, 4.8 and 32 mg/kg bw per day for males and 0, 0.78, 5.8 and 37 mg/kg bw per day for females, respectively, based on the 10-week premating feed intake in parental generation [F_0] animals).

Male and female rats were administered ethiprole in the feed for 10 weeks (premating period). Body weights and feed consumption were recorded weekly, and clinical signs were recorded at least once daily. Vaginal cytology was evaluated for the last 3 weeks of the prebreeding period. Animals were then randomly mated (one male with one female) within treatment groups for a 2-week mating period to produce the F_1 generation, with exposure continuing. F_0 males were necropsied after the delivery period, with histological evaluation of reproductive and other organs and andrological assessments (reproductive organ weights, epididymal sperm number, motility and morphology, testicular homogenization-resistant spermatid head counts, daily sperm production and efficiency of daily sperm production). The dams were allowed to rear their young to day 21 postpartum. F_1 litters were culled to 10 pups on postnatal day (PND) 4 and weaned on PND 21. At weaning, up to three weanlings of each sex per litter were necropsied, and 30 of each sex per dose were selected as F_1 parents of the F_2 generation. F_0 females were then necropsied, with measurement of organ weights, stage of estrus at necropsy, enumeration of ovarian primordial follicles, and histopathology of reproductive and other selected organs. Selected F_1 weanlings were administered ethiprole in the diet for a 10-week prebreeding exposure period; they were then mated for a 2-week period, as described above. The dams were allowed to rear their young to day 21 postpartum. On day 21 postpartum, each litter was weaned, and up to three weanlings of each sex per litter were necropsied. F_1 males were necropsied after the delivery period, with histopathology and andrological assessments (as described above). At weaning of the F_2 litters, parental F_1 females were then necropsied, with histopathology, as described above, and F_2 weanlings, up to three of each sex per litter, were necropsied.

There were no treatment-related deaths or clinical signs. At the start of the premating period, the body weights of F_1 animals at 500 ppm were significantly reduced (−13% for males and −17% for females, compared with controls), and body weight gains were reduced (−13%) in 500 ppm F_0 females in the premating period. Feed consumption was significantly lower for variable time points in F_0 males at 75 and 500 ppm and in F_0 females at 500 ppm.

At necropsy, F_0 parental absolute and relative liver weights (> +30% in males and > +70% in females) and thyroid weights (+28% in males and > +17% in females) and F_0 maternal adrenal weights (+11%) were significantly increased at 500 ppm. At 75 ppm, absolute and relative liver weights of F_0 females were slightly (+14%), but statistically significantly, increased. In F_1 animals, absolute and relative liver weights (> +26% in males and > +70% in females) and thyroid weights (> +32% in males and > +11% in females) were increased at 500 ppm. At 75 ppm, liver weights of F_1 females were slightly (+16% and +20% for absolute and relative weights, respectively), but statistically significantly, increased. At 500 ppm, histopathological findings were observed in the liver (hepatocyte cytoplasmic alteration in F_1 males, hypertrophy in F_0 and F_1 females) and thyroid (follicular cell hypertrophy in F_1 males and in F_0 and F_1 females). In the absence of concomitant histopathological findings, the increased liver weights at 75 ppm and increased adrenal weights at 500 ppm are considered not toxicologically relevant.

In general, JMPR considers increased liver weight with histopathological hypertrophy as an adaptive response in the absence of other liver effects. However, the liver weights were more than 170% of control values in both F_0 and F_1 females. Based on the rather large weight increase and the lack of blood parameter assessments, the liver effects at 500 ppm in females are considered adverse.

F_1 and F_2 pup body weights per litter were statistically significantly reduced at 500 ppm on PNDs 4, 7, 14 and 21. At necropsy at weaning, F_1 male and female pup body weights were statistically significantly reduced at 500 ppm. Absolute measured organ weights for F_1 pups were reduced at 500 ppm (gaining statistical significance for thymus, spleen, paired kidneys and brain, but not for liver); relative organ weights of spleen and kidneys were reduced, whereas relative liver and brain weights were increased. There were no treatment-related gross necropsy findings in either the F_1 males or the F_1 females at weaning.

During the postweaning/prebreeding exposure period of the selected F_1 offspring, the age at acquisition of female vaginal opening was statistically significantly delayed by approximately 2 days at 500 ppm. F_1 male preputial separation was delayed by approximately 3 days when expressed as age at acquisition or adjusted age at acquisition. Delay in acquisition of puberty for F_1 females was

associated with statistically significantly reduced body weights at acquisition at 500 ppm. These minimal effects on reproductive development in the F_1 offspring triggered measurement of anogenital distance in F_2 offspring at birth (PND 0), which showed no differences among groups.

At necropsy at weaning, F_2 male and female pup body weights were statistically significantly reduced at 500 ppm. Most likely due to reduced body weights, all absolute organ weights for F_2 pups were also reduced at 500 ppm. Relative organ weights for both sexes were either increased or unaffected (again, due to body weight reductions) at 500 ppm. There were no treatment-related gross necropsy findings in either the F_2 males or the F_2 females at weaning.

The NOAEL for parental toxicity was 75 ppm (equal to 4.8 mg/kg bw per day), based on reduced body weight and body weight gain and effects on liver and thyroid (histopathological and increased organ weights) at 500 ppm (equal to 32 mg/kg bw per day).

The NOAEL for offspring toxicity was 75 ppm (equal to 4.8 mg/kg bw per day), based on reduced body weight with associated delays in acquisition of puberty at 500 ppm (equal to 32 mg/kg bw per day).

The NOAEL for reproductive toxicity was 500 ppm (equal to 32 mg/kg bw per day), the highest dose tested (Tyl, Myers & Marr, 2001).

(b) Developmental toxicity

Rats

A dose range–finding developmental toxicity study was performed in groups of pregnant rats administered ethiprole by gavage at a dose of 0, 7.5, 25, 50 or 150 mg/kg bw per day from GD 6 to GD 20 (Foulon, 1999a). The results of this study were not remarkably different from those of the main developmental toxicity study described below.

In the main developmental toxicity study, ethiprole (batch no. CDR 9706; purity 92.7%) in 0.5% aqueous methylcellulose 400 was administered daily by gavage to groups of 23–25 mated Sprague Dawley (Crl: CD (SD) BR) rats from gestation day (GD) 6 to GD 20 at 0, 3, 10 or 30 mg/kg bw per day. All animals were examined twice daily except once daily on weekends, on public holidays and on GD 20. Body weight was determined on day 0 and on alternate days from GD 6 to GD 20. Feed consumption was determined during the intervals GDs 1–6, 6–8, 8–10, 10–12, 12–14, 14–16, 16–18 and 18–21. The fetuses were delivered by caesarean section on GD 21. The females were subjected to gross pathological examination. The gravid uterine weight was recorded. The dams were evaluated for the number of corpora lutea and the number and status of implantations (resorptions, dead and live fetuses). Live fetuses were removed from the uteri, counted, weighed, sexed and examined externally. Half of the fetuses were fixed in Bouin's solution and processed by scalpel and /or razor blade sectioning for visceral malformations. The other half of the fetuses were processed for skeletal and cartilage findings.

No deaths occurred in the study. Adverse maternal effects were noted at 30 mg/kg bw per day through body weight loss from GD 6 to GD 8 (Table 15) and reduced feed consumption from GD 6 to GD 12 (approximately 88% of control values).

The general conditions of females at 3 and 10 mg/kg bw per day were similar to those of controls. Macroscopic examination of females revealed increased mean liver weight at 10 and 30 mg/kg bw per day (111% and 115% of control values, respectively), and an accentuated lobular pattern was observed in 5/25 females at 30 mg/kg bw per day, compared with none in any of the other groups.

There were no significant effects of treatment on gestational parameters, including early and late resorptions, fetal death status and percentage of male fetuses. Analysis of mean fetal weights (calculated by sex and sexes combined) revealed no significant differences between treated and control groups. At 30 mg/kg bw per day, a few fetal findings were observed: increased incidences of enlarged thymus in male and female fetuses, of a slight ossification delay for a few bones (7th cervical centrum, 1st metatarsal and dumbbell at thoracic centrum) and of the anomalies at the 13th ribs (no, a short or a

discontinuous costal cartilage). No treatment-related malformations were observed. Minimal increased incidences in anomalies at the 4th and 7th costal cartilages and the thoracic centrum cartilage were observed at 30 mg/kg bw per day. At 10 and 30 mg/kg bw per day, one fetus per dose showed the presence of 25 presacral vertebrae, compared with none in the control and low-dose groups (Table 16).

Table 15. Developmental toxicity study in the rat: selected mean maternal body weights and maternal body weight change intervals

	0 ppm	3 ppm	10 ppm	30 ppm
Number of animals observed	25	23	23	25
Maternal body weight (g)				
Pretreatment: day 0	267.0	267.6	267.1	267.7
Pretreatment: day 6	301.0	299.4	301.1	300.6
Treatment day 8	307.0	305.0	304.5	300.0
Treatment day 21	438.8	439.9	443.7	430.5
Maternal body weight change (g)				
Pretreatment: days 0–6	34.1	31.8	34.0	32.9
Treatment days 6–8	6.0	5.6	3.4	−0.6***
Treatment days 8–10	10.7	10.1	11.3	9.7
Corrected body weight change[a]	65.8	64.0	66.8	56.8
Gravid uterine weight (g)	106.1	108.3	109. 8	106. 0
Liver weight (g)	14.2	14.8 (104%)	16.4*** (111%)	18.8*** (115%)

ppm: parts per million; ***: $P < 0.001$

[a] Corrected body weight change = (body weight on GD 21 − body weight on GD 0) − (gravid uterine weight).

Source: Foulon (2000)

Table 16. Developmental toxicity study in the rat: selected visceral and skeletal findings

	Number (%) of fetuses affected				Number (%) of litters affected			
	0 mg/kg bw per day	3 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	0 mg/kg bw per day	3 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day
Number of fetuses/litters examined	180	168	169	176	24	23	23	24
Visceral examinations								
Variations								
Thymus (unilateral/bilateral): enlarged	7 (3.8)	2 (1.2)	6 (3.6)	16 (9.3)	5 (20.8)	2 (8.7)	4 (17.4)	11 (44.0)
Skeletal examinations								
Variations								
7th cervical centrum: unossified/normal cartilage	6 (3.2)	4 (2.2)	14 (7.9)	13 (6.8)	5 (20.0)	3 (13.0)	7 (30.4)	9 (36.0)

	Number (%) of fetuses affected				Number (%) of litters affected			
	0 mg/kg bw per day	**3 mg/kg bw per day**	**10 mg/kg bw per day**	**30 mg/kg bw per day**	**0 mg/kg bw per day**	**3 mg/kg bw per day**	**10 mg/kg bw per day**	**30 mg/kg bw per day**
14th thoracic rib (unilateral)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
Thoracic centrum: dumbbell/normal cartilage	6 (3.2)	6 (3.5)	12 (7.6)	18 (9.6)	4 (16.0)	6 (26.1)	7 (30.4)	14 (56.0)
1st metatarsal (bilateral): unossified/normal cartilage	11 (5.6)	25 (14.0)	24 (13.8)	55 (29.1)	6 (24.0)	11 (47.8)	11 (47.8)	19 (76.0)
3rd and 4th proximal phalanges of forepaws (bilateral): unossified/normal cartilage	1 (0.6)	2 (1.3)	3 (1.6)	4 (2.4)	1 (4.0)	2 (8.7)	2 (8.7)	3 (12.0)
Anomalies								
13th rib(s) (unilateral/bilateral): short/discontinuous costal cartilage or costal cartilage absent	4 (2.1)	1 (0.6)	3 (1.7)	9 (5.0)	3 (12.0)	1 (4.3)	2 (8.7)	6 (24.0)
7th costal cartilages (bilateral) not attached to the sternum	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.0)
4th costal cartilage (unilateral): misshapen	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
Presence of 25 presacral vertebrae	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.5)	0 (0.0)	0 (0.0)	1 (4.3)	1 (4.0)
Thoracic centrum: bipartite and/or dumbbell or bipartite cartilage	2 (1.1)	2 (0.6)	2 (1.3)	5 (2.5)	2 (8.0)	1 (4.3)	2 (8.7)	5 (20.0)

bw: body weight
Source: Foulon (2000)

The NOAEL for maternal toxicity was 10 mg/kg bw per day, based on the slight decrease in body weight at GDs 6–8, decreased feed consumption and liver effects at 30 mg/kg bw per day.

The NOAEL for embryo/fetal toxicity was 10 mg/kg bw per day, based on the increased incidence of enlarged thymus and ossification delays in several bones at 30 mg/kg bw per day (Foulon, 2000).

Rabbits

A dose range–finding study was performed in which groups of eight pregnant rabbits were administered ethiprole by gavage at a dose of 0, 0.25, 0.75, 2 or 5 mg/kg bw per day from GD 6 to GD 28 (Foulon, 1999b). The results of this study were not remarkably different from those of the main developmental toxicity study described below.

In the main developmental toxicity study, groups of 30 artificially inseminated New Zealand white rabbits were exposed to ethiprole (batch no. CDR-9706; purity 92.7%) by gavage from GD 6 to GD 28. The doses given were 0, 0.5, 2.0 and 4.0 mg/kg bw per day in suspension in aqueous solution

of 0.5% methylcellulose 400. Two additional groups, a second control group and a low-dose group (0.25 mg/kg bw per day), were added to the study after a high abortion rate was observed at 4.0 mg/kg bw per day. The lower-dose group was added to ensure that at termination, there would be at least 20 pregnant females in each of the three treated groups. The additional control group was added to ensure that the newly added dose group (0.25 mg/kg bw per day) would have concurrent controls for statistical comparisons.

Maternal body weights were recorded for all females on GDs 0, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 29. Feed consumption was also measured daily from GD 1 to GD 29 for all females. Clinical observations were recorded daily. At scheduled termination, on GD 29, gravid uterine weight and number of ribs were recorded, and the dams were evaluated for number of corpora lutea and number and status of implantations (resorptions, dead and live fetuses). Live fetuses were removed from the uteri, counted, weighed and examined externally. The heads of fetuses from approximately half of each litter were immersed in Bouin fluid, and the internal structures were examined after fixation. The bodies of all fetuses were dissected for soft tissue anomalies. All the fetuses were sexed. Fetuses were eviscerated, skinned and fixed in absolute ethanol before staining. A subsequent skeletal examination was performed.

Three control rabbits and two rabbits dosed at 0.25 mg/kg bw per day died owing to gavage errors during the study. None of the deaths was considered to be related to treatment with ethiprole. No other unscheduled deaths occurred during the study. In the 2.0 mg/kg bw per day dose group, two females aborted (on GD 25 and GD 28) after body weight loss and reduced feed intake. Eleven females in the 4.0 mg/kg bw per day dose group aborted between GD 21 and GD 27. In all cases, this occurred after a severe reduction of feed intake since the start of dosing and a body weight loss. At necropsy, 10 out of these 11 females had a pale and/or accentuated lobular pattern of liver. Body weight changes and feed consumption at 2.0 and 4.0 mg/kg bw per day were statistically significantly reduced for many intervals during the dosing period, when compared with control values.

The mean number of late resorptions was slightly increased at 4.0 mg/kg bw per day. All the other litter parameters did not show any statistically significant treatment-related effects. Fetal weights were considered unaffected by treatment. No malformations at external, visceral or skeletal examination were considered to reflect any abnormal development linked to treatment, except for two fetuses at 4.0 mg/kg bw per day with a diaphragmatic hernia. Incomplete ossification was noted for several bones (pubis, metacarpal and/or middle phalanges) at 2.0 and 4.0 mg/kg bw per day. Increased incidences of enlarged fontanelles and the presence of 27th presacral vertebrae were also observed at 2.0 and 4.0 mg/kg bw per day. The single incidence of a 27th presacral vertebrae at 0.5 mg/kg bw per day is considered to be within the biological range (Table 17).

The NOAEL for maternal toxicity was 0.5 mg/kg bw per day, based on excessive maternal toxicity (abortion, decreased body weight and reduced feed consumption) at 2.0 mg/kg bw per day.

The NOAEL for embryo/fetal toxicity was 0.5 mg/kg bw per day, based on increased incidences of ossification delays in several bones (metacarpal, phalanges, pubis), enlarged fontanelles and the presence of 27 presacral vertebrae at 2.0 mg/kg bw per day (Foulon, 2002).

2.6 Special studies

(a) Acute neurotoxicity

The acute neurotoxicity of ethiprole (batch no. CD9706; purity 93.0%) was investigated in groups of 10 male and 10 female Crl:CD BR rats administered a single dose of 0, 100, 500 or 2000 mg/kg bw by oral gavage. Observations made at 4 hours, 7 days and 14 days after dosing included a functional observational battery (FOB) and motor activity assessment. At the end of the study period, all animals were killed and the tissues fixed by whole body perfusion. Examination was confined to designated tissues of the nervous system, which were subsequently examined microscopically.

Table 17. Developmental toxicity study in the rabbit: selected visceral and skeletal findings

	Number (%) of fetuses affected						Number (%) of litters affected					
Observation	**0 mg/kg bw per day**	**0 mg/kg bw per day**	**0.25 mg/kg bw per day**	**0.50 mg/kg bw per day**	**2.0 mg/kg bw per day**	**4.0 mg/kg bw per day**	**0 mg/kg bw per day**	**0 mg/kg bw per day**	**0.25 mg/kg bw per day**	**0.50 mg/kg bw per day**	**2.0 mg/kg bw per day**	**4.0 mg/kg bw per day**
Number of fetuses/litters examined	178	169	191	171	136	80	23	23	26	24	20	13
Number of heads/litters (heads) examined	83	80	90	78	63	38	22	23	26	23	20	12
Visceral examinations												
Malformations												
Diaphragmatic hernia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (15.4)
Skeletal examinations												
Variations												
Anterior and/or posterior fontanelle(s): enlarged	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (4.3)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.0)	1 (7.7)
Hyoid centrum: unossified	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	4 (6.2)	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	2 (15.4)
Presence of 27 presacral vertebrae	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	5 (2.6)	3 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)	2 (10.0)	2 (15.4)
1st metacarpal: incomplete ossification or unossified	18 (9.8)	19 (11.4)	20 (12.1)	17 (10.6)	39 (27.6)	31 (35.4)	6 (26.1)	12 (52.2)	13 (50.0)	11 (45.8)	13 (65.0)	8 (61.5)

Observation	Number (%) of fetuses affected						Number (%) of litters affected					
	0 mg/kg bw per day	**0 mg/kg bw per day**	**0.25 mg/kg bw per day**	**0.50 mg/kg bw per day**	**2.0 mg/kg bw per day**	**4.0 mg/kg bw per day**	**0 mg/kg bw per day**	**0 mg/kg bw per day**	**0.25 mg/kg bw per day**	**0.50 mg/kg bw per day**	**2.0 mg/kg bw per day**	**4.0 mg/kg bw per day**
Forepaws: 4th or/and 5th middle phalanges: unossified												
Pubis (unilateral/ bilateral): incomplete ossification	4 (3.1)	3 (1.2)	4 (2.5)	2 (1.0)	14 (8.2)	11 (11.0)	3 (13.0)	2 (8.7)	4 (15.4)	1 (4.2)	6 (30.0)	4 (30.8)

bw: body weight
Source: Foulon (2002)

There were no treatment-related clinical signs or changes in body weight noted at any dose. Decreased feed consumption was noted among females treated at 2000 mg/kg bw. Behavioural changes were confined to the day of treatment. Behavioural observations at 2000 mg/kg bw included licking and chewing behaviours, decreased activity and rearing counts in the arena, increased forelimb grip strength, decreased landing foot splay, decreased rectal temperature, and a lower level of locomotor activity when assessed in the Coulbourn system. At 500 mg/kg bw, behavioural observations were increased forelimb grip strength, decreased landing foot splay, decreased rectal temperature (in females only) and a lower level of locomotor activity when assessed in the Coulbourn system. Females were also observed in the arena as chewing and with lower activity and rearing counts. At 100 mg/kg bw, behavioural observations were limited to decreased landing foot splay in both males and females. Females also had a lower level of locomotor activity when assessed in the Coulbourn system. Females were also observed in the arena as chewing. All changes appeared to be fully reversible, as behavioural parameters, rectal temperature and activity counts were normal at later evaluation times. There was no evidence of neuropathology in this study.

No NOAEL was identified. The lowest-observed-adverse-effect level (LOAEL) was 100 mg/kg bw, the lowest dose tested, based on decreased landing foot splay (both sexes) and a lower level of activity compared with controls (females only) on the day of dosing only (Palmer, 2001a).

In a second acute neurotoxicity study, ethiprole (batch no. CDR 9706; purity 93.0%) was administered to groups of 10 male and 10 female Crl:CD BR rats as a single oral dose (by gavage) of 0, 10, 25, 35 or 250 mg/kg bw. All of these animals were subjected to an FOB prior to treatment, at 4 hours after dosing and at 7 and 14 days after treatment. Motor activity of each animal was also quantitatively assessed at the same intervals. Throughout the study, clinical signs, body weights and feed consumption were monitored. At the end of the observation period, all animals were killed. There were no further investigations.

There were no clinical signs of reaction to treatment following dosing. There were no differences in body weights, feed conversion ratios or feed consumption between treated and control groups. The FOB changes associated with treatment were confined to the day of treatment. There were no differences between groups 7 and 14 days after treatment. At 250 mg/kg bw, 4 hours after dosing, observations included lower levels of arousal, higher incidence of closure of eyes, and lower activity and rearing counts among females. Forelimb and hindlimb grip strengths were increased in males, and females showed a decreased hindlimb splay. Locomotor activity was statistically significantly decreased in females. At 35 mg/kg bw, very limited changes were observed: indications of a lower level of arousal and a higher incidence of closure of the eyes. No effects on motor activity or FOB assessments were seen at 10 or 25 mg/kg bw.

The NOAEL for acute neurotoxicity was 25 mg/kg bw, based on the lower level of arousal and a higher incidence of closure of the eyes at 35 mg/kg bw on the day of dosing only (Palmer, 2001b).

(b) Subchronic neurotoxicity

In a subchronic assessment of the potential neurotoxicity of ethiprole (batch no. CDR 9706; purity 93%), groups of 10 male and 10 female Crl:CD BR rats were given ethiprole by dietary administration at a concentration of 0, 20, 100 or 400 ppm (equal to 0, 1.4, 7.2 and 28.7 mg/kg bw per day for males and 0, 1.7, 8.4 and 33.0 mg/kg bw per day for females, respectively) for 13 weeks. All of these animals were subjected to an FOB prior to treatment and after 4, 8 and 13 weeks of treatment. Motor activity of each animal was also quantitatively assessed at the same intervals. Throughout the study, clinical signs, body weights and feed consumption were monitored. At the end of the treatment period, all animals were killed and the tissues fixed by whole body perfusion. Brain, thyroid and liver were weighed, but relative weights were not calculated. Examination was confined to designated tissues of the nervous system, which were subsequently examined microscopically in five males and five females per group in control and high-dose animals.

At 400 ppm, findings were limited to statistically significantly increased liver weights (122% and 165% of control values for males and females, respectively) and thyroid weights (144% and 135% of control values for males and females, respectively). At 100 ppm, only the thyroid weights among males were statistically significantly increased (124% and 114% of control values for males and females, respectively), whereas the liver weights showed a 104% and 128% increase relative to controls for males and females, respectively. There were no signs of toxicity at 20 ppm or effects noted in the behavioural or neuropathology assessment for any treatment group. At 20 ppm, the thyroid and liver weights were not statistically significantly increased (thyroid: 119% and 106% of control values for males and females, respectively; liver: 97% and 116% of control values for males and females, respectively).

The NOAEL for systemic toxicity was 20 ppm (equal to 1.4 mg/kg bw per day), based on a statistically significant increase in thyroid weight in males at 100 ppm (equal to 7.2 mg/kg bw per day). No signs of neurotoxicity were observed at 400 ppm (equal to 28.7 mg/kg bw per day), the highest dose tested (Palmer, 2001c).

In the public literature, two studies were found on the reproductive and neurobehavioural effects of maternal exposure in F_1 generation mice: Tanaka & Inomata (2017) and Tanaka, Suzuki & Inomata (2018).

In Tanaka & Inomata (2017), ethiprole (lot no. KPL1496; purity 99.5%) was administered to groups of 10 male and 10 female mice at a dietary concentration of 0%, 0.0003%, 0.0009% or 0.0027% (equal to 0, 0.48, 1.47 and 4.56 mg/kg bw per day for males and 0, 0.58, 1.75 and 4.96 mg/kg bw per day for females, respectively, based on feed intake during premating). F_0 animals were treated from 5 weeks of age onwards; at 9 weeks of age, each female was mated with one male for 5 days. Males were subsequently killed just after mating (at 9 weeks of age), and females were killed after weaning of the offspring. Ethiprole administration was continued in one male and one female offspring from each litter until the end of the study, when the selected offspring were 11 weeks of age. Body weight, exploratory behaviour, offspring parameters, and functional and behavioural developmental parameters were measured.

In Tanaka, Suzuki & Inomata (2018), the study design was similar to that described in Tanaka & Inomata (2017), except for slightly different doses, and mated females were used. Ethiprole treatment was stopped at weaning, and the study proceeded with one male and one female offspring from each litter until 11 weeks of age after weaning.

The authors reported several effects on functional and behavioural parameters and suggested that these could be caused by irreversible effects on the central nervous systems of the pups, due to exposure of the maternal animals until weaning. However, the authors did not take into account that none of the effects was consistent over time or sex, there was not a consistent dose–response relationship and/or the effects were not observed in the other study (Tanaka & Inomata, 2017; Tanaka, Suzuki & Inomata, 2018).

(c) Mechanistic studies

In a 28-day study in mice, the effect of ethiprole on liver proliferation and liver P450 enzymes was investigated (Langrand-Lerche, 2002). This study did not provide additional information relevant to the evaluation of ethiprole.

The mechanism of action of ethiprole on thyroid function in the rat was studied in 2-week studies (Webber, 2001a,b,c). These studies did not provide additional information relevant to the evaluation of ethiprole.

A single-dose toxicity study in non-pregnant rabbits was provided (Totis, 2011). The study was evaluated for the sake of completeness; however, as this study was performed in non-pregnant rabbits,

it cannot overrule the effects seen in the does in the developmental toxicity study (see section 2.5(b) above).

This study was designed specifically to investigate whether or not the findings seen in does in the rabbit developmental toxicity study (see section 2.5(b) above) were relevant to the establishment of an acute reference dose (ARfD). Ethiprole (batch no. 101016E021; purity 96.4%) was administered by oral gavage to groups of non-pregnant female New Zealand white rabbits (15 per group and subgroup) at a single dose of 0.85, 1.5 or 3.0 mg/kg bw. A similarly constituted group received the vehicle (0.5% aqueous methylcellulose 400) and acted as a control. Animals were terminated 24 hours (subgroup 1) or 14 days (subgroup 2) after dosing. Clinical observations were performed once for subgroup 1 and daily for subgroup 2. Body weight was measured during the acclimatization period and just before the oral administration in order to calculate the required dosing volume. Body weight and feed consumption were measured every 2 days for subgroup 2. On day 2 (subgroup 1) or day 14 (subgroup 2), blood samples were collected from the central ear artery of each animal. Serum was then separated from whole blood and used for thyroid hormone analysis (T_3 and T_4). Ten out of 15 animals randomly selected from each termination time group were subjected to necropsy, and liver and thyroid were weighed, fixed and examined microscopically. In addition, hepatic cytochrome P450 activities (total, pentoxyresorufin *O*-dealkylation, ethoxyresorufin *O*-deethylation, benzyloxyresorufin *O*-dealkylation) and uridine diphosphate glucuronosyltransferase isoenzymes were assessed in these selected animals.

Based on the absence of any clearly adverse effects in this study, the NOAEL was 3.0 mg/kg bw per day, the highest dose tested (Totis, 2011). It should be noted that comparison with the maternal effects in the rabbit developmental toxicity study (see section 2.5(b) above) is hampered by the fact that the animals in the current study were non-pregnant and the fact that the body weights were expressed to one significant figure only. Therefore, the effects seen at 2.0 mg/kg bw per day in the developmental toxicity study (abortions, body weight changes of <100 g) cannot be overruled by the results of the current study.

(d) Immunotoxic effects

No studies were submitted.

(e) Studies on metabolites

Acute oral toxicity

Acute oral toxicity studies with ethiprole metabolites are summarized in Table 18.

Table 18. Acute oral toxicity studies with ethiprole metabolites

Metabolite	Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	Reference
Amide metabolite of ethiprole (RPA 112916)	Rat	Wistar	M + F	Oral	94.5	>5 000	Dange (2001b)
Sulfone metabolite of ethiprole (RPA 097973)	Rat	Wistar	M + F	Oral	99.1	>2 000	Bigot (1999a)
Sulfide metabolite of ethiprole (RPA 107566)	Rat	Wistar	M + F	Oral	98.1	>2 000	Bigot (1999b)

bw: body weight; F: female; LD_{50}: median lethal dose; M: male

Short-term oral toxicity studies in rats

Groups of 10 male and 10 female Wistar (AF) rats received RPA 112916 (batch no. PAN01/99; purity 94.5%) in the diet at a concentration of 0, 50, 500, 5000 or 10 000 ppm (equal to 0, 5.2, 51.4, 515

and 983 mg/kg bw per day for males and 0, 5.2, 53.5, 512 and 993 mg/kg bw per day for females, respectively) for at least 28 days. Mortality and clinical signs were checked daily. Body weight and feed consumption were measured weekly throughout the study. A neurotoxicity assessment was performed on all animals during the acclimatization period and during week 4 of dosing; ophthalmic examinations were performed on all animals in the acclimatization period and on control and high-dose animals during week 4 of dosing. Clinical chemistry, haematology and urine analysis were performed towards the end of the dosing phase. In addition, a blood sample was collected during week 4 for T_3, T_4 and TSH analysis. All animals were subjected to necropsy, selected organs were weighed and designated tissues were sampled and examined microscopically.

RPA 112916 induced no treatment-related mortalities or clinical signs. Lower body weights (93% of control value) and body weight gains were noted in males at 10 000 ppm. Statistically significant decreases in mean feed consumption were observed in males at 10 000 ppm for the first 3 weeks and in females for the first week (82–88% of control values for males and 86% for females). Increases in prothrombin times were reported in males at 5000 and 10 000 ppm (107% and 115% of control values, respectively). At 5000 and 10 000 ppm, increases in mean total cholesterol (135% and 123% of control values, respectively) and triglyceride concentrations (150% and 110% of control values, respectively) were seen in females.

RPA 112916 induced an imbalance of thyroid hormones in animals treated at 5000 or 10 000 ppm, resulting in higher levels of TSH and lower levels of T_4. In females at 500 ppm, a statistically significant increase in TSH was also observed.

Significant increases in absolute and relative weights of liver, thyroid (males only) and adrenals were observed in males and females at 5000 and 10 000 ppm; at 500 ppm, increases in absolute and relative liver weights (males and females, <115% of controls) and absolute and relative adrenal weights (females) were observed. At histopathological examination, slight to mild centrilobular to panlobular hepatocellular hypertrophy was observed in all animals at 5000 and 10 000 ppm and in one male at 500 ppm. Thyroid follicular cell hypertrophy was found in six males and one female at 5000 ppm and in one male at 10 000 ppm. Slight adrenal microvacuolation of the zona fasciculata was observed at 500 (males only), 5000 and 10 000 ppm.

The NOAEL was 50 ppm (equal to 5.2 mg/kg bw per day), based on increased TSH and increased liver weight with associated histopathological changes at 500 ppm (equal to 51.4 mg/kg bw per day) (Bigot, 2000).

Genotoxicity

Genotoxicity data available for the amide metabolite (RPA 112916) and the sulfone metabolite (RPA 097973) of ethiprole are negative (Table 19).

Table 19. Overview of genotoxicity tests with ethiprole metabolites

Ethiprole metabolite	End-point	Test object	Concentration	Purity (%)	Results	Reference
Amide metabolite (RPA 112916)	Bacterial gene mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 *Escherichia coli* WP2 pKM101 and WP2 *uvr*A pKM101	Experiment 1: 0, 1.6, 8, 40, 200, 1 000 and 5 000 µg/plate in DMSO (± S9) Experiment 2: 0, 156.25, 312.5, 625, 1 250, 2 500 and 5 000 µg/plate in DMSO (± S9)	99.7	Negative	Ballantyne (2001)

Ethiprole metabolite	End-point	Test object	Concentration	Purity (%)	Results	Reference
Sulfone metabolite (RPA 097973)	Bacterial gene mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2 *uvr*A	Experiment 1: 0, 8, 40, 200, 1 000 and 5 000 µg/plate in DMSO (± S9) Experiment 2 (except TA100): 0, 20.48, 51.2, 128, 320, 800, 2 000 and 5 000 µg/plate in DMSO (± S9) Experiment 2 (TA100): 4.096, 10.24, 25.6, 64, 160, 400 and 1 000 µg/plate in DMSO (± S9)	99.1	Negative	Dawkes (1999)
Sulfide metabolite (RPA 107566)	Bacterial gene mutation	*S. typhimurium* TA98 and TA100	± S9 Non-GLP screening test, no further details provided	Un-known	Negative	Esdaile (2002)

DMSO: dimethyl sulfoxide; GLP: good laboratory practice; S9: 9000 × *g* supernatant fraction from rat liver homogenate

3. Observations in humans

No medical problems related to the handling of ethiprole at the Bayer AG (CropScience Division) plant in Hangzhou, China, were reported to plant or health and safety management since the start of production in 2010 until the end of 2017.

All employees at the Bayer AG (CropScience Division) plant in Dormagen, Germany, undergo annual examinations, not directly related to ethiprole handling.

No reports are available on any symptoms for researchers handling the product in field trials.

No published human poisoning cases or epidemiological studies have been identified.

Comments

Biochemical aspects

In metabolism studies conducted in rats, ethiprole was rapidly excreted (up to 86% at 48 hours) after administration of a single or repeated low dose of 5 mg/kg bw; at a single high dose of 1000 mg/kg bw, excretion (up to 77%) was slightly lower. After a single low dose, the majority of administered radioactivity was excreted in the faeces, with urinary excretion being 24–36% after 168 hours. Similar results were obtained after repeated low doses. At the high dose, faecal excretion was again the major route of excretion, and urine accounted for only 3–5% of excretion after 168 hours. After bile duct cannulation, the urinary excretion and biliary excretion were, respectively, approximately 11% and 67% in males and 30% and 52% in females at the low dose, and approximately 1% and 9% in males and 2% and 7% in females at the high dose. T_{max} values in whole blood and tissues were 8 and 48 hours at the low and high doses (5 and 1000 mg/kg bw, respectively). Tissue distribution was independent of sex, dose and duration of dosing. The highest radioactive residues were present in the liver and kidney (up to 12% and 1%, respectively, at the low dose, and up to 1% for both at the high dose) and glandular tissues, including thyroid.

Ethiprole is extensively metabolized. The pattern of metabolites in urine was independent of sex, dose and duration of dosing. The major components were the polar glucuronide conjugate of hydroxy-MB 45897, the sulfinic acid RPA 104615, as well as the less polar (non-conjugated) MB 45897 and, in female urine, the carboxylic acid RPA 112705. Three primary parallel metabolic pathways could be derived from the metabolites observed: 1) hydrolysis of the nitrile group to form the amide RPA

112916; 2) reduction of the sulfoxide group to form the sulfide RPA 107566, with subsequent alkyl oxidation; and 3) oxidation of the sulfoxide group to form the major metabolite (i.e. the sulfone RPA 097973), followed by further metabolic reactions, including conjugate formation (McCorquodale & Anderson, 1999).

Toxicological data

The oral median lethal dose (LD_{50}) for ethiprole in rats was greater than 7080 mg/kg bw (Steiblen, 1997).

In a 28-day toxicity study, mice were fed diets containing ethiprole at a concentration of 0, 50, 250, 1000 or 2500 ppm (equal to 0, 9.3, 47.4, 186.2 and 458.0 mg/kg bw per day for males and 0, 11.8, 57.9, 234.4 and 513.0 mg/kg bw per day for females, respectively). The NOAEL was 50 ppm (equal to 9.3 mg/kg bw per day), based on increased liver weights and histopathological changes in the liver at 250 ppm (equal to 47.4 mg/kg bw per day) (Dange, 1999).

In a 28-day toxicity study, rats were fed diets containing ethiprole at a concentration of 0, 20, 100, 500 or 2500 ppm (equal to 0, 1.8, 9.2, 46.1 and 219.3 mg/kg bw per day for males and 0, 2.0, 9.6, 46.3 and 220.2 mg/kg bw per day for females, respectively). The NOAEL was 20 ppm (equal to 1.8 mg/kg bw per day), based on adrenal effects (increased weight with slight increase in vacuolation) and slight effects on thyroid hormones at 100 ppm (equal to 9.2 mg/kg bw per day) (Dange, 2001a).

In a 90-day toxicity study, rats were fed diets containing ethiprole at a concentration of 0, 5, 20, 500 or 2500 ppm (equal to 0, 0.296, 1.17, 30.5 and 155 mg/kg bw per day for males and 0, 0.373, 1.50, 37.6 and 188 mg/kg bw per day for females, respectively). The NOAEL was 20 ppm (equal to 1.17 mg/kg bw per day), based on mortality, changes in thyroid hormone levels, changes in clinical chemistry parameters and increased liver and thyroid weights associated with microscopic changes in these target organs at 500 ppm (equal to 30.5 mg/kg bw per day) (Dange & Foulon, 2002a).

In a 90-day toxicity study, dogs received ethiprole at a dietary concentration of 0, 30, 90 or 200 ppm (equal to 0, 1.0, 3.2 and 7.6 mg/kg bw per day for males and 0, 1.1, 3.6 and 8.5 mg/kg bw per day for females, respectively). The NOAEL was 30 ppm (equal to 1.0 mg/kg bw per day), based on reduced body weight gain and decreased thymus weight with atrophy at 90 ppm (equal to 3.2 mg/kg bw per day) (Bigot, 2002).

In a 1-year toxicity study, dogs received ethiprole at a dietary concentration of 0, 9, 30 or 90 ppm (equal to 0, 0.27, 0.70 and 2.73 mg/kg bw per day for males and 0, 0.22, 0.76 and 2.51 mg/kg bw per day for females, respectively). The NOAEL was 30 ppm (equal to 0.70 mg/kg bw per day), based on overall reduced body weight gain at 90 ppm (equal to 2.51 mg/kg bw per day) (Chevalier, 2001).

The overall NOAEL for oral toxicity in dogs was 30 ppm (equal to 1.0 mg/kg bw per day), based on reduced body weight gain at 90 ppm (equal to 2.51 mg/kg bw per day).

In a 78-week chronic toxicity and carcinogenicity study in mice, ethiprole was administered in the diet at a concentration of 0, 10, 50, 150 or 300 ppm (equal to 0, 1.7, 8.6, 25.6 and 50.8 mg/kg bw per day for males and 0, 1.7, 12.5, 36.3 and 73.5 mg/kg bw per day for females, respectively). The NOAEL for toxicity was 150 ppm (equal to 36.3 mg/kg bw per day), based on a decrease in survival rate in females at 300 ppm (equal to 73.5 mg/kg bw per day). The NOAEL for carcinogenicity was 150 ppm (equal to 36.3 mg/kg bw per day), based on an increase in the incidence of hepatocellular adenomas in females at 300 ppm (equal to 73.5 mg/kg bw per day) (Richard, 2002).

In a 104-week combined chronic toxicity and carcinogenicity study in rats, ethiprole was administered in the diet at 0, 5, 20, 75 or 250 ppm (equal to 0, 0.22, 0.85, 3.21 and 10.8 mg/kg bw per day for males and 0, 0.29, 1.17, 4.40 and 14.7 mg/kg bw per day for females, respectively). The NOAEL for toxicity was 20 ppm (equal to 0.85 mg/kg bw per day), based on effects in the thyroid and/or liver (histopathological changes, increased organ weights and/or altered thyroid hormone or bilirubin levels) at 75 ppm (equal to 3.21 mg/kg bw per day). At 250 ppm (equal to 10.8 mg/kg bw per day), increased incidences of tumours (subcutaneous lipoma in males, hepatocellular adenoma in males and thyroid

adenoma in males and females) were noted, and the slight increase in ovary sex cord tumours was considered equivocal. The NOAEL for carcinogenicity was 75 ppm (equal to 3.21 mg/kg bw per day) (Dange & Foulon, 2002b).

The Meeting concluded that ethiprole is carcinogenic in mice and rats.

Ethiprole was tested for genotoxicity in an adequate range of in vitro and in vivo assays. No evidence of genotoxicity was found (Ballantyne, 1998; Marshall, 1998; Burman, 1999; Fellows, 1999; Howe, 2001).

The Meeting concluded that ethiprole is unlikely to be genotoxic.

In view of the lack of genotoxicity and the fact that tumours were observed only at doses unlikely to occur in humans, the Meeting concluded that ethiprole is unlikely to pose a carcinogenic risk to humans via exposure from the diet.

In a two-generation reproductive toxicity study, ethiprole was administered to rats in the diet at a concentration of 0, 10, 75 or 500 ppm (equal to 0, 0.66, 4.8 and 32 mg/kg bw per day for males and 0, 0.78, 5.8 and 37 mg/kg bw per day for females, respectively, based on the 10-week premating feed intake in F_0 generation animals). The NOAEL for parental toxicity was 75 ppm (equal to 4.8 mg/kg bw per day), based on decreased body weight and body weight gain and effects on liver and thyroid (histopathological effects and increased organ weights) at 500 ppm (equal to 32 mg/kg bw per day). The NOAEL for offspring toxicity was 75 ppm (equal to 4.8 mg/kg bw per day), based on reduced body weight with associated delays in acquisition of puberty at 500 ppm (equal to 32 mg/kg bw per day). The NOAEL for reproductive toxicity was 500 ppm (equal to 32 mg/kg bw per day), the highest dose tested (Tyl, Myers & Marr, 2001).

In a developmental toxicity study in rats, ethiprole was administered by oral gavage at a dose of 0, 3, 10 or 30 mg/kg bw per day on days 6–20 of gestation. The NOAEL for maternal toxicity was 10 mg/kg bw per day, based on the slight decrease in body weight at GDs 6–8, decreased feed consumption and liver effects at 30 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 10 mg/kg bw per day, based on the increased incidence of enlarged thymus and ossification delays in several bones at 30 mg/kg bw per day (Foulon, 2000).

In a developmental toxicity study in rabbits, ethiprole was administered orally by gavage from GDs 6 through 28 at a dose of 0, 0.25, 0.5, 2.0 or 4.0 mg/kg bw per day. The NOAEL for maternal toxicity was 0.5 mg/kg bw per day, based on excessive maternal toxicity (abortion, decreased body weight and reduced feed consumption) at 2.0 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 0.5 mg/kg bw per day, based on increased incidences of ossification delays in several bones (metacarpal, phalanges, pubis), enlarged fontanelles and the presence of 27 presacral vertebrae (variation) at 2.0 mg/kg bw per day (Foulon, 2002).

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

In an acute neurotoxicity study in rats, ethiprole was administered orally by gavage at a dose of 0, 100, 500 or 2000 mg/kg bw. No NOAEL was identified. The LOAEL was 100 mg/kg bw, the lowest dose tested, based on decreased landing foot splay (both sexes) and a lower level of activity compared with controls (females only) on the day of dosing only. There was no evidence of neuropathology after 14 days (Palmer, 2001a).

In a second acute neurotoxicity study in rats, ethiprole was administered orally by gavage at a dose of 0, 10, 25, 35 or 250 mg/kg bw. The NOAEL for acute neurotoxicity was 25 mg/kg bw, based on the lower level of arousal and a higher incidence of closure of the eyes at 35 mg/kg bw on the day of dosing only. There was no evidence of neuropathology after 14 days (Palmer, 2001b).

In a 90-day dietary neurotoxicity study, rats were fed ethiprole at a concentration of 0, 20, 100 or 400 ppm (equal to 0, 1.4, 7.2 and 28.7 mg/kg bw per day for males and 0, 1.7, 8.4 and 33.0 mg/kg bw per day for females, respectively). The NOAEL for systemic toxicity was 20 ppm (equal to 1.4 mg/kg bw per day), based on an increase in thyroid weight in males at 100 ppm (equal to 7.2 mg/kg bw

per day). No signs of neurotoxicity were observed at 400 ppm (equal to 28.7 mg/kg bw per day), the highest dose tested. There was no evidence of neuropathology at any dose (Palmer, 2001c).

The Meeting concluded that ethiprole induces transient neurobehavioural effects.

No studies on immunotoxic effects were submitted.

Toxicological data on metabolites and/or degradates

RPA 112916 (plant and rat metabolite)

The acute oral LD_{50} for the amide metabolite of ethiprole (RPA 112916) in rats was greater than 5000 mg/kg bw (Dange, 2001b).

In a 28-day dietary toxicity study, rats were fed RPA 112916 at a concentration of 0, 50, 500, 5000 or 10 000 ppm (equal to 0, 5.2, 51.4, 515 and 983 mg/kg bw per day for males and 0, 5.2, 53.5, 512 and 993 mg/kg bw per day for females, respectively). The NOAEL was 50 ppm (equal to 5.2 mg/kg bw per day), based on increased TSH and increased liver weight with associated histopathological changes at 500 ppm (equal to 51.4 mg/kg bw per day) (Bigot, 2000).

RPA 112916 was not mutagenic in an Ames test (Ballantyne, 2001).

RPA 112916 is found in bile of rats in low amounts (2–3% of the total radioactive residues) and is structurally very similar to its parent compound.

These findings indicate that the toxicological profile of RPA 112916 is very similar to that of its parent, ethiprole. It is concluded that RPA 112916 is not more potent than ethiprole.

RPA 097973 (plant metabolite)

The oral LD_{50} for the sulfone metabolite of ethiprole (RPA 097973) in rats was greater than 5000 mg/kg bw (Bigot, 1999a).

RPA 097973 was not mutagenic in an Ames test (Dawkes, 1999).

RPA 097973 is found in urine at over 10% of the dose, and its toxicity is considered to be covered by the parent compound.

***N-Glucuronide of RPA 107566 (goat metabolite*)**

The *N*-glucuronide of RPA 107566 is not found in the rat. RPA 107566 is a minor metabolite in rat, found in faeces at less than 5%. The acute oral LD_{50} for RPA 107566 is greater than 2000 mg/kg bw (Bigot, 1999b), and RPA 107566 is negative in a screening Ames test (Esdaile, 2002). The *N*-glucuronide of RPA 107566 is expected to be less toxic than RPA 107566.

For chronic toxicity, the threshold of toxicological concern approach (Cramer class III) could be applied to the *N*-glucuronide of RPA 107566.

Human data

In reports on manufacturing plant personnel, no adverse health effects were noted. No information on accidental or intentional poisoning in humans was identified.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.005 mg/kg bw, based on the NOAEL of 0.5 mg/kg bw per day for maternal (abortion, decreased body weight and reduced feed consumption) and embryo/fetal toxicity (ossification delays in several bones, enlarged fontanelles and the presence of 27 presacral vertebrae) in the developmental toxicity study in rabbits and using a safety factor of 100. The margin between the upper bound of the ADI and the LOAEL for liver, thyroid and skin tumours in rats is approximately 2000.

The Meeting established an ARfD of 0.005 mg/kg bw, based on the NOAEL of 0.5 mg/kg bw per day for maternal toxicity (decreased body weight and reduced feed consumption) in the developmental toxicity study in rabbits and using a safety factor of 100.

The Meeting concluded that the metabolites ethiprole-amide (RPA 112916) and ethiprole-sulfone (RPA 097973) would be covered under the ADI and the ARfD for ethiprole.

Levels relevant to risk assessment of ethiprole

Species	Study	Effect	NOAEL	LOAEL
Mouse	Seventy-eight-week study of toxicity and carcinogenicity[a]	Toxicity	150 ppm, equal to 36.3 mg/kg bw per day	300 ppm, equal to 73.5 mg/kg bw per day
		Carcinogenicity	150 ppm, equal to 36.3 mg/kg bw per day	300 ppm, equal to 73.5 mg/kg bw per day
Rat	Acute neurotoxicity study[b]	Neurotoxicity	25 mg/kg bw	35 mg/kg bw
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	20 ppm, equal to 0.85 mg/kg bw per day	75 ppm, equal to 3.21 mg/kg bw per day
		Carcinogenicity	75 ppm, equal to 3.21 mg/kg bw per day	250 ppm, equal to 10.8 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	500 ppm, equal to 32 mg/kg bw per day[c]	–
		Parental toxicity	75 ppm, equal to 4.8 mg/kg bw per day	500 ppm, equal to 32 mg/kg bw per day
		Offspring toxicity	75 ppm, equal to 4.8 mg/kg bw per day	500 ppm, equal to 32 mg/kg bw per day
	Developmental toxicity study[b]	Maternal toxicity	10 mg/kg bw per day	30 mg/kg bw per day
		Embryo and fetal toxicity	10 mg/kg bw per day	30 mg/kg bw per day
Rabbit	Developmental toxicity study[b]	Maternal toxicity	0.5 mg/kg bw per day	2.0 mg/kg bw per day
		Embryo and fetal toxicity	0.5 mg/kg bw per day	2.0 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
Dog	Thirteen-week and 1-year studies of toxicity[a,d]	Toxicity	30 ppm, equal to 1.0 mg/kg bw per day	90 ppm, equal to 2.51 mg/kg bw per day

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

Acceptable daily intake (ADI) (applies to ethiprole, ethiprole-amide and ethiprole-sulfone, expressed as ethiprole)

0–0.005 mg/kg bw

Acute reference dose (ARfD) (applies to ethiprole, ethiprole-amide and ethiprole-sulfone, expressed as ethiprole)

0.005 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 ethiprole

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; extensive (~80%) at 5 mg/kg bw; low (~10%) at 1 000 mg/kg bw
Dermal absorption	No data
Distribution	Highest residues in liver, kidney and glandular tissues, including thyroid
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Largely complete within 48 hours, primarily in bile and faeces
Metabolism in animals	Extensive, independent of sex, dose and duration of dosing; hydrolysis of nitrile group, reduction of sulfoxide group, oxidation of sulfoxide group
Toxicologically significant compounds in animals and plants	Ethiprole
Acute toxicity	
Rat, LD_{50}, oral	>7 080 mg/kg bw
Rat, LD_{50}, dermal	No data
Rat, LC_{50}, inhalation	No data
Rabbit, dermal irritation	No data
Rabbit, ocular irritation	No data
Guinea-pig, dermal sensitization	No data

Short-term studies of toxicity	
Target/critical effect	Body weight gain
Lowest relevant oral NOAEL	1.0 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Thyroid and liver
Lowest relevant NOAEL	0.85 mg/kg bw per day (rat)
Carcinogenicity	Carcinogenic in mice and rats[a]
Genotoxicity	
	No evidence of genotoxicity in vitro or in vivo[a]
Reproductive toxicity	
Target/critical effect	Body weight, liver, thyroid
Lowest relevant parental NOAEL	4.8 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	4.8 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	32 mg/kg bw per day, highest dose tested (rat)
Developmental toxicity	
Target/critical effect	Abortion, body weight, feed consumption, ossification delays, several skeletal variations
Lowest relevant maternal NOAEL	0.5 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	0.5 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	25 mg/kg bw
Subchronic neurotoxicity NOAEL	28.7 mg/kg bw per day, highest dose tested
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity	No data
Studies on toxicologically relevant metabolites	
RPA 112916	Acute oral LD_{50}: >5 000 mg/kg bw (rat) 28-day dietary toxicity NOAEL: 5.2 mg/kg bw per day (rat) Ames: Not mutagenic
RPA 097973	Acute oral LD_{50}: >5 000 mg/kg bw (rat) Ames: Not mutagenic
Human data	
	No adverse effects in manufacturing personnel

[a] Unlikely to pose a carcinogenic risk to humans via exposure from the diet.

Summary

	Value	Study	Safety factor
ADI	0–0.005 mg/kg bw[a]	Developmental toxicity study in rabbits	100
ARfD	0.005 mg/kg bw[a]	Developmental toxicity study in rabbits	100

[a] Applies to ethiprole, ethiprole-amide and ethiprole-sulfone, expressed as ethiprole.

References

Ballantyne M (1998). RPA107382: reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan-requiring strain of *Escherichia coli*. Unpublished report no. 198/115-D5140/R016832. Covance Laboratories Ltd, Harrogate, United Kingdom. Bayer File No. M-192448-01-1. Submitted to WHO by Bayer.

Ballantyne M (2001). RPA112916 (metabolite of ethiprole): reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli*. Unpublished report no. 1905/64-D6171/C016325. Covance Laboratories Ltd, Harrogate, United Kingdom. Bayer File No. M-202186-01-1. Submitted to WHO by Bayer.

Bigot D (1999a). Acute oral toxicity in the rat: RPA097973. Unpublished report no. SA 98546/R016844. Rhone-Poulenc Agro, Sophia Antipolis, France. Bayer File No. M-192471-01-1. Submitted to WHO by Bayer.

Bigot D (1999b). Acute oral toxicity in the rat: RPA107566. Unpublished report no. SA 98545/R016845. Rhone-Poulenc Agro, Sophia Antipolis, France. Bayer File No. M-192473-01-1. Submitted to WHO by Bayer.

Bigot D (2000). RPA112916 metabolite of ethiprole (RPA107382): 28-day toxicity study in the rat by dietary administration. Unpublished report no. SA 99387/R016688. Aventis CropScience S.A., Sophia Antipolis, France. Bayer File No. M-192137-01-1. Submitted to WHO by Bayer.

Bigot D (2002). RPA 107382: 90-day toxicity study in the dog by dietary administration. Amended report. Unpublished report no. SA 98340, C023975/C014168. Aventis CropScience S.A., Sophia Antipolis, France. Bayer File No. M-206098-02-1. Submitted to WHO by Bayer.

Burman M (1999). RPA 107382: induction of micronuclei in the bone marrow of treated mice. Unpublished report no. 198/119-D5140/R000181. Covance Laboratories Ltd, Harrogate, United Kingdom. Bayer File No. M-158055-01-1. Submitted to WHO by Bayer.

Chevalier G (2001). Ethiprole (RPA107382): 52-week dietary toxicity in beagle dogs. Unpublished report no. 17445 TCC, C013286. CIT Centre Internationale de Toxicologie, Evreux, France. Bayer File No. M-204326-01-1. Submitted to WHO by Bayer.

Dange M (1999). RPA 107382: preliminary 28 day toxicity study in the C57 B1/6N mice by dietary administration. Unpublished report no. SA 98331, R005957. Rhone-Poulenc Agro, Sophia Antipolis, France. Bayer File No. M-171883-01-1. Submitted to WHO by Bayer.

Dange M (2001a). Ethiprole RPA 107382: 28-day toxicity study in the rat by dietary administration. Unpublished report no. SA 99383, C011658. Aventis CropScience S.A., Sophia Antipolis, France. Bayer File No. M-201261-01-1. Submitted to WHO by Bayer.

Dange M (2001b). Acute oral toxicity in the rat: RPA 112916. Unpublished report no. SA 01040/C012671. Aventis CropScience S.A., Sophia Antipolis, France. Bayer File No. M-203164-01-1. Submitted to WHO by Bayer.

Dange M, Foulon O (2002a). RPA 107382: 90-day toxicity study in the rat by dietary administration. Unpublished report no. SA 97233, C021079/C016682. Aventis CropScience S.A., Sophia Antipolis, France. Bayer File No. M-192116-02-1. Submitted to WHO by Bayer.

Dange M, Foulon O (2002b). RPA 107382: chronic toxicity and carcinogenicity study of ethiprole (RPA 107382) in the Wistar rat by dietary administration. Unpublished report no. SA 97593, C021077/C012607. Aventis CropScience S.A., Lyon, France. Bayer File No. M-203024-02-1. Submitted to WHO by Bayer.

Dawkes N (1999). RPA097973: reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan-requiring strain of *Escherichia coli*. Amended final report. Unpublished report no. 198/126-D5140/R016656. Covance Laboratories Ltd, Harrogate, United Kingdom. Bayer File No. M-192043-01-1. Submitted to WHO by Bayer.

Esdaile DJ (2002). RPA 107566: intermediate of RPA 107382. Industrial hygiene hazard identification screen. Unpublished report no. SA 98065. Bayer CropScience, Sophia Antipolis, France. Bayer File No. M-217373-01-1. Submitted to WHO by Bayer.

Fellows M (1999). RPA 107382: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the microtitre fluctuation technique. Unpublished report no. 198/114-D5140/R000204. Covance Laboratories Ltd, Harrogate, United Kingdom. Bayer File No. M-158105-01-1. Submitted to WHO by Bayer.

Foulon O (1999a). Ethiprole (RPA107382). Range-finding study for developmental toxicity in the rat by gavage. Unpublished report no. SA 99036, R016879. Aventis CropScience S.A., Sophia Antipolis, France. Bayer File No. M-192545-01-1. Submitted to WHO by Bayer.

Foulon O (1999b). Ethiprole (RPA107382). Range-finding study for developmental toxicity in the rabbit by gavage. Unpublished report no. SA 99078, R016881. Aventis CropScience S.A., Sophia Antipolis, France. Bayer File No. M-192549-01-1. Submitted to WHO by Bayer.

Foulon O (2000). Ethiprole (RPA107382). Developmental toxicity study in the rat by gavage. Unpublished report no. SA 99146, R018797. Aventis CropScience S.A., Sophia Antipolis, France. Bayer File No. M-196111-01-1. Submitted to WHO by Bayer.

Foulon O (2002). Ethiprole (RPA107382). Developmental toxicity study in the rabbit by gavage. Unpublished report no. SA 99088, R016684. Aventis CropScience S.A., Sophia Antipolis, France. Bayer File No. M-192124-02-1. Submitted to WHO by Bayer.

Howe J (2001). Ethiprole (RPA 107382): measurement of unscheduled DNA synthesis in rat liver using an in vivo/in vitro procedure. Unpublished report no. 2014/31-D6173/C021030. Covance Laboratories Ltd, Harrogate, United Kingdom. Bayer File No. M-210455-01-1. Submitted to WHO by Bayer.

Langrand-Lerche C (2002). 28-day explanatory toxicity study in the C57BL/6 mouse. Ethiprole. Unpublished report no. SA 01416/C026077. Bayer CropScience S.A., Sophia Antipolis, France. Bayer File No. M-210729-01-1. Submitted to WHO by Bayer.

Marshall R (1998). RPA107382: induction of chromosome aberrations in cultured human peripheral blood lymphocytes. Unpublished report no. 198/116-D5140/R016593. Covance Laboratories Ltd, Harrogate, United Kingdom. Bayer File No. M-191907-01-1. Submitted to WHO by Bayer.

McCorquodale GY, Anderson AR (1999). [^{14}C]-RPA 107382: absorption, distribution, metabolism and excretion in the rat. Unpublished report no. C025330. Inveresk Research International Ltd, Tranent, United Kingdom. Bayer File No. M-192087-02-1. Submitted to WHO by Bayer.

Palmer HA (2001a). RPA107382: neurotoxicity to rats by acute oral administration. Unpublished report no. RNP/558, C016157. Huntingdon Life Sciences Ltd, Huntingdon, United Kingdom. Bayer File No. M-201848-01-1. Submitted to WHO by Bayer.

Palmer HA (2001b). RPA107382: neurotoxicity study by a single oral gavage administration to CD rats followed by a 14-day observation period. Unpublished report no. RNP/608, C016526. Aventis CropScience S.A., Sophia Antipolis, France. Bayer File No. M-202573-01-1. Submitted to WHO by Bayer.

Palmer HA (2001c). RPA107382: 13 week neurotoxicity study in rats by dietary administration. Unpublished report no. RNP/610/992922, C016595. Huntingdon Life Sciences Ltd, Huntingdon, United Kingdom. Bayer File No. M-202708-01-1. Submitted to WHO by Bayer.

Richard J (2002). Ethiprole (RPA107382): carcinogenicity study by oral route (dietary admixture) in C57BL/6 mice. Amended report. Unpublished report no. 17351 TCS, C023452/C014046. CIT Centre International de Toxicologie, Evreux, France. Bayer File No. M-205769-02-1. Submitted to WHO by Bayer.

Steiblen G (1997). RPA107382: acute oral toxicity in the rat. Unpublished report no. SA 97421, R016801. Rhone-Poulenc Agro, Sophia Antipolis, France. Bayer File No. M-192389-01-1. Submitted to WHO by Bayer.

Tanaka T, Inomata A (2017). Reproductive and neurobehavioral effects of ethiprole administered to mice in the diet. Birth Defects Res. 109:1568–85.

Tanaka T, Suzuki T, Inomata A (2018). Reproductive and neurobehavioral effects of maternal exposure to ethiprole in F_1-generation mice. Birth Defects Res. 110(3):259–75.

Totis M (2011). Ethiprole – Acute toxicity study in the rabbit by gavage. Unpublished report no. SA 10376. Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France. Bayer File No. M-416191-01-1. Submitted to WHO by Bayer.

Tyl RW, Myers CB, Marr MC (2001). Ethiprole 2-generation reproductive toxicity evaluation of ethiprole (RP107382) administered in the feed to CD (Sprague-Dawley) rats. Unpublished report no. 65C.07407.300, B003509. Research Triangle Institute, Research Triangle Park, NC, USA. Bayer File No. M-240522-01-1. Submitted to WHO by Bayer.

Webber C (2001a). Ethiprole investigation into potential effects on thyroid function after two weeks of treatment in male rats using the perchlorate discharge test. Unpublished report no. RNP/613 / C017842. Huntingdon Life Sciences Ltd, Huntingdon, United Kingdom. Bayer File No. M-205038-01-1. Submitted to WHO by Bayer.

Webber C (2001b). The effect of repeated oral doses of ethiprole on the biliary excretion of intravenously administered ^{125}I-thyroxine (T_4) in male rats. Unpublished report no. C017804. Huntingdon Life Sciences Ltd, Huntingdon, United Kingdom. Bayer File No. M-204974-01-1. Submitted to WHO by Bayer.

Webber C (2001c). The effect of repeated oral doses of ethiprole on the pharmacokinetics of intravenously administered ^{125}I-thyroxine (T_4) in male rats. Unpublished report no. C016605. Huntingdon Life Sciences Ltd, Huntingdon, United Kingdom. Bayer File No. M-202728-01-1. Submitted to WHO by Bayer.