Evaluation Report

ETHIPROLE

July 21, 2004

Food Safety Commission

Pesticides Expert Committee

(Progress of Evaluation)

January 15, 2003	Registration application submitted
October 29, 2003	The Ministry of Health, Labour and Welfare requests a health risk assessment in line with the establishment of maximum residue limits.
November 6, 2003	Meeting of the Food Safety Commission (explanation of MHLW's request outline)
December 3, 2003	3rd meeting of the Pesticides Expert Committee
June 2, 2004	Additional references accepted
June 9, 2004	12th meeting of the Pesticides Expert Committee
June 17, 2004	49th meeting of the Food Safety Commission (reporting of the discussion results from the Pesticides Expert Committee meeting)
June 17 to July 14, 2004	Public comments
July 21, 2004	Finalized

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Summary

Ethiprole (IUPAC: 5-amino-1-(2,6-dichloro-α, α, α-trifluoro-*p*-tolyl)-4-ethylsulfinylpyrazole-3-carbonitrile), belonging to the phenylpyrazole class of insecticides, was evaluated based on various tests.

The test results used for the evaluation were animal metabolism (rats), plant metabolism (rice, cotton, sweet peppers), soil degradation, photolysis in water, residues in crops, residues in soils, acute toxicity (rats), subchronic toxicity (rats, dogs), chronic toxicity (dogs), chronic toxicity/carcinogenicity (rats), carcinogenicity (mice), two-generation reproductive toxicity (rats), developmental toxicity (rats, rabbits), genotoxicity, etc.

The compound did not show any genotoxicity, teratogenicity or neurotoxicity. In the carcinogenicity tests, thyroid and liver tumors were observed in rats and in mice, respectively. The neoplasms were developed through non-genotoxic mechanisms, so the no observed adverse effect levels (NOAELs) were determined.

Based on the lowest value among NOAELs, which was 0.5 mg/kg bw/day in the rabbit developmental toxicity test, and after dividing this lowest value by a safety factor of 100, the ADI was settled as 0.005 mg/kg bw/day.

I. Outline of the Pesticide to be Evaluated

1. Usage

Insecticide

2. Common name (ISO name)

Ethiprole

3. Chemical name

IUPAC name:

5-amino-1-(2,6-dichloro-α, α, α-trifluoro-*p*-tolyl)-4-ethylsulfinylpyrazole-3-carbonitrile

CAS chemical name (No. 181587-01-9)

5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)-1*H*-pyrazole-3-carbonitrile

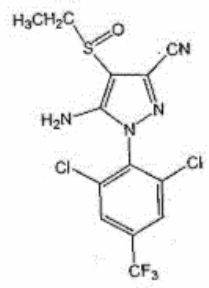
4. Chemical formula

$C_{13}H_9Cl_2F_3N_4OS$

5. Relative molecular mass

397.2

6. Chemical structure



7. Background of development

Ethiprole is a phenylpyrazole insecticide, and was discovered by Bayer CropScience Japan (former Rhone-Poulenc Agrochemicals) in 1994. The compound acts on the γ-aminobutyric acid-dependent neurotransmission system in insects.

Ethiprole has been registered in Indonesia as an insecticide applied to paddy rice.

In January 2003, Bayer CropScience Japan (hereinafter "The Applicant") applied for the registration of the compound according to the Agricultural Chemicals Regulation Law. Refs. 1-16 and 20-62 were submitted (Ref. 1).

II. Summary of the Tests Results

1. Absorption, distribution, metabolism, and excretion

Various studies were conducted using Ethiprole labeled with ^{14}C at the phenyl ring (hereinafter "^{14}C-Ethiprole").

The fate of Ethiprole was examined in SD rats (male and female) with a single gavage administration of 5 mg/kg bw (hereinafter "Single Low") or 1000 mg/kg bw (hereinafter "Single High") of ^{14}C-Ethiprole, and repeated oral administration of the non-labeled compound for 14 days followed by a single oral administration of 5 mg/kg bw of ^{14}C-Ethiprole (hereinafter "Repetitive").

In the treatment with Single Low and Single High, rats eliminated from 23.5 to 36.4% (Single Low) and from 2.96 to 5.13% (Single High) of the dosage in the urine and from 54.9 to 67.3% (Single Low) and from 87.5 to 88.4% (Single High) of the dosage in the feces by 168 hours, respectively. The compound was mainly eliminated in the feces both in Single Low and Single High and rarely in the breath.

In treatment with Repetitive, less than 0.9% of the radioactivity remained in the carcass, which was equivalent to those in the rats treated with Single Low and Single High. Therefore, the compound would not be accumulated in rats.

In rats (male and female) treated with Single Low, the radioactivity (from 54.5 to 66.7%) in the feces by 96 hours was substantially equivalent to that (from 51.6 to 67.2%) in the bile. This suggests that most of the radioactivity found in the feces had once been absorbed in the body, metabolized by the liver, excreted into the bile, and finally into the feces. Decrease in radioactivity in the urine (reduced from 23.3 to 11.0% in male, from 36.2 to 30.4% in female) could be explained by the reabsorption of the compound via enterohepatic circulation, followed by the excretion of the reabsorbed metabolite into the urine.

The plasma concentration of the compound reached the maximum at 8 hours after the administration in males and females treated with Single Low, and at 24 hours (38.7 μg eq/g) in males and at 48 hours (29.8 μg eq/g) in females treated with Single High. The plasma concentration subsequently exhibited a biphasic reduction, reaching 0.09, 0.17, 2.9 and 3.0 μg eq/g by 168 hours after the administration in males and females treated with Single Low and Single High, respectively.

The half-lives greatly varied among female rats treated with Single Low (113.8 h) but were within the range of 44.3 to 49.2 hours in other groups, showing no dosage effect. The longer half-life of the plasma concentration in females treated with Single Low was attributed to the slightly gentler gradient of the concentration curve in the β-phase where the plasma concentration was originally low. In terms of $T_{1/2}$ against C_{max}, no difference could be found in the time course of the plasma concentration among different treatment groups. Therefore, the actual time courses of the

plasma concentration seemed to be substantially identical in all treatment groups.

The residual radioactivity levels in major tissues in rats treated with Single Low and Single High are presented in Table 1. In Single High, the elimination of the radioactivity from tissues was slower in females than in males. Considering the facts that the initial absorption rate was slightly lower in the females and that the tissue concentrations in females decreased to levels not significantly different from those in the males by 168 hours, the slow decrease in tissue concentrations observed in the females before 96 hours seems to have no influence on the development of toxicity of Ethiprole.

Table 1. Residual Radioactivity in Major Tissues (μg eq/g)

Dose	Sex	8 hours*	48 hours	
Single Low	Male	liver (14.5), renal fat (11.7), adrenal gland (7.92), pancreas (6.42), kidney (5.36), thyroid gland (5.32), lung (4.25)	liver (1.61), plasma (0.81), kidney (0.50)	
	Female	liver (13.3), renal fat (11.4), adrenal gland (9.81), pancreas (7.56), kidney (5.87), thyroid gland (5.85), ovary (5.23), lung (4.45)	liver (0.77), renal fat (0.37), kidney (0.33), adrenal gland (0.31), plasma (0.30)	
Dose	Sex	48 hours*	96 hours	168 hours
Single High	Male	renal fat (208), thyroid gland (192), liver (161), adrenal gland (120), pancreas (92.9), kidney (65.6)	liver (14.5), skin/hair (11.8), plasma (7.9)	skin/hair (9.9), liver (1.8), thyroid gland (1.8), kidney (1.6)
	Female	renal fat (138), liver (138), adrenal gland (123), pancreas (86.1), brain (68.4), thyroid gland (64.6)	liver (56.3), renal fat (30.7), adrenal gland (27.6), ovary (27.5), pancreas (23.7), thyroid gland (20.0), kidney (19.7), lung (16.2)	thyroid gland (3.4), skin/hair (2.3), liver (1.7), adrenal gland (1.7), kidney (1.3)

* Around the time when the maximum blood concentration was reached.

The main metabolites detected in the urine were Q[1], R, I and J, while other metabolites such as F, S, U and V were also detected. Metabolites Q and S were assumed to be glucuronate conjugation and sulfate conjugation, respectively. Metabolite U is probably a parathiazine derivative formed through cyclization of I, and V is assumed to be a sulfate conjugation of H. The kinds of metabolites were not significantly different between Repetitive and Single, suggesting that repetitive administration of the compound does not affect the metabolic pathway. Similar kinds of metabolites were detected in the urine of males and females; however, metabolite I was more abundant in females than in males, and metabolite V was detected only in females.

Compared to the urine, fewer kinds of metabolites were detected in the feces. In Single Low, the main metabolite detected in the feces was I, both in males and females. In contrast to the urine, metabolite I was more abundant in the feces of males (22%) than of females (10%). Other metabolites such as H, B, D (in females only), J and E were also detected at lower levels. Only a trace amount (0.2-0.3%) of Ethiprole was detected in the feces. Meanwhile, in Single High, a large amount of unabsorbed Ethiprole was found in males (72.2%) and in females (77.0%). In males, the kinds of metabolites detected in Single High were the same as those detected in Single Low, indicating that the metabolic pathway was unaffected by the dosage. The metabolite composition was more simple in females, with only small amounts of metabolites J and E being detected other than Ethiprole. As with the case of the urine, repetitive administration of the compound did not affect the metabolic pathway. In a biliary excretion test using rats with and without bile duct cannulation, metabolite I was frequently detected in the feces in the former but not in the latter. Therefore, it is considered that the metabolite was excreted into the feces via the bile.

The main metabolic pathways of Ethiprole in rats could be ① the hydrolysis of the carbonitrile group, ② the reduction of the sulfoxide group followed by the oxidation of the alkyl group and ③ the oxidation of the sulfoxide group into a sulfone followed by either (a) the hydroxylation of the alkyl group followed by oxidation, sulfate conjugation or cyclization to produce a parathiazine derivative, and reduction of the sulfone, (b) oxidative dealkylation, substitution of the sulfone group with a hydroxyl group, reduction, sulfate conjugation and glucuronate conjugation or production of a sulfinic acid form via reduction, or (c) the oxidation of the carbonitrile group (Refs. 2-3).

2. Plant metabolism

(1) Rice

A total of 0.67 kg a.i./ha (1-fold treatment) or 3.35 kg a.i./ha (5-fold treatment) of

[1] See Appendix 1 for the abbreviations for the metabolites.

^{14}C-Ethiprole was applied to rice (*Oryza sativa*) twice (i.e., on 26 days and 14 days before harvest). The rice leaves and ears were sampled after the first application, before and after the second application, and on the harvest day.

The radioactivity distribution in rice straw, paddy, chaff and husked rice were from 89.3 to 93.4%, from 6.6 to 10.7%, from 5.6 to 9.4% and from 1.0 to 1.3%, respectively. Most of the radioactivity was detected in the straw, and the radioactivity detected in the husked rice was only about 10% of the total radioactivity detected in the paddy. With 1-fold treatment, Ethiprole accounted for 67% and 75.0% of the total residual radioactivity (TRR) in the husked rice and straw, respectively. The main metabolite was metabolite B, which was 20% and 34.6% of TRR in the husked rice and straw, respectively.

The main metabolic pathway of the compound in rice could be the production of a sulfone form (B) via the oxidation of the sulfoxide group (Ref. 4).

(2) Cotton

A total of 0.67 kg a.i./ha or 6.7 kg a.i./ha of ^{14}C-Ethiprole was applied to cotton (*Gossypium hirsutum*) twice (i.e., on 61 days and 48 days before harvest). The cotton leaves and pods (only on the harvest day) were sampled after the first application, before and after the second application, and on the harvest day.

The radioactivity in the cottonseeds was 0.2% of the total radioactivity in the pod. In the cottonseeds, Ethiprole was detected in the range of 1.4 to 7.0% TRR. The main metabolite detected in the cottonseeds was metabolite B, accounting for 2.1 to 2.9% TRR. Trace amounts of metabolites F, K and L were also detected.

The main metabolic pathways of Ethiprole in cotton could be the production of a sulfone form (B) via the oxidation of the sulfoxide group, further oxidation resulting in the production of a sulfonic acid form (F) and the dechlorination (K) of the sulfone form (Ref. 5).

(3) Sweet peppers

A total of 0.67 kg a.i./ha or 3.35 kg a.i./ha of ^{14}C-Ethiprole was applied to sweet pepper (*Capsicum annum*) twice (i.e., on 26 days and 14 days before harvest). The pepper leaves and fruits were sampled after the first application (foliage only), before and after the second application, and on the harvest day.

Substantially all radioactivity was detected from the leaves. Radioactivity detected in the fruit did not exceed 1% of the total radioactivity in the whole plant at any point in time. In fruits collected on the harvest day, Ethiprole accounted for 60% TRR, while metabolites B, C and F accounted for 16.4%, 5.3% and 2.6% of TRR, respectively.

The main metabolic pathways of the compound in sweet peppers could be the production of a

sulfone form (B) via the oxidation of the sulfoxide group and the production of an amide form (C) via the hydrolysis of the carbonitrile group (Ref. 6).

3. Fate in soil

(1) Degradation in flooded aerobic soil

Water was added to sandy loam soil in a weight ratio of 4:1, based on the dry weight of the soil to prepare the flooded aerobic soil. ^{14}C-Ethiprole was applied to the soil at 0.52 kg a.i./ha or 5.2 kg a.i./ha. The test was carried out through 12 months incubation at 20±1℃ in the dark.

As for the radioactivity distribution, no volatile radioactivity was detected throughout the test, and most of the total applied radioactivity (TAR) was distributed in the flooded soil. At the end of the test, Ethiprole was 11.3% TAR and major degradates B and E were 11.5% and 52.3% of TAR, respectively. The half-life of Ethiprole in the flooded soil was 5 days.

The main degradation pathways of Ethiprole could be the reduction (in the anaerobic layer) (E) and the oxidation (in water or the surface oxidizing layer) (B) of the sulfoxide group (Ref. 7).

(2) Degradation in aerobic soil

^{14}C-Ethiprole was applied to silt loam soil or sandy loam soil at 0.68 kg a.i./ha or 6.8 kg a.i./ha. The test was carried out through 12 months incubation at 25±1℃ in the dark.

As for the radioactivity distribution, volatile radioactivity was not detected throughout the test in the silt loam soil and slightly detected (0.02% TAR) after 365 days in the sandy loam soil. At the end of the test, Ethiprole was 1.7% of TAR, and degradates B, C, D and F were 34.6%, 19.0%, 27.3% and 3.7% of TAR, respectively. The half-lives of Ethiprole in the silt loam soil and the sandy loam soil were 71 and 30 days, respectively.

The main degradation pathways of Ethiprole could be ① the production of a sulfone form (B) via the oxidation of the sulfoxide group, ② the production of an amide form (C) via the hydrolysis of the carbonitrile group and ③ the production of D via the hydrolysis of the carbonitrile group in B or via the oxidation of C (Ref. 8).

(3) Degradation in anaerobic soil

Deionized water was added to loam soil to achieve a water depth of 2 cm or more, and ^{14}C-Ethiprole was applied to the soil at 0.59 kg a.i./ha. The test was carried out through 118 days incubation at 20±1℃ under an anaerobic condition.

As for the radioactivity distribution, volatile radioactivity was slightly detected (0.04% TAR or less) from 6 hours to 57 days after treatment. At the end of the test, Ethiprole was 2.21% of TAR, and degradates C, E and M were 5.75%, 67.0% and 9.11% of TAR, respectively. The half-life of Ethiprole in the flooded soil was 11.2 days.

The main degradation pathways of Ethiprole could be the reduction of the sulfoxide group (E) and the hydrolysis of the carbonitrile group (C) (Ref. 9).

(4) Degradation in anaerobic soil (degradate B)

Deionized water was added to sandy loam soil, and degradate B labeled with ^{14}C at the phenyl ring was applied to the soil at 0.53 kg a.i./ha. The test was carried out through 365 days incubation at 20±1°C under an anaerobic condition.

As for the radioactivity distribution, no volatile radioactivity was detected throughout the test. At the end of the test, degradates B and D were 58.1% and 27.7% of TAR, respectively. The half-life of degradate B in the flooded soil was 535 days.

The main degradation pathway of degradate B could be the production of an amide form (D) via the hydrolysis of the carbonitrile group (C) (Ref. 10).

(5) Adsorption to soils

An adsorption test in soils was performed using clay loam (Hatzenbeler), silty loam (Oregon), volcanic ash soil (Tochigi), and sandy soil (Miyazaki).

The adsorption rate at adsorption equilibrium was in the range of 24 to 53%, the adsorption coefficient (K) was in the range of 1.56 to 5.56 (the adsorption coefficient (K_{oc}) based on the organic carbon content was in the range of 50.5 to 163), and the adsorption coefficient (K_F) determined by adsorption isotherm equation was in the range of 1.48 to 5.93 (the adsorption coefficient (K_{Foc}) based on the organic carbon content was in the range of 53.9 to 158) (Ref. 11).

4. Fate in water

(1) Hydrolysis in water

^{14}C-Ethiprole was dissolved in buffer solutions of pH 4.0, 5.0, 7.0 and 9.0 to prepare Ethiprole solutions of about 3 μg/L. The solutions were incubated for 31 days at 25±1°C in the dark.

Ethiprole was stable against hydrolysis and showed no significant degradation at pH 4.0, 5.0 and 7.0. The compound was gradually hydrolyzed (83% remaining by 31 days) at pH 9.0. The half-life of Ethiprole in the buffer solution of pH 9.0 was 121 days.

The main degradation pathway could be the production of an amide form (C) via the hydrolysis of the carbonitrile group (Ref. 12).

(2) Photolysis in water (sterilized buffer solution)

^{14}C-Ethiprole was dissolved in a sterilized buffer solution of pH 5.0 to prepare an Ethiprole solution of about 3 μg/L. The solution was exposed to light (730 W/m^2 measured in the range of 290-800 nm wavelength) for 16 hours at 25±1°C to study photolytic degradation.

At the end of the test, Ethiprole accounted for 18.6% TAR, and the major degradates detected were N, P (including the predicted degradate X) and O, which accounted for 18.5%, 37.2% and 7.5% of TAR, respectively. The half-life of the compound under natural sunlight in spring at 35 degrees north latitude was 2.0 days.

The main degradation pathways could be the cyclization between the pyrazole ring and the phenyl ring (N) and the following hydroxylation (P, O) (Ref. 13).

(3) Photolysis in water (sterilized natural water)

^{14}C-Ethiprole was dissolved in a sterilized natural water buffer solution to prepare an Ethiprole solution of about 4.4 μg/L. The solution was exposed to light (765 W/m^2 measured in the range of 300-800 nm wavelength) for 96 hours at 25±0.2℃ to study photolytic degradation.

At the end of the test, Ethiprole accounted for 2.0% of TAR, and the major degradates detected were N, P and $^{14}CO_2$, which accounted for 1.0%, 4.9% and 14.7% of TAR, respectively. The half-life of the compound under natural sunlight in spring at 35 degrees north latitude was 1.3 days.

The main degradation pathways could be the cyclization between the pyrazole ring and the phenyl ring (N) and the following hydroxylation (P) (Ref. 14).

5. Residues in crops

Rice, apples and tea plants were used for residual tests in crops for Ethiprole (parent compound) and metabolite B.

As indicated in the results shown in Table 2, the highest residual value was 3.18 mg/kg in tea leaves (unrefined), which were collected 7 days after single application of 200 g a.i./ha of the compound. The values decreased to 2.45 and 0.35 mg/kg on 14 and 21 days after the application, respectively. The residual values of Ethiprole and metabolite B detected in husked rice did not exceed 0.05 mg/kg under all tested conditions (Refs. 15-16).

Table 2. Results of Residual Tests in Crops

Crops (parts analyzed) Year	Number of fields	Formulations	Amount used (g a.i./ha)	Applications (times)	PHI (days)	Residues (ppm)			
						Ethiprole		Metabolite B	
						Highest	Average	Highest	Average
Paddy rice (husked rice) 2000	2	P	200	1	14	<0.005	<0.005	<0.005	<0.005
				1	21	0.010	0.006*	0.006	0.005*
				1	28	0.009	0.006*	0.007	0.006*
				2	14	0.008	0.006*	0.005	0.005*
				2	21	0.012	0.008*	0.008	0.006*
				2	28	0.014	0.009*	0.010	0.007*
Paddy rice (straw) 2000	2	P	200	1	14	0.13	0.08	0.10	0.07
				1	21	0.10	0.07	0.17	0.11
				1	28	0.10	0.06*	0.18	0.11

				2	14	0.22	0.14	0.19	0.15
				2	21	0.10	0.07	0.17	0.12
				2	28	0.07	0.05	0.14	0.10
Paddy rice	2			2	14	0.026	0.020	0.016	0.012
(husked	1			2	19	0.03	0.028	0.016	0.013
rice)	2	WP	200	2	28	0.05	0.039	0.030	0.023
2002	2			2	42	0.015	0.011*	0.017	0.011*
	2			2	56	<0.01	<0.008	<0.01	<0.008
Paddy rice	2			2	14	0.8	0.48	0.8	0.53
(straw)	1			2	19	0.5	0.48	0.52	0.46
2002	2	WP	200	2	28	0.80	0.55	1.10	0.74
	2			2	42	0.28	0.21	0.55	0.38
	2			2	56	0.22	0.16*	0.41	0.28
Apple				2	14	0.398	0.186	0.031	0.019
(fruit)				2	21	0.145	0.074	0.020	0.015
2001	2	WP	400	2	28	0.031	0.025	0.012	0.009
				2	42	0.035	0.025	0.013	0.011
				2	56	0.012	0.009	0.007	0.006*
Tea plant				1	7	3.18	2.21	0.88	0.59
(unrefined	2	WP	200	1	14	2.45	1.36	1.19	0.67
leaf)				1	21	0.35	0.19	0.43	0.21
2001									
Tea plant				1	7	2.28	1.60	0.51	0.37
(leachate)	2	WP	200	1	14	1.59	0.98	0.72	0.44
2001				1	21	0.13	0.10	0.12	0.09

ai: Active ingredient
PHI: Pre-harvest interval
P: Powder preparation
WP: Wettable Powder
*For data including values below the detection limit, the average was calculated using the detection limit values and was marked *.
*In cases where all data were below the detection limit, the average of the detection limit values was taken and was marked <.
*The analytical values for metabolite B were expressed in terms of Ethiprole.
Ethiprole/metabolite B=397.2/412.2=0.96 was used as the conversion factor.

Based on the results of the residual tests in crops, the estimated intakes of Ethiprole (parent compound only) through cultivated agricultural products are shown in Table 3. The estimated amounts were calculated based on the assumption that Ethiprole was applied to all of the applicable crops under conditions that would give the highest residual value of Ethiprole among the applied methods, and that processing or cooking of the crops would not affect the amounts of residual pesticides.

Table 3. Estimated Amounts of Ethiprole exposure through Food (unit: μg/person/day)

Crops	Residues (mg/kg)	National average		Infants (ages 1 to 6)		Pregnant women		Elderly (age 65 or above)	
		ff	Intake	ff	Intake	ff	Intake	ff	Intake
Rice	0.039	185.1	7.22	97.7	3.81	139.7	5.45	188.8	7.36

Apple	0.074	35.3	2.61	36.2	2.68	30.0	2.22	35.6	1.89
Tea plant	2.21	3.0	6.63	1.4	3.09	3.5	7.74	4.3	9.50
Total			16.5		9.6		15.4		18.8

・The residual values listed here are the maximum values among the averages for individual test groups treated with the compound for various periods and frequencies registered (see Table 2).
・"ff": Agricultural product consumptions (g/person/day) based on the results of the National Nutrition Surveys in 1998-2000 (Refs. 17-19).
・"Intake": Estimated intakes (μg/person/day) of Ethiprole calculated from residual values and agricultural product consumptions.

6. Transfer to milk

Two Holstein cows were continuously given Ethiprole (4 mg/cow/day) and metabolite B (2.8 mg/cow/day) for 7 days by gavage to study the transfer of the compound to milk.

Neither Ethiprole nor metabolite B was detected from the milk samples taken from 1 day after the first administration to 5 days after the final administration (Ref. 20).

7. Residues in soils

Residual values of Ethiprole in soils were studied (both in the vessel and the field) using volcanic ash soil and mineral soil.

The assumed half-lives under the conditions tested are shown in Table 4. The half-life for Ethiprole alone was from 3.9 to 28 days, which was rather short, but the total of the half-lives of Ethiprole and degradates B, E, C and D was up to 254 days (Ref. 21).

Table 4. Results of Residual Tests in Soils (Assumed half-life)

Type of tests	Conditions	Concentration*	Kinds of soil	Ethiprole	Ethiprole + degradates B and E	Ethiprole + degradates B, E, C and D
Vessel	Paddy field	0.2 mg/kg	Volcanic ash soil	3.9 days	231 days	-
			Mineral soil	4.6 days	219 days	-
	Dry field	0.8 mg/kg	Volcanic ash soil	25 days	109 days	254 days
			Mineral soil	9.2 days	82 days	148 days
Field	Paddy field	200 g a.i./ha	Volcanic ash soil	4.2 days	54 days	-
			Mineral soil	3.9 days	5.4 days	-
	Dry field	700 g a.i./ha	Volcanic ash soil	18 days	32 days	39 days
			Mineral soil	28 days	83 days	88 days

* Pure form, wettable powder and granules were used in the vessel, paddy field and dry field tests, respectively.

8. Acute toxicity

Acute oral and dermal toxicities of Ethiprole were studied using Wistar rats, and the acute inhalation toxicity was studied using SD rats.

Wistar rats (groups of 5 males and 5 females) were given single oral administration of Ethiprole at doses of 5000 or 7080 mg/kg bw. One male and two females died at 5000 mg/kg bw. In this group, there were a decrease in locomotor activity, drooping eyelid and hunchback position, which probably reflected central nervous system depression. None of the males died at 7080 mg/kg bw, while a female died with no clinical symptom. Experiment was repeated under the same condition at 2000 and 5000 mg/kg bw. Both in males and females, one out of 5 rats and 2 out of 5 rats died at 2000 and 5000 mg/kg bw, respectively. In these groups, neurological symptoms such as sedation, muscle tone, hunchback position and irritation were observed. Based on these results, the acute oral LD_{50} was >7080 mg/kg bw in male and female rats. The absence of dose relationship was attributed to the saturation of systemic absorption. The acute dermal LD_{50} was >2000 mg/kg bw and the acute inhalation LD_{50} was >5.2 mg/L in male and female rats (Refs. 22-25).

Acute oral toxicities of 8 metabolites were also studied using Wistar and SD rats.

The acute oral LD_{50}s of metabolites C, D and K were >5000 mg/kg bw in male and female rats. The acute oral LD_{50}s of metabolites B, E, P and F were >2000 mg/kg bw in male and female rats. The acute oral LD_{50} of metabolite N was from 423 to 439 mg/kg bw in rats (Refs. 26-33).

9. Skin and eye irritation, and skin sensitization

A test on New Zealand White rabbits revealed no sign of irritation (Refs. 34-35).

Skin sensitization potential was assessed in guinea pigs by the Maximization method. No skin sensitization was observed (Ref. 36).

10. Subchronic toxicity

(1) Subchronic toxicity test for 90 days in rats

Wistar rats (groups of 10 males and 10 females) were fed diets containing Ethiprole, technical grade at 0, 5, 20, 500, or 2500 ppm for 90 days.

Eight males died at 2500 ppm. And one male and 3 females died at 500 ppm. At this dose, piloerection, change in the motor activity, and increases in the platelet count, triglyceride, blood potassium and T_3[2] and a decrease in MCHC were observed in males and females. Piloerection, change in the motor activity, low body weight, decreases in food consumption, hematocrit, hemoglobin and total cholesterol, an increase in ALT and hypertrophy and necrosis of hepatocytes in males, and yellow-brown pigmentation in the kidney in females were detected. At this dose

[2] See Appendix 2 for the abbreviations for the test values, etc.

and higher, decreases in MCV, MCH and T_4, and increases in total protein, blood calcium, TSH and the relative liver and thyroid weights (relative weight against body weight will be described as relative weight hereafter), hypertrophy in the liver and thyroid gland, darkening of the liver and kidney, centrilobular hypertrophy of hepatocytes, hypertrophy/hyperplasia of the thyroid follicular epithelial cells and hypertrophy of hepatocytes (significant only in males at 2500 ppm) in males and females. Prothrombin time was extended in males and hematocrit, hemoglobin, total cholesterol and blood chlorine were decreased in females.

The causes of death of 8 males at 2500 ppm were considered to be that the over dose treatment with Ethiprole above the maximum tolerance dose damaged the liver and consequently the blood coagulating system, showing hemorrhage in various organs and severe hepatocellular necrosis in the dead animals and extension of the prothrombin time in the survivors, resulting in the deterioration of the physical status.

The NOAEL would be 20 ppm (1.2 mg/kg bw/day for males, 1.5 mg/kg bw/day for females) (Refs. 3, 37).

(2) Subchronic toxicity test for 90 days in dogs

Beagle dogs (groups of 4 males and 4 females) were fed diets containing Ethiprole, technical grade at 0, 30, 90, or 200 ppm for 90 days.

At 200 ppm, there were depletion of hepatic glycogen in males and females, and a fatal case, suppression in body weight gain (no significant difference) and an increase in ALP in females. At 90 ppm and higher, there were suppression in body weight gain (no significant difference at the dose of 90 ppm), a decrease in the absolute testis weight, centrilobular hypertrophy of hepatocytes, thymus atrophy and prostatic immaturity in males. There were a decrease in the relative prostate weight and absence of epididymal sperm in males at 30 ppm and higher. In all groups including the control group, immature testis and a decrease in the number of epididymal sperms were observed.

The decreases in the prostate and testis weights and the absence of epididymal sperm observed in 90 ppm and higher groups were not detected in the dogs treated with 30 and 90 ppm Ethiprole in a chronic toxicity study, in which same age (about 6-month-old) dogs were used at the start. In addition, the decreases were within the range of the historical background data (except for the prostate weight in 1 case at 200 ppm). Therefore, these changes were considered to be a delay in sexual development due to the suppressed body weight gain in these dose groups.

No histopathological change accompanied the decrease in the relative prostate weight, which were within the range of the historical background and the absence of sperm in the epididymis (1 case) at 30 ppm. The administration period (starting from 5- to 6-month-old) of this study corresponds to the developing period of the reproductive system in dogs, therefore, these changes

observed at 30 ppm were considered to be incidental, or a mild delay in sexual development, but were not toxicologically significant.

The NOAEL would be 30 ppm (1.0 mg/kg bw/day for males, 1.1 mg/kg bw/day for females) (Refs. 3, 38).

(3) Subchronic neurotoxicity test for 90 days in rats

CD rats (groups of 10 males and 10 females) were fed diets containing Ethiprole, technical grade at 0, 20, 100, or 400 ppm for 90 days.

At 400 ppm, there were an increase in the liver weight in males and females and an increase in the thyroid weight in females. There was an increase in the thyroid weight in males at 100 ppm and higher. At the highest dose, there was a slight axonal degeneration in peripheral nerves, which was within the range of the background control data. Similar lesions to the degeneration were not detected in the combined study of chronic toxicity and carcinogenicity, suggesting that the lesion was not caused by the Ethiprole administration. Therefore, the compound showed no neurotoxicity.

The NOAELs would be 20 ppm (1.4 mg/kg bw/day) for males and 100 ppm (8.4 mg/kg bw/day) for females (Refs. 3, 39).

11. Chronic toxicity/carcinogenicity study

(1) Chronic toxicity study for 1 year in dogs

Beagle dogs (groups of 5 males and 5 females) were fed diets containing Ethiprole, technical grade at 0, 9, 30, or 90 ppm for 1 year. At 90 ppm, there was suppression in body weight gain in males and females.

The NOAEL would be 30 ppm (0.70 mg/kg bw/day for males, 0.76 mg/kg bw/day for females) (Ref. 40).

(2) Combined study of chronic toxicity for 52 weeks and carcinogenicity for 104 weeks in rats

Wistar rats (carcinogenicity test groups: 60 males and 60 females, satellite groups: 10 males and 10 females, recovery groups: 15 males and 15 females) were fed diets containing Ethiprole, technical grade at 0, 5, 20, 75, or 250 ppm for 52 and 104 weeks for studying chronic toxicity and carcinogenicity, respectively.

At 250 ppm, there were a decrease in MCHC, increases in total protein and the relative thyroid weight, hypertrophy and focal hyperplasia (not significant) of the thyroid follicular cell and thyroid follicular cell adenoma (not significant) in males and females (see Table 5). In males at the same dose, decreases in hemoglobin and T_4, and increases in albumin, TSH, the relative liver weight, mineral deposition in thyroid colloid and basophilic altered foci in the liver, acidophilic

altered foci and progressive chronic nephropathy were observed. In females, decreases in MCV and MCH, and increases in RBC, platelet count, cholesterol and blood calcium, centrilobular hypertrophy of hepatocytes, fibrosis in bile ducts, diffuse hypertrophy of the thyroid follicular cell, focal sinusoidal dilation in the liver, renal arteritis/periarteritis and alveolar macrophage aggregation were observed. At 75 ppm and higher, there were an increase in MCV, extension of prothrombin time and fibrosis in bile ducts in males and decreases in prothrombin time and T_4, increases in TSH and the relative liver weight, mineral deposition in thyroid colloid and bile duct proliferation in females. The incidences of neoplastic lesions were not significantly different from those in the control groups (see Table 5).

In the present study, there were focal hyperplasia of the thyroid follicular cell and thyroid follicular cell adenoma. The test results and those obtained from other toxicity tests in **14 (1)** suggest that the administration of Ethiprole induces hepatic drug-metabolizing enzymes such as β-glucuronyltransferase, showing similarity to the induction by phenobarbital. The induction might promote the excretion of T_4 into the bile and reduce its blood concentration, which alter the hypothalamus-piruitary-thyroid axis and control to increase the blood TSH concentration. The TSH increase seems to be a trigger to stimulate the thyroid gland continuously. Taken together, Ethiprole treatment indirectly exerts an adverse effect on the thyroid via excessive stimulation.

The incidence of myelin degeneration in the sciatic nerve increased in females that died or were humanely killed in the middle of the study in the carcinogenicity test groups at 20 ppm and higher, although the incidence was not significantly different from that in the control group, neither in the terminal sacrifice nor in all animals examined. The increase in degenerative lesion in the sciatic nerve might be related to the earlier death and/or humanely killed animals in the control group, as the lesion had insufficient time to develop. Therefore, the increase is not considered to be treatment related.

The NOAEL would be 20 ppm (0.85 mg/kg bw/day for males, 1.17 mg/kg bw/day for females) (Refs. 41, 59-61).

Table 5. Incidence of thyroid tumor in rats administered Ethiprole for 104 weeks

Sex	Male					Female				
Dose (ppm)	0	5	20	75	250	0	5	20	75	250
Number of rats	60	60	59	60	59	59	59	60	60	60
Focal hyperplasia of the thyroid follicular cell	2	1	0	1	5	0	1	0	1	2
Thyroid follicular cell adenoma	0	0	0	0	4	0	0	0	0	2
Thyroid follicular cell carcinoma	0	0	0	0	0	0	0	1	1	0
Total of focal proliferative lesions	2	1	0	1	9	0	1	1	2	4

Not significantly different in Fisher's exact test.

(3) Carcinogenicity test for 78 weeks in mice

C57BL/6 mice (groups of 50 males and 50 females) were fed diets containing Ethiprole, technical grade at 0, 10, 50, 150, or 300 ppm for 78 weeks.

There were increases in ALT and the relative liver weight, clear altered foci in the liver and fatty change of hepatocytes in males, and hepatic cell adenoma in females at 300 ppm (see Table 6). There was an increase in the relative liver weight in males at 150 ppm and higher.

Test results obtained from other toxicity tests including the "Liver toxicity test" (see **14 (2)**) suggest that Ethiprole has a tumor promoting activity, which mechanism is similar to that of phenobarbital, which had caused the hepatic cell adenoma in females at 300 ppm.

The NOAELs would be 150 ppm (25.6 mg/kg bw/day) for males and 50 ppm (12.5 mg/kg bw/day) for females (Refs. 42, 62).

Table 6. Incidence of liver tumor in rats administered Ethiprole for 78 weeks

Sex	Male					Female				
Dose (ppm)	0	10	50	150	300	0	10	50	150	300
Number of rats	49	50	50	50	50	50	50	50	50	50
Hepatic cell adenoma	5	5	4	1	1	0	2	1	2	6*
Hepatic cell carcinoma	0	3	1	0	1	0	0	0	0	0

* Fisher's exact test, $P<0.05$

12. Reproductive/developmental toxicity

(1) Two-generation reproductive toxicity study in rats

SD rats (groups of 30 males and 30 females) were fed diets containing Ethiprole, technical grade at 0, 10, 75, or 500 ppm for two generations.

In the parent animals, there were increases in the relative liver and thyroid weights in P males and females, suppression in body weight gain, an increase in the relative adrenal weight, hypertrophy of hepatocytes and thyroid follicular cells and darkening of the liver and kidney in P females, decreases in the relative liver, thyroid and pituitary weights and hypertrophy of thyroid follicular cells in F_1 males and females, hypertrophy of hepatocytes in F_1 males, and suppression in body weight gain, an increase in the absolute spleen weight, hypertrophy of hepatocytes and darkening of the liver and kidney in F_1 females at 500 ppm. Also, there were a delay in preputial separation in F_1 males and a delay in vaginal opening in F_1 females at 500 ppm.

In the offspring, there were low body weight, decreases in the absolute thymus and spleen weights and the relative kidney weight and increases in the relative liver and brain weights in males and females of F_1 and F_2 rats at 500 ppm.

The NOAELs in this study for parent animals and offspring would be 75 ppm (P male: 4.77 mg/kg bw/day, P female: 5.82 mg/kg bw/day, F_1 male: 6.03 mg/kg bw/day, F_1 female: 6.76 mg/kg bw/day) for both sexes of rats (Refs. 3, 43).

(2) Developmental toxicity study in rats

A developmental toxicity study was conducted in SD rats (groups of 25 females). The animals were given Ethiprole on days 6-21 of pregnancy at 0, 3, 10, or 30 mg/kg bw/day by gavage.

In dams, there were suppression in body weight gain, a decrease in food consumption and clear image of the hepatic lobule at 30 mg/kg bw/day. There was an increase in the liver weight at 10 mg/kg bw/day and higher. In fetuses, there were increases in incidences of dumbbell ossification of thoracic centrum and incomplete ossification of the metatarsal at 30 mg/kg bw/day.

The NOAELs in this study would be 3 mg/kg bw/day for dams and 10 mg/kg bw/day for fetuses. No teratogenicity of Ethiprole was evident (Ref. 44).

(3) Developmental toxicity study in rabbits

New Zealand White rabbits (groups of 30 females) were given Ethiprole on days 6-28 of pregnancy at 0, 0.25, 0.5, 2.0, or 4.0 mg/kg bw/day by gavage.

In dams, there were abortion, suppression in body weight gain and decrease in food consumption at 2.0 mg/kg bw/day and higher. In fetuses, there were increases in incidences of unossification/incomplete ossification of metacarpal and unossification of phalanx of the forepaw at 2.0 mg/kg bw/day and higher.

The NOAELs for dams and fetuses in this study would be 0.5 mg/kg bw/day. No teratogenicity of Ethiprole was evident (Ref. 45).

13. Genotoxicity

Ethiprole has been tested in a range of *in vitro* and *in vivo* following standard protocols (Table 7). It was not mutagenic in bacteria with and without an exogenous metabolic activation system. It did not induce chromosomal aberrations in primary human peripheral blood lymphocyte culture. Ethiprole did not induce *in vivo*/*in vitro* unscheduled DNA synthesis in rat hepatocyte. It did not induce micronucleus in mouse bone marrow cells. Although the micronucleus test had technical shortcomings, the committee concluded that the chemical did not induce micronuclei in mouse bone marrow cells (Refs. 46-50). Ethiprole was not genotoxic.

Table 7. Summary of Genotoxicity Test Results (Ethiprole)

Test systems		Cells/animals	Dose	Results
in vitro	Bacterial reverse mutation (+/-S9 mix)	S. typhimurium TA98, TA100, TA1535, TA1537, E. coli WP2*uvrA*		Negative
	Chromosome aberration (+/-S9 mix)	Human lymphocyte culture		Negative
in vivo/in vitro	Unscheduled DNA synthesis	Wistar rat, 3 males	800, 2000 (mg/kg bw, single oral administration)	Negative
in vivo	Rodent micronucleus	CD-1 mice, 5 males and 5 females	500, 1000, 2000 (mg/kg bw, single oral administration)	Negative

+/-S9 mix: with/without exogenous metabolic activation.

The metabolites B, C, D, E, F, K, N, and P have been tested in microbial reverse mutation tests (Table 8) and were not mutagenic (Ref. 50).

The test for metabolite B lacked the positive controls for all bacterial strains in the presence of an exogenous metabolic activation system. However, this was not considered a problem, since its parent compound was not mutagenic (Refs. 51-58).

Table 8. Summary of Genotoxicity Test (Bacterial Reverse Mutation Test) Results (Metabolites)

Test substance (Metabolite)	Test systems	Cells	Results
B	Bacterial reverse mutation (+/-S9 mix)	S. typhimurium TA98, TA100, TA1535, TA1537, E. coli WP2*uvrA*	Negative
C	Bacterial reverse mutation (+/-S9 mix)	S. typhimurium TA98, TA100, TA1535, TA1537, E. coli WP2/pKM101, WP2*uvrA*/pKM101	Negative
D	Bacterial reverse mutation (+/-S9 mix)	S. typhimurium TA98, TA100, TA1535, TA1537, E. coli WP2/pKM101, WP2*uvrA*/pKM101	Negative
E	Bacterial reverse mutation (+/-S9 mix)	S. typhimurium TA98, TA100, TA1535, TA1537, E. coli WP2*uvrA*	Negative
F	Bacterial reverse mutation (+/-S9 mix)	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Negative
K	Bacterial reverse mutation (+/-S9 mix)	S. typhimurium TA98, TA100, TA1535, TA1537, E. coli WP2/pKM101, WP2*uvrA*/pKM101	Negative
N	Bacterial reverse mutation (+/-S9 mix)	S. typhimurium TA98, TA100, TA1535, TA1537, E. coli WP2/pKM101, WP2*uvrA*/pKM101	Negative
P	Bacterial reverse mutation (+/-S9 mix)	S. typhimurium TA98, TA100, TA1535, TA1537, E. coli WP2*uvrA*/pKM101	Negative

+/-S9 mix: with/without exogenous metabolic activation.

14. Other toxicity tests

(1) Thyroid tumorigenesis mechanism study in rats

① Perchlorate release test for evaluating thyroid toxicity

Wistar rats (groups of 24 males) were given Ethiprole, technical grade at 0 or 20 mg/kg bw/day for 14 days by gavage. Twenty-four hours after the last administration, ^{125}I-labeled sodium iodide and potassium perchlorate ($KClO_4$) were administered into the caudal vein and peritoneal cavity, respectively, to perform a perchlorate release test for measuring iodine (^{125}I) intake by the thyroid gland. (Positive control drug; PTU: 200 mg/kg bw/day by gavage)

The radioactivity in the thyroid gland increased in the Ethiprole-treated group compared to the control group, but no difference was observed in the thyroid weight. After the perchlorate administration, neither the thyroid weight nor the total blood radioactivity level changed in the Ethiprole-treated group, while the thyroid radioactivity level decreased and the total blood radioactivity level increased in the PTU-treated group. Therefore, the pathway of effects of Ethiprole on the thyroid was different from PTU, the positive control drug, and Ethiprole had no direct influence on the thyroid gland (Ref. 59).

② Effects on T_4 blood kinetics

Wistar rats (groups of 8 males) were given Ethiprole, technical grade at 0 or 20 mg/kg bw/day for 14 days by gavage. After the last administration, ^{125}I-T_4 was administered into the caudal vein to study the effects of the compound on T_4 blood kinetics. (Control drug; phenobarbital: 80 mg/kg bw/day via intraperitoneal administration)

The blood kinetics in the Ethiprole-treated group was similar to that in the Phenobarbital-treated group. In the Ethiprole-treated group, there were increases in clearance and steady-state distribution volume compared to those in the control group, but the effects were smaller than those observed in the Phenobarbital-treated group.

Ethiprole would act as a β-glucronyltransferase inducer like phenobarbital, but its action would be weaker than that of phenobarbital (Ref. 60).

③ Effects on T_4 bile excretion

Wistar rats (groups of 7 males) were given Ethiprole, technical grade at 0 or 20 mg/kg bw/day for 14 days by gavage. After the last administration, ^{125}I-T_4 was administered into the caudal vein to study the effects on T_4 bile excretion. (Control drug; phenobarbital: 80 mg/kg bw/day via intraperitoneal administration)

Compared with those in the control group, there were increases in the liver weight, the amount of radioactivity eliminated through the bile and the kinetic constant in Ethiprole-treated and

Phenobarbital-treated groups, and increases in the liver radioactivity level and the total radioactivity in the Phenobarbital-treated group. In each group, from 50 to 60% of the radioactivity was assigned to conjugates of ^{125}I-T_4, and about 20% was assigned to free ^{125}I or unidentified metabolites of ^{125}I-T_4.

Ethiprole administration promoted the elimination of ^{125}I-T_4 through the bile, with about 60% of the bile radioactivity assigned to ^{125}I-T_4 conjugates. Therefore, Ethiprole would induce hepatic drug-metabolizing enzymes such as β-D-glucronyltransferase (Ref. 61).

(2) Liver toxicity test in mice

C57BL/6 mice (groups of 15 males, interim sacrifice: groups of 15 males) were fed diets containing Ethiprole, technical grade at 0, 100, 300, or 1000 ppm for 28 days to study the liver toxicity. (Control drug; phenobarbital: 80 mg/kg bw/day by gavage)

An increase in the relative liver weight, diffuse and all lobular hypertrophy of hepatocytes and swelling and darkening of the liver were observed in the interim (Day 8) and terminal (Day 29) sacrifice groups, and a decrease in water consumption in the interim sacrifice group at 1000 ppm. EROD activity increased in the liver toxicity test by measuring the enzymatic activity of CYP molecule species. BrdU labeling index in the hepatocytes by immunohistological staining significantly increased in the interim sacrifice group but not in the terminal sacrifice group as compared to that in the control group.

There were increases in total cytochrome P450 content and BROD and PROD activities at 300 ppm and higher.

There were increases in total cytochrome P450 content and BROD, EROD and PROD activities in the Phenobarbital-treated group, in which BROD and PROD were markedly induced.

Ethiprole was a drug-metabolizing enzyme activity inducer as well as phenobarbital, and promoted hepatocyte proliferation activity at the early stage of administration though temporarily. These results suggests that an increase in hepatocellular adenoma in females at 300 ppm in the carcinogenicity study in mice was due to the tumor promotion effects of Ethiprole, which mechanism is similar to that of phenobarbital (Ref. 62).

III. Evaluation

Absorption, distribution, metabolism, and excretion of Ethiprole were studied in rats. The plasma concentration of the compound reached the maximum concentration at 8 hours and from 24 to 48 hours after the administration of Single Low and Single High, respectively. The main elimination pathway was fecal excretion. The tissue concentrations were relatively high in the liver, kidney, renal fat, thyroid gland, adrenal gland, and skin/hair. Metabolites F, I, J, Q, R and S were detected in the urine, and Ethiprole and metabolites B, E, H, I and J were detected in the feces. The main metabolic pathways of Ethiprole in rats could be the oxidation or reduction of the sulfonyl group and the oxidation of the alkyl group.

Plant metabolism of Ethiprole was studied in rice, cotton, and sweet peppers. Ethiprole, metabolite B, etc., were detected in husked rice, cottonseeds and pepper fruits. The main metabolic pathway of the compound in plants could be the production of a sulfone form (B) via the oxidation of the sulfoxide.

Half-lives of Ethiprole and metabolite B in soils were 5-71 days and 535 days, respectively.

The half-life of Ethiprole under natural sunlight in spring at 35 degrees north latitude was 1.3-2.0 days.

The residual levels of Ethiprole and metabolite B were examined in paddy rice, apples, and tea plants. The highest residual value of the compound (3.18 mg/kg) was observed in tea leaves cropped 7 days after a single application of 200 g a.i./ha, but the values diminished to 2.45 and 0.35 mg/kg 14 and 21 days after the application, respectively. The residual values of Ethiprole and metabolite B detected in husked rice did not exceed 0.05 mg/kg under all conditions tested.

Ethiprole and metabolite B were not detected in the milk taken from female Holstein given these drugs continuously for 7 days by gavage.

Residual values of Ethiprole in soils were studied using volcanic ash soil and mineral soil. The half-life of Ethiprole alone was 3.9-28 days, and the total of the half-lives of Ethiprole and degradates B, C, D and E was up to 254 days.

The LD_{50} of Ethiprole was >7080 mg/kg bw by oral dose, >2000 mg/kg bw by dermal treatment, and >5.2 mg/L by inhalation.

The NOAELs of Ethiprole were 1.2 mg/kg bw/day in rats and 1.0 mg/kg bw/day in dogs in subchronic toxicity tests. The compound showed no neurotoxicity.

Since thyroid tumor was observed in the chronic toxicity/carcinogenicity tests in rats and liver tumor was observed in the carcinogenicity test in mice, the mechanisms of the thyroid and liver tumors were studied.

The administration of Ethiprole would act as a hepatic drug metabolizing enzyme inducer,

which promotes excess excretion of T_4 into the bile and modulates in the hypothalamus-piruitary-thyroid axis to increase TSH secretion. The resulting increase in TSH concentration would trigger a continuous, excessive stimulation of the thyroid gland, which would indirectly cause the thyroid tumor. Moreover, Ethiprole would act as a tumor promoter via the same mechanism as that of phenobarbital, which would cause the liver tumor.

The mechanism of thyroid and liver tumor induction by Ethiprole treatment was described above. In addition, the compound showed no particular genotoxicity in the genotoxicity test, so theses tumors were developed through a non-genotoxic mechanism. Therefore, the threshold doses for the compound could be determined.

The NOAELs were 0.85 mg/kg bw/day in rats, 12.5 mg/kg bw/day in mice, and 0.70 mg/kg bw/day in dogs in the chronic toxicity and carcinogenicity tests.

In the two-generation reproductive toxicity study in rats, the NOAEL was 4.77 mg/kg bw/day for parent animals and offspring. The compound showed no reproductive toxicity.

The developmental toxicity studies were performed in rats and rabbits, and the NOAELs were 3 mg/kg bw/day for dams and 10 mg/kg bw/day for fetuses in rats and 0.5 mg/kg bw/day for dams and fetuses in rabbits. Ethiprole showed no teratogenicity in either rats or rabbits.

Ethiprole was not mutagenic in bacteria with and without an exogenous metabolic activation system. It did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes. Ethiprole did not induce *in vivo*/*in vitro* unscheduled DNA synthesis in rat hepatocytes. It did not induce micronucleus in mouse bone marrow cells.

The NOAELs in the evaluated toxicological tests are shown in Table 9.

Table 9. The NOAELs Determined in Several Toxicity Evaluating Tests

Species	Evaluating test	NOAEL (mg/kg bw/day)	Note
Mouse	78 weeks, carcinogenicity	Male: 25.6 Female: 12.5	
Rat	90 days, subchronic toxicity	Male: 1.2 Female: 1.5	
	90 days, subchronic neurotoxicity	Male: 1.4 Female: 8.4	No neurotoxicity
	Combined study of chronic toxicity (52 weeks) and carcinogenicity (104 weeks)	Male: 0.85 Female: 1.17	
	Two-generation reproductive toxicity study	Parent animals and offsprings: P male: 4.77 P female: 5.82 F_1 male: 6.03 F_1 female: 6.76	
	Developmental toxicity study	Dam: 3 Fetus: 10	No teratogenicity
Rabbit	Developmental toxicity study	Dam and fetus: 0.5	No teratogenicity
Dog	90 days, subchronic toxicity	Male: 1.0 Female: 1.1	
	1 year, chronic toxicity	Male: 0.70 Female: 0.76	

Based on the evaluation, the Pesticides Expert Committee of the Food Safety Commission settled the ADI value of Ethiprole as:

ADI:	0.005 mg/kg bw/day
(Referred data for ADI)	Developmental toxicity test
Laboratory animal tested:	Rabbit
Duration:	23 days
Administration route:	Gavage administration
NOAEL:	0.5 mg/kg bw/day
Safety factor:	100
Residue definition for exposure assessment:	
	Ethiprole (parent chemical only)

(Appendix 1. Abbreviations for metabolites/degradates)

Abbreviation	Chemical name
B	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)-1*H*-pyrazole-3-carbonitrile
C	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)-1*H*-pyrazole-3-carboxyamide
D	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfonyl)-1*H*-pyrazole-3-carboxyamide
E	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylthio)-1*H*-pyrazole-3-carbonitrile
F	5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-pyrazole-4-sulfonic acid
H	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(2-hydroxyethylsulfonyl)-1*H*-pyrazole-3-carbonitrile
I	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(carboxymethylsulfonyl)-1*H*-pyrazole-3-carbonitrile
J	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1*H*-pyrazole-3-carbonitrile
K	5-amino-[2-chloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfonyl)-1*H*-pyrazole-3-carbonitrile
L	5-formylamino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1*H*-pyrazole-3-carbonitrile
M	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylthio)-1*H*-pyrazole-3-carboxyamide
N	8-chloro-3-ethylsulfinyl-6-trifluoromethyl-4*H*-pyrazolo[1,5-α]benzimidazole-2-carbonitrile
O	2-cyano-8-hydroxy-6-trifluoromethyl-4*H*-pyrazolo[1,5-α]benzimidazole-3-sulfonic acid
P	3-ethylsulfinyl-8-hydroxy-6-trifluoromethyl-4*H*-pyrazolo[1,5-α]benzimidazole-2-carbonitrile
Q	Glucuronic acid conjugate of J
R	5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole-4-sulfinic acid
S	Sulfuric acid conjugate of J
U	3-cyano-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-1,5,6,7-tetrahydro-pyrazolo[4,3-b][1,4]thiazin-6-one-4,4-dioxide
V	Sulfuric acid conjugate of H
W	5-amino-3-cyano-1-(2-chloro-4-trifluoromethylphenyl)pyrazole-4-sulfonic acid
X	7-chloro-5-trifluoromethyl-1*H*-indazole-3-carboxyamide

(Appendix 2. Abbreviations for test values, etc.)

Abbreviation	Name
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
BrdU	5-bromo-2-deoxyuridine
BROD	Benzyloxyresorufin-*O*-debenzylase
EROD	Ethoxyresorufin-*O*-deethylase
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
PROD	Pentoxyresorufin-*O*-depentylase
PTU	Propylthiouracil
T_3	Triiodothyronine
T_4	Thyroxine
TSH	Thyroid-stimulating hormone

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