Evaluation Report

TOLFENPYRAD

October 6, 2004

Food Safety Commission

Pesticides Expert Committee

(Progress of Evaluation)

April 24, 2003	First registration in Japan
September 11, 2003	Registration application submitted (expansion of application)
July 12, 2004	The Ministry of Health, Labour and Welfare requests a health risk assessment in line with the establishment of maximum residue limits.
July 15, 2004	54th meeting of the Food Safety Commission (explanation of MHLW's request outline)
July 21, 2004	14th meeting of the Pesticides Expert Committee
September 2, 2004	60th meeting of the Food Safety Commission
September 2 to September 29, 2004	Public comments
October 6, 2004	Finalized

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Summary

Tolfenpyrad (IUPAC: 4-chloro-3-ethyl-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxa mide), belonging to the pyrazole class of insecticides, was evaluated based on various tests.

The test results used for the evaluation covered animal metabolism (rats), plant metabolism (eggplants, cabbages, peaches), soil degradation, photolysis in water, residues in crops, residues in soils, acute toxicity (rats, mice), subchronic toxicity (rats, mice, 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 carcinogenicity, teratogenicity or genotoxicity.

Based on the lowest value among NOAELs, which was 0.56 mg/kg bw/day in the rat combined study of chronic toxicity and carcinogenicity, and after dividing this lowest value by a safety factor of 100, the ADI was settled as 0.0056 mg/kg bw/day.

I. Outline of the Pesticide to be Evaluated

1. Usage

Insecticide

2. Common name (ISO name)

Tolfenpyrad

3. Chemical name

IUPAC name:

4-chloro-3-ethyl-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide

CAS chemical name (No. 129558-76-5)

4-chloro-3-ethyl-1-methyl-*N*-[[4-(4-methylphenoxy)phenyl]methyl]-1*H*-pyrazole-5-carboxamide

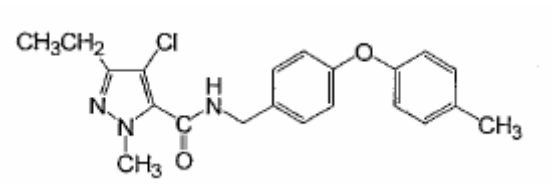
4. Chemical formula

$C_{21}H_{22}ClN_3O_2$

5. Relative molecular mass

383.9

6. Chemical structure



7. Background of development

Tolfenpyrad is a pyrazole insecticide, and was discovered by Mitsubishi Chemical Corporation in 1991. The compound acts mainly through the inhibition of the mitochondrial electron transport system.

Tolfenpyrad has not been registered overseas. In Japan, the compound was first registered for its application to vegetables, tea plants, etc., on April 24, 2003. Twenty-eight tons of technical grade compound were produced in pesticide fiscal year 2002 (Ref. 1). In September 2003, Nihon Nohyaku Co., Ltd. (hereinafter “The Applicant”) applied for the registration of the compound’s expanded application according to the Agricultural Chemicals Regulation Law. Refs. 2-24 and 28-82 were submitted (Ref. 2).

II. Summary of the Tests Results

1. Absorption, distribution, metabolism, and excretion

Various studies were conducted using tolfenpyrad labeled with ^{14}C at the pyrazol ring (hereinafter "Py-^{14}C-tolfenpyrad") or at the tolyl ring (hereinafter "To-^{14}C-tolfenpyrad"). Unless otherwise noted, radioactivity concentrations and metabolite concentrations were calculated in terms of tolfenpyrad (the procedure was also applied to all other metabolism tests).

(1) Metabolism test in rats (single dose)

The distribution of tolfenpyrad was examined in rats after a single oral administration of 1 mg/kg bw (hereinafter "Py Low") or 20 mg/kg bw (hereinafter "Py High") of Py-^{14}C-tolfenpyrad or 1 mg/kg bw (To Low) of To-^{14}C-tolfenpyrad.

The transition of the total blood radioactivity is shown in Table 1.

Table 1. Transition of the total blood radioactivity

Dose	Low Dose (1 mg/kg bw)		High Dose (20 mg/kg bw)	
Sex	Male	Female	Male	Female
T_{max} (hr)*	2	4～6	6～8	4～12
C_{max} (μg/mL)*	0.27～0.30	0.25～0.28	1.93～2.22	2.23～2.37
$T_{1/2}$ (hr)*	12.1～16.4	11.0～27.6	12.6～16.3	11.5～14.2

* T_{max}: Time when the maximum total blood radioactivity was reached

C_{max}: Maximum total blood radioactivity

$T_{1/2}$: Half-life

Eighty-percent or more of the compound was eliminated within 72 hours after administration. Rats eliminated from 88.2 to 93.2%, from 1.7 to 3.0% and less than 0.1% of the total applied radioactivity (TAR) in the feces, the urine and the breath by 168 hours, respectively. By 48 hours after the administration of Py-^{14}C-tolfenpyrad, rats eliminated from 51.3 to 69.5% of TAR in the bile.

The residual radioactivity levels in major tissues are shown in Table 2 (Ref. 3).

Table 2. Residual radioactivity levels in major tissues after a single dose (μg/g organ)

Administration conditions	Around the time when the maximum total blood concentration was reached*	168 hours after administration

Py Low	Male	liver (5.40), stomach (1.92), small intestine (1.68), kidney (1.35), heart (0.8), plasma (0.46)	0.08 or lower in all tissues
	Female	liver (5.70), stomach (1.96), small intestine (1.46), kidney (1.38), brown adipose tissue (1.11), heart (0.88), plasma (0.65)	0.08 or lower in all tissues
To Low	Male	liver (5.56), stomach (2.47), small intestine (1.84), kidney (1.65), brown adipose tissue (0.93), heart (0.89)	0.08 or lower in all tissues
	Female	liver (5.74), stomach (2.08), small intestine (1.48), kidney (1.41), brown adipose tissue (1.39), heart (0.88)	
Py High	Male	stomach (25.2), liver (18.6), small intestine (13.4), large intestine (5.85), kidney (4.88), plasma (4.14), brown adipose tissue (3.12), heart (2.79)	bone marrow (1.6), adipose tissue (1.27), brown adipose tissue (1.11), skin (0.99)
	Female	stomach (22.0), liver (20.0), small intestine (12.7), large intestine (6.92), plasma (5.50), brown adipose tissue (5.17), kidney (4.95), heart (3.06)	bone marrow (2.6), skin (1.64), fat (1.42)

* Low: 4 hours after administration (around T_{max})
  High: 6 hours after administration (around T_{max})

Tolfenpyrad and higher than 1.0% of TAR of individual metabolites were not detected in the urine by 48 hours after administration. In the feces, tolfenpyrad was detected at from 4.1 to 15.1% of TAR of the administered radioactivity, and metabolites PT-CA, Sul-OH-PT-CA and OH-PT-CA were detected at from 23.9 to 48.9%, from 5.3 to 11.7% and from 6.4 to 12.9% of TAR, respectively. In the bile, the amount of tolfenpyrad ranged from N.D. to 0.7% of the administered radioactivity, and the amounts of metabolites PT-CA-TA・PT-CA-GA・PT-CA (total of these metabolites), Sul-OH-PT-CA and CO-PT ranged from 31.3 to 42.9%, from 4.7 to 7.7% and from 3.7 to 7.4% of TAR, respectively.

Metabolite concentrations in the plasma, liver, kidney and white adipose tissue were determined in rats treated with Py Low and Py High. Tolfenpyrad was not detected in these tissues, and the main metabolite was PT-CA, accounting for about 90% of the total amount of metabolites

detected. No difference in the metabolite production profile was observed among different doses or sexes.

The main metabolic pathways of tolfenpyrad in rats could be the oxidation of the methyl group on the tolyloxy ring (PT-CA) and the following oxidation of the ethyl group on the pyrazole ring (OH-PT-CA), and conjugation (Sul-OH-PT-CA). The cleavage of the C-N bond in the benzylamine moiety seems to be rare (Ref. 4).

(2) Plasma concentration and residual in gastrointestinal tract with oral high dose

The plasma concentration and residual of tolfenpyrad in the gastrointestinal tract were examined in SD rats (groups of 5 males) with a single gavage administration of high doses (160 or 320 mg/kg bw) of Py-^{14}C-tolfenpyrad.

The plasma concentrations of the compound 6 hours after administration were from 4.08 to 8.34 μg/mL (with the exception of 16.71 μg/mL in a rat that died after 7 hours) in rats at 160 mg/kg bw and from 5.18 to 6.97 μg/mL (excluding the fatal cases) in those at 320 mg/kg bw. At 160 mg/kg bw, the plasma concentration reached from 10.3 to 18.0 μg/mL by 72 hours and hardly decreased by 168 hours. At 320 mg/kg bw, the plasma concentration reached from 11.8 to 19.1 μg/mL by 168 hours.

The residual radioactivity in gastric contents after 168 hours was from 48.4 to 53.5% of TAR at 320 mg/kg bw, and greatly varied among rats at 160 mg/kg bw (from 0.2 to 29.7%). The residual radioactivity in small intestinal contents was from 1.9 to 4.8% of TAR at both dosages.

The delayed elimination of radioactivity from the stomach could be explained by the fact that the residual radioactivity in gastric contents could be easily recovered by washing with physiological saline; the radioactive substances were not fixed onto the walls of the gastrointestinal tract but were mixed into the contents. Moreover, the low residual radioactivity in small intestinal contents (about 3%) suggests that the gastric mobility was suppressed by the administration of a lethal dose of the compound (Ref. 5).

(3) Metabolism test in rats (Repetitive doses)

The metabolism of tolfenpyrad was examined in rats with a repetitive gavage administration of 1 mg/kg bw/day of Py-^{14}C-tolfenpyrad or To-^{14}C-tolfenpyrad (males only) for 14 days.

The T_{max}, C_{max} and $T_{1/2}$ of the whole-blood concentration of the compound after 14 days of repetitive doses were 8 hours, from 0.26 to 0.30 μg/mL and from 18.6 to 20.7 hours in males, respectively, and 12 hours, 0.51 μg/mL and 45.8 hours in females, respectively.

By 168 hours after the last administration, rats eliminated from 92.1 to 94.9% and from 2.2 to 3.4% of the compound in the feces and urine, respectively.

The residual radioactivity levels in major tissues in rats treated with repetitive doses are

presented in Table 3. The distribution of the radioactivity among the major tissues was similar to that achieved with a single dose (Ref. 6).

Table 3. Residual radioactivity levels in major tissues after repetitive doses (μg/g organ)

Administration conditions		Around the time when the maximum total blood concentration was reached*	168 hours after administration
Py-^{14}C-tolfenpyrad	Male	liver (7.77), kidney (2.98), brown adipose tissue (3.01), large intestine (1.87), small intestine (1.86), adipose tissue (1.38), bone marrow (1.48), heart (0.95), skin (0.75)	adipose tissue (0.89), bone marrow (0.76), skin (0.57)
	Female	liver (11.3), brown adipose tissue (7.27), bone marrow (3.06), kidney (2.88), large intestine (2.16), adipose tissue (1.66), small intestine (1.35), heart (0.91)	bone marrow (1.20), adipose tissue (0.86), skin (0.62)
To-^{14}C-tolfenpyrad	Male	liver (8.88), kidney (3.55), brown adipose tissue (3.02), large intestine (1.75), small intestine (1.39), bone marrow (1.28), adipose tissue (1.40), skin (0.80)	adipose tissue (0.95), bone marrow (0.63), skin (0.55)

* 12 hours after administration (around T_{max})

Tolfenpyrad and higher than 1.0% of TAR of individual metabolites were not detected in the urine within 24 hours after the last administration. In the feces, the amount of tolfenpyrad ranged from 0.6 to 1.1% of the administered radioactivity, and the amounts of metabolites PT-CA, Sul-OH-PT-CA and OH-PT-CA ranged from 57.2 to 65.2%, from 12.5 to 16.4% and from 11.1 to 13.8% of TAR, respectively. Other metabolites detected were each at less than 2% of TAR.

After repetitive administration of Py-^{14}C-tolfenpyrad for 14 days, no tolfenpyrad was detected in the plasma, and most of the radioactivity recovered in the plasma was PT-CA.

No difference in the metabolite profile or the metabolite distribution in the urine or feces was observed between repetitive and single oral doses (Ref. 7).

(4) Placental transfer and transfer to milk in rats

SD rats (groups of 4 pregnant females) were orally given Py-^{14}C-tolfenpyrad (3 mg/kg bw) to study the placental transfer of the compound and its transfer to milk (measured until 24 hours after administration).

The radioactivity levels in maternal plasma and in fetuses reached their maximum (2.90 μg/mL in maternal plasma and 0.87 μg/mL in embryo homogenate) 12 hours after administration.

The major metabolite detected in maternal plasma and embryonic homogenate was PT-CA.

The radioactivity in milk reached its maximum level (0.82 μg/mL in maternal plasma and 23.2 μg/mL in milk) 12 hours after administration. Most of the metabolite excreted in milk was a methyl ester form of PT-CA (PT-CA-Me).

The radioactivity level in infant plasma increased over time, exceeding the maternal plasma level at 12 hours after the administration of Py-^{14}C-tolfenpyrad to dams. Most of the metabolite detected in infant plasma was PT-CA (Refs. 8-11).

(5) *In vitro* metabolism test in the rat liver S-9 system

Py-^{14}C-tolfenpyrad (0.1 mg or 1 mg), To-^{14}C-tolfenpyrad (0.1 mg) and unlabeled compound (1 mg) were added to an *in vitro* metabolism system containing rat liver S-9 (4 ml) and incubated at 37℃ for 3 hours to perform the *in vitro* metabolism test of tolfenpyrad.

The compound was detected at from 10.2 to 12.4% of TAR. Major metabolites detected were OH-PT-CA, PT-CA and CO-PT-CA, accounting for from 24.5 to 32.4%, from 13.4 to 16.2% and from 9.3 to 13.2% of TAR, respectively. Twelve other metabolites were detected and identified, but each of them accounted for 8% or less of TAR.

The major metabolic pathways in the *in vitro* liver system would be the oxidation at position ω-1 of the ethyl group on the pyrazole ring, the oxidation of the methyl group on the tolyloxy ring, the cleavage of the benzylamine moiety, the demethylation of the *N*-methyl moiety, and the conversion of the ethyl group on the pyrazole ring to a vinyl group (Ref. 12).

2. Plant metabolism

(1) Eggplants

A metabolic test of the compound in plants was conducted using eggplants (variety: Senryo-Nigo) based on the experimental design summarized below.

Labeled compound	To-^{14}C-tolfenpyrad		To- or Py-^{14}C-tolfenpyrad
Plot	①	②	③
Treatment method	Addition to water culture medium	Application on leaf surface	Application on fruits and leaves
Developmental stage of plants under treatment	3 weeks after sowing	10 weeks after sowing	10 weeks after sowing
Part of plants treated	Absorbed via root	Applied in strips perpendicular to the costa at the center of	Applied on fruit and both sides of leaves positioned right below the fruit-bearing

		the leaves	part
Sampling date	1, 2 and 4 days after treatment	Immediately following 7 and 28 days after application	3, 7, 14 and 28 days after application
Dose	1 μg/mL each	7.5 μg/mL each	60 μg/mL each

In plot ①, the amount of radioactivity that migrated to the plants increased over time, although only a small portion of the radioactivity in the roots migrated to the stems or leaves. By Day 4, 53.9%, 0.4% and 0.2% of the treated radioactivity were recovered in the roots, leaves and stems, respectively.

In plot ②, the radioactivity applied to the center of the leaves traveled distally along the vein and was distributed throughout the distal half of the leaves by Day 28. However, proximal transfer of the radioactivity was hardly observed.

In plot ③, the radioactivity remained on the surface of the treated leaves or fruits, and recovered from 87.1 to 91.8% of the treated radioactivity being distributed on the surface by Day 28. The radioactivity in non-treated leaves or fruits was less than 0.1%, indicating that the radioactivity did not migrate to the non-treated parts of the plants.

In leaves, tolfenpyrad residue ranged from 89.5 to 93.6% (132-206 μg/g) of the treated radioactivity. Major metabolites detected were PT-OH, OH-PT, PT-CA and DM-PT, but each of their levels only ranged from 0.2 to 0.3% (0.3-0.7 μg/g) each. None of the other metabolites identified in the leaves exceeded 0.2% (0.4 μg/g). Metabolite T-AM was uniquely found in plants treated with To-^{14}C-tolfenpyrad, but was only detected at 0.4% (0.4 μg/g) of the treated radioactivity by Day 28.

In fruits, tolfenpyrad was detected from 92.2 to 93.6% (0.76-0.80 μg/g) of the treated radioactivity. Major metabolites were PT-OH, OH-PT, PT-CA and CO-PT, but each of their levels only ranged from 0.2 to 0.4% (0.002-0.003 μg/g) None of the other metabolites identified in the fruits exceeded 0.3% (0.002 μg/g). Metabolite T-AM was uniquely found in plants treated with To-^{14}C-tolfenpyrad, but was only detected at 0.1% (0.001 μg/g) of the treated radioactivity by Day 28.

Tolfenpyrad would not be extensively metabolized in eggplants, and the major metabolic pathways of the compound in eggplants would be the hydroxylation of the methyl group on the tolyl ring (PT-OH), hydroxylation (OH-PT) and oxidation (CO-PT) at position ω-1 of the ethyl group on the pyrazole ring, the cleavage of the C-N bond in the benzylamino moiety (T-AM), and the demethylation at 1-position on the pyrazole ring (DM-PT) (Ref. 13).

(2) Cabbages

Solution containing To-^{14}C-tolfenpyrad or Py-^{14}C-tolfenpyrad (0.5 mg/mL) was sprayed onto the entire aerial part of cabbages (variety: Akitoku) at the head-fill stage at 8 mL per pot. Samples were collected immediately and 7, 14 and 28 days after treatment (28 days only for Py-^{14}C-tolfenpyrad).

The total residual radioactivity (TRR) of To-^{14}C-tolfenpyrad in cabbages was 80.0% of the treated radioactivity immediately after treatment, which reduced to 58.9% by Day 28. The distributions of the radioactivity in the outer leaves and heads were 90.6% and 9.4%, respectively, immediately after treatment, and 99.7% and 0.3%, respectively, by Day 28. In the outer leaves, tolfenpyrad was detected at 55.0% (4.63 μg/g) of TRR, and major metabolites OH-PT, OH-T-CA, OH-T-OH and CA-T-AM were detected at 6.4% (0.54 μg/g), 3.9% (0.33 μg/g), 3.7% (0.31 μg/g) and 2.4% (0.20 μg/g) of TRR, respectively, by Day 28. Other metabolites detected in the outer leaves each accounted for 1.9% (0.16 μg/g) or less of TRR. In the heads, tolfenpyrad and metabolites each accounted for less than 0.1% (0.03 μg/g) of TRR by Day 28.

The TRR of Py-^{14}C-tolfenpyrad in cabbages was 89.4% of the treated radioactivity by Day 28. The distributions of the radioactivity residues in the outer leaves and heads were 97.2% and 2.8%, respectively. In the outer leaves, tolfenpyrad was detected at 49.8% (4.71 μg/g) of TRR, and major metabolites OH-PT, OH-PT-OH, OH-PT-CA and PCA were 7.89% (0.75 μg/g), 3.40% (0.32 μg/g), 2.93% (0.27 μg/g) and 2.11% (0.20 μg/g) of TRR, respectively, by Day 28. Other metabolites detected in the outer leaves each accounted for 1.6% (0.15 μg/g) or less of TRR. In the heads, tolfenpyrad was detected at 0.41% (0.034 μg/g) of TRR, and metabolites were detected at each less than 0.2% (0.03 μg/g) of TRR by Day 28.

Tolfenpyrad would be relatively easily absorbed by cabbages and degraded into a number of metabolites, but rarely migrate to the heads. The major metabolic pathways of the compound in cabbages would be the hydroxylation at position ω-1 of the ethyl group on the pyrazole ring (OH-PT), the oxidative degradation of the bond between the pyrazole ring and the tolyloxybenzyl group and the hydrolysis of the amide bond (T-AM) and the oxidation of the alkyl group in the tolyloxybenzyl moiety following cleavage (T-CA) (Refs. 14-15).

(3) Peaches

Solution containing To-^{14}C-tolfenpyrad or Py-^{14}C-tolfenpyrad (1.0 mg/mL) was sprayed onto the entire surface of the fruit-bearing branches of peaches (variety: Benishimizu) at 4 mL. Leaves, stems and fruits were sampled immediately, and 14, 28 and 56 days after treatment with To-^{14}C-tolfenpyrad. From plants treated with Py-^{14}C-tolfenpyrad, leaves and stems were sampled on Day 56 and fruits were sampled on Day 53.

The TRR of To-^{14}C-tolfenpyrad recovered in peaches was 32.6% and 32.8% of the treated radioactivity immediately after treatment and by Day 56, respectively, showing a small temporal

change. The distributions of the radioactivity residues in the treated leaves, stems and fruits were 83.1%, 7.5% and 9.3%, respectively. Ninety-five percent or more of the radioactivity residue present in the fruits was detected in the pericarp. The radioactivity level in the non-treated leaves was less than 0.1%.

In the leaves, tolfenpyrad was 20.0% (12.4 μg/g) of TRR, and major metabolites PT-CA, CA-T-CA and T-CA were 9.1% (5.6 μg/g) (including the conjugate, same hereinafter), 9.1% (5.7 μg/g) and 5.1% (3.2 μg/g) of TRR, respectively, by Day 56. Other metabolites identified in the leaves each accounted for 2.0% (1.2 μg/g) or less of TRR. In the fruits, radioactive residues of 1.02 μg/g were recovered, of which tolfenpyrad accounted for 0.79 μg/g and each metabolite accounted for 0.02 μg/g or less by Day 56. Tolfenpyrad was not detected in the sarcocarp by Day 56, and a conjugate of CA-T-CA was detected at 0.02 μg/g as a metabolite.

The TRR of Py-^{14}C-tolfenpyrad in peaches was 23.5% of the treated radioactivity by Day 56. The distribution of the radioactive residue in the treated leaves, stems and fruits was 86.1%, 7.3% and 6.6%, respectively, and 86.4% of the radioactive residue present in the fruits was recovered in the pericarp. In the leaves, tolfenpyrad was 28.1% (21.1 μg/g) of TRR, and major metabolites PT-CA, OH-PAM, PT-OH and OH-PT-CA were 14.6% (11.0 μg/g) (including the conjugate, same hereinafter), 7.8% (5.82 μg/g), 3.8% (2.83 μg/g) and 2.8% (2.06 μg/g) of TRR, respectively. Other metabolites detected in the leaves each accounted for 1.87% (1.40 μg/g) or less of TRR. In the pericarp, tolfenpyrad was 4.28% (8.24 μg/g) of TRR, and the identified metabolites were each 0.06% (0.12 μg/g) or less of TRR. Tolfenpyrad was hardly recovered in the flesh, accounting for 0.02% (0.003 μg/g) of TRR. The metabolite OH-PAM was detected at 0.26% (0.035 μg/g) of TRR, and other identified metabolites each accounted for 0.02% (0.003 μg/g) or less of TRR.

The major metabolic pathways of the compound in peaches would be the oxidation of the methyl group on the tolyloxy ring (PT-CA), the oxidative degradation of the bond between the pyrazole ring and the tolyloxybenzyl group and the hydrolysis of the amide bond (OH-PAM) and the oxidation of the alkyl group in the tolyloxybenzyl moiety resulting from cleavage (CA-T-CA) (Refs. 16-17).

3. Fate in soil

(1) Degradation in soil (aerobic, anaerobic, and sterilized soils)

Py-^{14}C-tolfenpyrad or To-^{14}C-tolfenpyrad was mixed into light clay (Ibaraki soil, Kochi soil) at 0.75 μg/g per dry weight. The test was carried out after incubating the soils at 30℃ under an aerobic condition for 91 days (Ibaraki soil) or for 183 days (Kochi soil), or under an anaerobic or sterilized condition for 28 days.

The elimination rates of tolfenpyrad hardly varied among the kinds of soils tested, and the

half-lives of the compound ranged from 3 to 5 days and from 127 to 179 days under aerobic and anaerobic conditions, respectively. The 90% decay time was from 29 to 34 days under the aerobic condition. The major degradate found in the aerobic soil was PT-CA, reaching the maximum levels of from 29.5 to 31.9% (0.22-0.24 μg/g) of the total applied radioactivity (TAR) by Days 7 through 14 in Ibaraki soil and from 14.9 to 15.1% (0.114-0.468 μg/g) of TAR by Day 3 in Kochi soil. In addition, PCA and PT(A)-4OH were detected at maximum levels of from 12.5 to 15.8% (from 0.094 to 0.119 μg/g) of TAR and from 4.5 to 4.6% (from 0.034 to 0.035 μg/g) of TAR, respectively. Other degradates did not exceed 2% (0.015 μg/g) of TAR. As for volatile substances, CO_2 was from 12.9 to 42.1% of TAR and from 39.8 to 72.2% of TAR in Ibaraki and Kochi soils, respectively, by the end of the study. No volatile organic substance was detected. The un-extracted residue level was from 30.7 to 50.9% of TAR by Day 91 in Ibaraki soil and from 14.6 to 32.6% by Day 183 in Kochi soil, and was higher for the Py-labeled form than for the To-labeled form.

The major degradate in anaerobic soil was PT-CA, detected at from 2.3 to 7.5% of TAR on Day 28. Only tolfenpyrad was detected in sterilized soil.

The main degradation pathways of tolfenpyrad would be the oxidation of the methyl group on the tolyloxy ring (PT-CA) and the following cleavages of the tolyl ring (PT-OH) and the amide bond (PCA, PAM), ultimately resulting in CO_2. The degradation of the compound in soil would involve aerobic microbes (Ref. 18).

(2) Adsorption to soils

Adsorption tests in Japanese soil were made using clay loam and three kinds of light clay.

The adsorption coefficient (K) was in the range of 722 to 1522, and the adsorption coefficient based on the organic carbon content (K_{oc}) was in the range of from 15.1×10^3 to 149×10^3 (average: 63.3×10^3) (Ref. 19).

4. Fate in water

(1) Hydrolysis

Non-labeled tolfenpyrad was dissolved in buffer solutions of pH 4, 7 and 9 to prepare tolfenpyrad solutions of 0.04 mg/L. The solutions were incubated for 5 days at 50±1℃.

The half-lives of tolfenpyrad under all tested conditions were 1 year or longer, suggesting that the compound is stable against hydrolysis (Ref. 20).

(2) Photolysis in water (purified water and river water)

To-^{14}C-tolfenpyrad was dissolved in purified water and river water to prepare tolfenpyrad solutions of 20 μg/L. The solutions were exposed to light (765 W/m^2 ±10℃, measured in the

range of 300-800 nm wavelength) for 58 hours at 25±1℃.

After 58 hours, tolfenpyrad was from 30 to 31% of TAR, and CA-T-NH_2 was detected at from 23.2 to 23.3% of TAR as the major degradate in purified water and river water. Other degradates such as PT-OH and PT-CHO were also detected at 5% or less of TAR. Tolfenpyrad was hardly degraded in the dark, accounting for from 87.3 to 89.1% of TAR in purified water and river water, even after 58 hours.

The compound is photolytically degraded, and the half-lives were 35.2 and 35.0 hours in purified water and river water, respectively, which were calculated as 11.4 and 11.3 days, respectively, under natural sunlight in spring in Tokyo (35 degrees north latitude).

The main degradation pathways would be the oxidation of the methyl group on the tolyloxy ring (PT-OH, PT-CHO and PT-CA) and the following cleavage of the amide bond in PT-CA (CA-T-NH_2) (Ref. 21).

5. Residues in crops

Vegetables, fruits and tea crops were used for residual tests in crops for tolfenpyrad (parent compound) and six metabolites (PT-CA, OH-PT and T-CA (studied using cucumbers, tomatoes, eggplants, cabbages and Chinese cabbages), OH-PAM, OH-T-CA and CA-T-CA (studied using eggplants)).

As indicated in the results shown in Table 4, the highest residual value was 23.3 mg/kg from tea leaves (unrefined), which were collected 7 days after a single application of from 300 to 450 g a.i./ha of the compound. The values decreased to 7.17, 0.83 and 0.18 mg/kg by Days 14, 21 and 30, respectively. Metabolites other than PT-CA were not detected under all tested conditions (Refs. 22-24).

Table 4. Results of residual tests in crops

Crops (Parts analyzed) Year	Number of fields	Formulations	Amount used (g ai/ha)	Applications (times)	PHI (days)	Residues (mg/kg)			
						Tolfenpyrad		Metabolite PT-CA	
						Highest	Average	Highest	Average
Japanese radish (field) (leaf) 1996	1	EC	120～300	4	7 14 21	7.06 3.59 1.37	6.78 3.26 1.33		
Japanese radish (field) (root) 1997	2	EC	300	2	7 14 21	0.05 0.03 0.03	0.05 0.02 0.01		
Japanese radish (field)	3	EC	195～300	2	7 14 21	9.97 5.37 2.09	7.30 3.03 1.39		

(leaf) 1996 1997									
Turnip (greenhouse) (root) 2003	2	EC	300～ 375	2	7 14 21 28	0.29 0.18 0.11 0.07	0.18 0.13 0.05 0.03		
Turnip (greenhouse) (leaf) 2003	2	EC	300～ 375	2	7 14 21 28	19.7 5.83 1.89 0.50	13.1 4.79 0.84 0.22		
Chinese cabbage (field) (forage) 1997	2	EC	300～ 375	2	7 14 21	0.34 0.14 0.09	0.22 0.11 0.05	<0.02 <0.02 <0.02	<0.02 <0.02 <0.02
Cabbage (field) (head) 1997	2	EC	300	2	7 14 21	0.29 0.08 0.04	0.14 0.04 0.02*	<0.02 <0.02 <0.02	<0.02 <0.02 <0.02
Broccoli (field) (floral bud) 2002	2	EC	300	2	3 7 14 21	0.51 0.27 0.16 0.11	0.44 0.21 0.09 0.05*		
Lettuce (greenhouse) (forage) 2002	2 2 2 2 1	EC	225～ 300	2	3 7 14 21 28	1.48 1.98 0.82 0.72 0.25	1.14 1.35 0.69 0.51 0.20		
Welsh onion (field) (forage) 2002	2	EC	225～ 300	2	3 7 14 21	1.77 0.86 0.39 0.18	1.25 0.55 0.28 0.10		
Tomato (greenhouse) (fruit) 1997	2	EC	300	2	1 3 7	0.37 0.48 0.47	0.33 0.37 0.34	<0.02 <0.02 <0.02	<0.02 <0.02 <0.02
Tomato (greenhouse) (fruit) 2000 2001	2	EC	300～ 480	2	1 7 14 21 28	0.56 0.74 0.54 0.54 0.51	0.43 0.55 0.42 0.42 0.32		
Eggplant (greenhouse) (fruit) 1997	2	EC	300～ 450	2	1 3 7	0.68 0.58 0.16	0.58 0.45 0.14	<0.02 <0.02 <0.02	<0.02 <0.02 <0.02
Cucumber (greenhouse) (fruit) 1996 1997[a]	3	EC	300	2	1 3 7	0.30 0.08 0.01	0.21 0.05 0.01*	0.02 0.03 <0.02	0.02* 0.02* <0.02
Cucumber (greenhouse) (fruit)	1	EC	300	4	1 3	0.12 0.04	0.12 0.04		

1996					7	0.02	0.02		
Watermelon (greenhouse) (pulp) 2001	2	EC	300	2	1 3 7	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01		
Mandarin orange (greenhouse) (fruit flesh) 2001	2	SC	600～750	2	1 3 7	0.02 0.02 0.03	0.01* 0.01* 0.02*		
Mandarin orange (greenhouse) (peel) 2001	2	SC	600～750	2	1 3 7	6.17 7.11 5.80	4.19 4.01 3.78		
Chinese citron (field) (fruit) 2002	2	SC	750	2	1 3 7	0.78 0.93 1.09	0.53 0.62 0.69		
Chinese citron (field) (fruit flesh) 2002	2	SC	750	2	1 3 7	0.06 0.06 0.07	0.04 0.04* 0.04		
Chinese citron (field) (peel) 2002	2	SC	750	2	1 3 7	2.21 2.59 3.44	1.46 2.05 2.15		
Citron (field) (fruit) 2001	1	SC	750	2	1 3 7	0.42 0.57 0.39	0.41 0.51 0.36		
Citrus sphaerocarpa (field) (fruit) 2001	1	SC	960	2	1 3 7	0.61 0.59 0.03	0.56 0.47 0.03		
Pear (field) (fruit) 2000	2	SC	525～600	2	7 14 21	1.26 0.93 0.69	0.93 0.70 0.63		
Peach (unbagged) (sarcocarp) 2002	2	SC	525～600	2	1 3 7	0.04 0.03 0.02	0.02* 0.02* 0.01*		
Peach (unbagged) (epicarp) 2002	2	SC	525～600	2	1 3 7	22.75 16.01 8.84	9.56 7.46 5.39		
Tea plant (covered with shade) (unrefined leaf)	2	EC	300～450	1	7 14 21 30	23.3 7.17 0.83 0.18	19.7 5.67 0.72 0.14		

1997									
Tea plant (covered with shade) (leachate) 1997	2	EC	300～450	1	7 14 21 30	0.21 0.08 0.01 <0.01	0.20 0.08 0.01 <0.01		

ai: Active ingredient

PHI: Pre-harvest interval

EC: Emulsion concentrate

SC: Flowable

For data including values below the detection limit, the average was calculated using the detection limit values and marked.

*In cases where all data were below the detection limit, the average of the detection limit values was taken and marked <.

*The analytical values for metabolite PT-CA were expressed in terms of tolfenpyrad.

Tolfenpyrad/PT-CA=383.9/413.9=0.93 was used as the conversion factor.

a: Test for the metabolite was conducted in two fields (in 1997 only).

Based on the results of the residual tests in crops, the estimated tolfenpyrad (parent compound only) intake through domestically cultivated agricultural products are shown in Table 5. The estimated amounts were calculated based on the assumption that tolfenpyrad was applied to all of the applicable crops under conditions giving the highest residual value of tolfenpyrad among the applied methods, and that processing or cooking of the crops would not affect the amounts of residual pesticides.

Table 5. Estimated amounts of tolfenpyrad 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
Japanese radishes (root)	0.02	45.0	0.9	18.7	0.37	28.7	0.57	58.5	1.17
Japanese radishes (leaf)	3.03	2.2	6.67	0.5	1.52	0.9	2.73	3.4	10.3
Turnips (root)	0.18	2.6	0.47	0.7	0.13	0.7	0.13	4.2	0.76
Turnips	13.1	0.5	6.55	0.1	1.31	0.3	3.93	1.1	14.4

(leaf)									
Chinese cabbage	0.11	29.4	3.23	10.3	1.13	21.9	2.41	29.9	3.29
Cabbage	0.04	22.8	0.91	9.8	0.39	22.9	0.92	23.1	0.92
Broccoli	0.21	4.5	0.95	2.8	0.59	46.7	9.81	4.1	0.86
Lettuce	1.35	6.1	8.24	2.5	3.38	6.4	8.64	4.2	5.67
Welsh onion	0.55	11.3	6.22	4.5	2.48	8.2	4.51	11.5	6.33
Tomato	0.55	24.3	13.4	16.9	9.30	24.5	13.5	18.9	10.4
Eggplant	0.58	4.0	2.32	0.9	0.52	3.3	1.91	5.7	3.31
Cucumber	0.21	16.3	3.42	8.2	1.72	10.1	2.12	16.6	3.49
Mandarin orange	0.02	41.6	0.83	35.4	0.71	45.8	0.92	42.6	0.85
Chinese citron (whole)	0.69	0.1	0.069	0.1	0.069	0.1	0.069	0.1	0.069
Other citruses	0.56	2.5	1.4	1.5	0.84	3.5	1.96	2.3	1.29
Japanese pear	0.7	5.1	3.57	4.4	3.08	5.3	3.71	5.1	3.57
Peach	0.02	0.5	0.01	0.7	0.014	4	0.08	0.1	0.002
Tea plant	5.67	3	17.0	1.4	7.938	3.5	19.8	4.3	24.4
Total			76.2		35.5		77.7		91.1

- The residual values listed here are the maximum values among the averages for individual test groups treated with the compound over the various periods and frequencies registered (see Table 4).
- “ff”: Agricultural product consumptions (g/person/day) based on the results of the National Nutrition Surveys in 1998-2000 (Refs. 25-27)
- “Intake”: Estimated intakes (μg/person/day) of tolfenpyrad calculated from residual values and agricultural product consumptions.
- The predicted intake for “Other citruses” including Citrus sphaerocarpa and Citrus sudachi was calculated using the residual value for Citrus sphaerocarpa (0.56 mg/kg), which was the highest.
- The predicted consumption for watermelon was not calculated, since all the data were below the detection limit.

6. Residues in soils

Residual values of tolfenpyrad and various degradates in soils were studied (both in the vessel and the field) using volcanic ash light clay soil and alluvium clay loam.

The assumed half-lives under the conditions tested are shown in the Table 6. The half-life for tolfenpyrad alone was 3-34 days, and the total length of half-lives of tolfenpyrad and degradates PT-CA and PCA was 3-47 days (Ref. 28).

Table 6. Results of residual tests in soils (assumed half-life)

Type of tests	Concentration*	Kinds of soil	Tolfenpyrad	Tolfenpyrad + degradates PT-CA and PCA
Vessel	0.3 mg/kg	Volcanic ash light clay	6 days	9 days
		alluvium clay loam	34 days	47 days
Field	300g ai/ha	Volcanic ash light clay	5 days	10 days
		alluvium clay loam	3 days	3 days

* Pure form and SC were used in the vessel and field tests, respectively.

7. Acute toxicity

Acute oral, dermal and inhalation toxicities of tolfenpyrad were studied using SD rats, and acute oral toxicity was studied using ICR rats.

The acute oral LD_{50} values are shown in Table 7.

Table 7. The acute oral LD_{50} of tolfenpyrad (mg/kg bw)

Animals tested	Kind of solvent	Sex	
		Male	Female
Rats	Aqueous CMC-Na solution	260～386	113～150
	Olive oil	86	75
Mice	Aqueous CMC-Na solution	114	107
	Olive oil	80～100	50～80

The acute dermal LD_{50}s of tolfenpyrad in rats were >2000 mg/kg bw in males and >3000 mg/kg bw in females, and the acute inhalation LC_{50}s of tolfenpyrad in rats were >2.21 mg/L in males and >1.50 mg/L in females (Refs. 29-35).

Acute oral toxicities of 8 metabolites were also studied using SD rats. The results are shown in Table 8 (Refs. 36-45).

Table 8. The acute oral LD_{50} of metabolites of tolfenpyrad (mg/kg bw)

Metabolite	Kind of solvent	Sex	
		Male	Female
PT-CA	Aqueous CMC-Na solution	27.4	15.4
	Olive oil	62	54
OH-PT	Aqueous CMC-Na solution	70.8	35.5
	Olive oil	30～60	
T-CA	Aqueous CMC-Na solution	600～2000	>2000
T-AM		>2000	
CA-T-CA			
PCA			
OH-T-CA		2024	>2000
OH-PAM		1095	

8. Skin and eye irritation, and skin sensitization

Tolfenpyrad displayed a mild irritation of the eye and the skin in New Zealand rabbits (Refs. 46-47).

Skin sensitization potential was assessed in Hartley guinea pigs by the Maximization method. No skin sensitization was observed (Ref. 48).

9. Subchronic toxicity

(1) Subchronic toxicity test for 90 days in rats

Fischer rats (groups of 10 males and 10 females) were fed diets containing tolfenpyrad, technical grade at 0, 15, 80, or 160 ppm for 90 days.

The major findings for individual treatment groups are shown in Table 9.

Table 9. Findings of subchronic toxicity test for 90 days in rats

160 ppm Males and females	Increase in serum inorganic phosphate, darkening of the Harderian gland, diffuse hypertrophy of pancreatic acinar cells
Males	Decrease in food consumption, increases in MCV, MCH and reticulocyte, decrease in TG, increases in the brain, heart, spleen, relative adrenal and testis weights, darkening of the liver, hyaline droplet accumulation in the renal

	proximal tubular epithelium, increased secretion from the Harderian gland
Females	Decrease in thrombocyte, increases in serum γ-GTP and urea nitrogen, decrease in the relative ovary weight, decreases in hemotopoietic cells in the femur and sternum, hypertrophy of acinar cells in the submandibular gland, atrophy of the ovary and uterus
80 ppm and higher Males and females	Suppression in body weight gain, increases in serum potassium and the relative lung weight, diffuse hypertrophy of hepatocytes and increase in the number of mast cells in the mesenteric lymph node
Males	Increase in the relative kidney weight
Females	Decrease in food consumption, increases in MCV, serum ALP and glucose, decreases in WBC, serum TG, total protein and albumin, increases in the relative brain, heart, liver and spleen weights, hypertrophy of the renal proximal tubular epithelium, increased secretion from the Harderian gland
15 ppm and higher Males	Increase in the relative liver weight
Females	Increase in the relative kidney weight

The NOAEL would be <15 ppm (<0.91 mg/kg bw/day for males, <1.01 mg/kg bw/day for females) (Refs. 11, 49-50).

(2) Subchronic toxicity test for 2 weeks in rats – Effects on mitochondrial function and morphology

Fischer rats (groups of 7 males and 7 females) were fed diets containing tolfenpyrad, technical grade at 0, 15, 100, or 200 ppm for 2 weeks.

There were hepatocellular hypertrophy and increased hepatic mitochondria in males and females, and an increase in L-lactic acid concentration in whole blood in females at 200 ppm. A suppression in body weight gain, a decrease in food consumption and an increase in the relative liver weight in males and females, and an increase in L-lactic acid concentration in whole blood in males were observed at 100 ppm and higher.

The increases in L-lactic acid concentration in whole blood and mitochondria in hepatocytes were assumed to be the results of abnormal energy metabolism in mitochondria caused by the administration of tolfenpyrad.

The NOAEL would be 15 ppm (1.32 mg/kg bw/day for males, 1.27 mg/kg bw/day for females) (Refs. 10, 51).

(3) Subchronic toxicity test for 90 days in mice

ICR mice (groups of 10 males and 10 females) were fed diets containing tolfenpyrad,

technical grade at 0, 15, 100, or 300 ppm for 90 days.

There were an increase in the relative liver weight in males and females, a decrease in food consumption and increases in GOT and the relative heart weight in males, and a decrease in MCHC in females at 300 ppm.

The NOAEL would be 100 ppm (15.9 mg/kg bw/day for males, 20.2 mg/kg bw/day for females) (Ref. 52).

(4) Subchronic toxicity test for 90 days in dogs

Beagle dogs (groups of 4 males and 4 females) were given tolfenpyrad, technical grade at 0, 1, 5, or 10 mg/kg bw/day for 90 days by gavage.

At 10 mg/kg bw/day, there were loose stool, mucous stool and an increase in serum potassium concentration in females. There were vomiting in males and females, a decrease in urine volume in females, and loose stool and mucous stool (only at 5 mg/kg bw/day) in males at 5 mg/kg bw/day and higher.

The NOAELs would be 1 mg/kg bw/day for males and females (Ref. 53).

(5) Subchronic toxicity test for 90 days in dogs (additional test)

Beagle dogs (groups of 4 males and 4 females) were given tolfenpyrad, technical grade at 0, 10, 30, or 100 mg/kg bw/day for 90 days by gavage to confirm the findings of the toxicity test.

At 100 mg/kg bw/day, 5 out of 8 dogs either died (1 male and 2 females) or were humanely killed in moribund state (1 male and 1 female) by Day 41. Further administration was considered difficult for the surviving 3 dogs, which showed absence of defecation, extreme weight loss and decrease in food consumption, so they were humanely killed on Day 49.

There were decreases in body weight and food consumption in males and females at 100 mg/kg bw/day. Also, there were an increase in the ratio of segmented neutrophils and a decrease in the ratio of acidophils in males, and an increase in serum free fatty acid, a decrease in the spleen weight, thymic atrophy and centrilobular vacuolation of hepatocytes in females. An increase in acidophils in liver cytoplasm in males and females, and a fatal case, increases in serum GPT and urea nitrogen, decreases in urine volume and testis weight, atrophy of seminiferous tubule and thymus and centrilobular vacuolation in males were observed at 30 mg/kg bw/day and higher. There were vomiting, loose stool, mucous stool and salivation in males and females, and decreases in white blood cells, total serum cholesterol, TG and phospholipids in females at 10 mg/kg bw/day and higher (Ref. 54).

(6) Subchronic neurotoxicity test for 90 days in rats

SD rats were fed diets containing tolfenpyrad, technical grade at 0, 15, 40, or 80 ppm.

At 80 ppm, there were suppression in body weight gain in males and females, and a decrease in food consumption in females. The compound showed no neurotoxicity.

The NOAEL for general toxicity would be 40 ppm (2.7 mg/kg bw/day for males, 3.2 mg/kg bw/day for females) (Ref. 55).

(7) Subchronic toxicity test of tolfenpyrad and metabolites PT-CA and OH-PT for 4 weeks in rats

Fischer rats (groups of 5 males and 5 females) were fed diets containing tolfenpyrad or metabolite PT-CA or OH-PT, technical grade at 0, 3, 10, 30, or 100 ppm (3 ppm not tested for tolfenpyrad) for 4 weeks.

In rats treated with tolfenpyrad, there were suppression in body weight gain and a decrease in food consumption in males and females, a decrease in total serum protein, an increase in the relative brain weight and hyaline droplet accumulation in the renal proximal tubular epithelium in males, and diffuse hypertrophy of hepatocytes and hypertrophy of pancreatic acinar cells in females at 100 ppm. At 30 ppm and higher, there were an increase in the relative liver weight in males and females, and an increase in the relative kidney weight in males.

In rats treated with PT-CA, there were suppression in body weight gain, increases in the relative brain and kidney weights and diffuse hypertrophy of hepatocytes in males at 100 ppm. At 30 ppm and higher, there were increases in the relative kidney weight and relative liver weight in males and females, respectively.

In rats treated with OH-PT, there was an increase in the relative kidney weight in males at 100 ppm.

The NOAELs would be 10 ppm (0.9 mg/kg bw/day both for males and females) for tolfenpyrad, 10 ppm (0.8 mg/kg bw/day for males, 0.9 mg/kg bw/day for females) for PT-CA, and 30 ppm (2.5 mg/kg bw/day) for males and 100 ppm (8.8 mg/kg bw/day) for females for OH-PT (Ref. 56).

10. Chronic toxicity/carcinogenicity study

(1) Chronic toxicity study for 1 year in dogs

Beagle dogs (groups of 4 males and 4 females) were given tolfenpyrad, technical grade at 0, 1, 5, or 10 mg/kg bw/day for 1 year by gavage. Dogs in the 10 mg/kg bw/day group were given 20 mg/kg bw/day for the first 5 weeks.

At 10 mg/kg bw/day, there were fatal cases (1 male and 1 female), decreases in body weight and food consumption and an increase in acidophilic alterations in liver cytoplasm in males and females, and vomiting, loose stool and pigmentations of hepatocytes and Kupffer cells in females. There were salivation and decreases in total serum cholesterol and phospholipids in males and females, vomiting in males, and increases in serum A/G ratio and albumin in females at 5 mg/kg

bw/day and higher.

The NOAEL would be 1 mg/kg bw/day for males and females (Ref. 57).

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

Fischer rats (groups of 50 males and 50 females, interim sacrifice: groups of 10 males and 10 females) were fed diets containing tolfenpyrad, technical grade at 0, 15, 40, or 80 ppm for 104 weeks.

At 80 ppm, there were increases in the relative brain, lung and heart weights and an increase in secretion from the Harderian gland in males and females, suppression in body weight gain, decreases in food consumption and WBC, hypertrophy of the renal proximal tubular epithelium and an increased number of mast cells and histiocytes in the sinus of the mesenteric lymph node in males, and increases in the relative liver, kidney and adrenal weights in females. There were increases in the relative liver and kidney weights and hyaline droplet accumulation in the renal proximal tubular epithelium in males, and suppression in body weight gain, decreases in food consumption and WBC, darkening of the Harderian gland, hypertrophy of the renal proximal tubular epithelium, increased basophilic altered foci in the liver and increased histiocytes in the sinus of the mesenteric lymph node in females at 40 ppm and higher.

The NOAEL would be 15 ppm (0.56 mg/kg bw/day for males, 0.69 mg/kg bw/day for females). The compound showed no carcinogenicity (Refs. 11, 58).

(3) Carcinogenicity test for 78 weeks in mice

ICR mice (groups of 50 males and 50 females) were fed diets containing tolfenpyrad, technical grade at 0, 15, 150, or 500/400/300* ppm for 78 weeks.

At 500/400/300 ppm, there were an increase in the relative liver weight in males and females, increases in the relative brain and adrenal weights and decreases in the relative testis and epididymis weights in males, and suppression in body weight gain and atrophy of the ovary, uterine horns and cervix in females. At 150 ppm and higher, there were a decrease in food consumption in males and females, and suppression in body weight gain and a decrease in the relative spleen weight in males.

The NOAEL in this study would be 15 ppm (2.2 mg/kg bw/day for males, 2.8 mg/kg bw/day for females). The compound showed no carcinogenicity (Ref. 59).

* “500/400/300 ppm” represents the highest dose; the compound was first administered to this group at 500 ppm but was decreased to 400 ppm in Week 13, because suppression in body weight gain, a decrease in food consumption and severe symptoms were observed in males and females, and was further decreased to 300 ppm in Week 20 due to the persistence of these symptoms.

11. Reproductive/developmental toxicity

(1) Two-generation reproductive toxicity study in rats

SD rats (groups of 30 males and 30 females) were fed diets containing tolfenpyrad, technical grade at 0, 0.75, 1.5, or 3 mg/kg bw/day for two generations.

In the parent animals, there were a decrease in food consumption in P males and females, 3 fatal cases (2 died from dystocia, 1 killed in moribund state), increased gestation length, abnormal delivery and a decrease in fertility rate in P females, a decrease in food consumption in F_1 males, and suppression in body weight gain and a decrease in the number of implantations in F_1 females at 3 mg/kg bw/day. At 1.5 mg/kg bw/day and higher, there were suppression in body weight gain in P females, and a decrease in food consumption in F_1 females.

In offspring, there were delays in pinna detachment and eye opening in F_1 and F_2 males and females, a decrease in viability of offspring, a delay in righting reflex and decreases in the spleen, relative brain and thymus weights in F_1 generation, and suppression in body weight gain and blackening of the abdominal cavity resulting from accumulation of dark green contents in the small intestine in F_2 generation at 3 mg/kg bw/day. There were suppression in body weight and blackening of the abdominal cavity resulting from accumulation of dark green contents in the small intestine in F_1 generation at 1.5 mg/kg bw/day and higher. There was a decrease in the relative thymus weight in F_2 generation at 0.75 mg/kg bw/day and higher.

Abnormal delivery was observed in P dams, but neither in F_1 dams nor in other similar tests. The long-term administration of this compound, beginning from pre-mating and throughout the pregnancy, caused general toxicity, as evidenced by a decrease in food consumption and low body weight; such toxicological effects were combined with bleeding and other loads at parturition and induced hyposthenia. Therefore, the abnormal delivery might have occurred as a secondary phenomenon to such hyposthenia rather than as a direct influence of this compound on the endocrine or nervous system, or the uterine muscle of the dams.

In offspring, there was a decrease in the relative thymus weight observed in F_2 generation at 0.75 mg/kg bw/day and higher. Immunological functions in F_1 and F_2 generations were studied in a next-generation immunological toxicity test (see (2) in 11. Developmental toxicity), but no change was found in the humoral or cellular immunological function. Therefore, the decrease in the relative thymus weight seems to have little toxicological significance.

The NOAELs in this study for parent animals and offspring would be 0.75 mg/kg bw/day for both sexes of rats (Refs. 10, 60).

(2) Two-generation reproductive toxicity study - Second-generation immunological toxicity test in rats

SD rats (groups of 15 pregnant females) were given tolfenpyrad, technical grade at 0, 0.75, or 3 mg/kg bw/day, from pregnancy and lactation periods of the P parents to maturation of the F_2 offspring by gavage.

In the parent animals, there were suppression in body weight gain and a decrease in food consumption in P generation, decreases in body weight and food consumption and suppression in body weight gain in F_1 generation, and decreases in food consumption and the relative spleen weight in F_2 generation at 3 mg/kg bw/day.

In offspring, there were suppression in body weight gain, a decrease in the relative thymus weight (also in 4-day-old males at 0.75 mg/kg bw/day) and blackening of the abdominal cavity resulting from accumulation of dark green contents in the small intestine in F_1 generation, and decreases in food consumption, the relative thymus weight, numbers of thymocytes and splenocytes, blackening of the abdominal cavity resulting from accumulation of dark green contents in the small intestine and changes in lymphocyte subset (increased proportion of CD3-/CD45RA+ splenocytes at 4 days after birth, decreased proportions of CD3+/CD45RA- splenocytes and CD4+/CD8- splenocytes at 21 days after birth, and decreased proportion of CD3+/CD45RA- splenocytes at 10 weeks after birth) in F_2 generation at 3 mg/kg bw/day.

Despite such changes, humoral or cellular immunological functions were not affected in mature rats. Therefore, this compound would have no immunological toxicity to the next generation (Refs. 10, 61).

(3) Developmental toxicity study in rats

A developmental toxicity study was conducted in SD rats (groups of 24 females). The animals were given tolfenpyrad on days 6-15 of pregnancy at 0, 1, 3, or 4.5 mg/kg bw/day by gavage.

In dams, there were suppression in body weight gain and a decrease in food consumption at 3 mg/kg bw/day. In fetuses, there were low body weight and an increased incidence of lumbar rib at 4.5 mg/kg bw/day.

Most of the lumbar ribs were rudimentary, which have little significance as an indicator of teratogenicity, and were not accompanied by any change in the number of lumbar vertebrae. Therefore, this phenomenon would not show the teratogenicity of tolfenpyrad.

The NOAELs in this study would be 1 mg/kg bw/day for dams and 3 mg/kg bw/day for fetuses. No teratogenicity of tolfenpyrad was evident (Refs. 10, 62).

(4) Developmental toxicity study in rabbits

Japanese White rabbits (groups of 16 females) were given tolfenpyrad on days 6-18 of pregnancy at 0, 1, 3, or 6 mg/kg bw/day by gavage.

In dams, there were abortions, a decrease in food consumption, premature labor (1 case) and death of all embryos (1 case) at 6 mg/kg bw/day, and a fatal case at 3 mg/kg bw/day.

In fetuses, there were increased incidences of skeletal variations (lumbar ribs, extra sternebral ossification site) at 1 and 6 mg/kg bw/day. However, the incidence of extra sternebral ossification site was not correlated with the dose, and the incidence of lumbar ribs, which was within the range of the background control data, was not accompanied by a change in the number of lumbar vertebrae. Therefore, these skeletal variations would not indicate the teratogenicity of tolfenpyrad.

One case of death was observed in the dams at 3 mg/kg bw/day. A histopathological examination of this rat revealed pulmonary congestion, fatty changes of hepatocytes and the renal proximal tubular epithelium, circulatory failures such as atrophy of the spleen and changes derived from undernutrition or asthenia, suggesting that its death was caused by the deterioration in physical status resulting from body weight loss and prolonged absence or suppression of food consumption.

The NOAELs for dams and fetuses in this study would be 1 mg/kg bw/day for dams and 6 mg/kg bw/day for fetuses. No teratogenicity of tolfenpyrad was evident (Refs. 10, 63).

12. Genotoxicity

Tolfenpyrad has been tested *in vitro* and *in vivo* following standard protocols (Table 10). It was not mutagenic in DNA repair and reverse mutation tests in bacteria with and without an exogenous metabolic activation system. Tolfenpyrad induced chromosomal aberrations in cultured Chinese hamster cells (CHL) but it was negative in the mouse micronucleus test.

In the in vitro chromosomal aberration test, numerical aberrations (i.e., induction of polyploids) were observed but no structural aberration was observed. Since the compound did not induce micronuclei *in vivo*, even at the maximum tolerated doses, the committee concluded that tolfenpyrad was not hazardous at least in vivo (Refs. 64-68).

Table 10. Summary of genotoxicity test results (tolfenpyrad)

Test systems		Cells/animals	Dose	Results
in vitro	DNA repair	*Bacillus subtilis* H17, M45 strains		Negative
	Bacterial reverse mutation (+/-S9 mix)	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 strains *E. coli* WP2*uvrA* strain		Negative

	Chromosome aberration (+/-S9 mix)	Cultured Chinese hamster cells (CHL)		Positive (-S9)
in vivo	Rodent micronucleus	ddY mice (6 males and 6 females)	male: 0, 3, 6, 12, 24 female: 0, 1.8, 3.5, 7, 14 (mg/kg bw/day, intraperitoneal administration for two consecutive days)	Negative

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

The metabolites PT-CA, OH-PT, T-CA, T-AM, CA-T-CA, OH-T-CA, OH-PAM, and PCA were tested in bacterial reverse mutation tests (Table 11) and were not mutagenic. The metabolites PT-CA and OH-PT did not induce chromosomal aberration in cultured Chinese hamster cells (CHL) or micronucleus in rats (Refs. 69-80).

Table 11. Summary of genotoxicity test results (metabolites)

Test systems	Test substance (Metabolite)	Cells/animals	Dose	Results
Bacterial reverse mutation (+/-S9 mix)	PT-CA	*S. typhimurium* TA98, TA100, TA1535, TA1537 strains *E. coli* WP2*uvrA* strain		Negative
	OH-PT			Negative
	T-CA			Negative
	T-AM			Negative
	CA-T-CA			Negative
	OH-T-CA			Negative
	OH-PAM			Negative
	PCA			Negative
Chromosome aberration (+/-S9 mix)	PT-CA	Cultured Chinese hamster cells (CHL/IU)		Negative
	OH-PT			Negative
Rodent	PT-CA	SD rats	0, 5, 10, 20	Negative

micronucleus	OH-PT	6 males and 6 females*	(mg/kg bw/day, oral administration by gavage for two consecutive days)	Negative

* 10 males and 10 females for high dose.

13. Other toxicity tests

(1) *In vitro* respiratory inhibition in animal mitochondrial systems

① Study of inhibitory effect on respiration in the rat liver mitochondrial system (electron transfer system)

In vitro inhibitory effect of tolfenpyrad on respiration in the rat liver mitochondrial system (electron transfer system) was studied.

Tolfenpyrad strongly inhibited respiration in the rat liver mitochondrial system (IC_{50}=0.0078 μg/mL). Complex I was considered to be the major site of action (Ref. 81).

② Study of inhibitory effects on respiration in bovine myocardial mitochondria Complex I

Inhibitory effects of tolfenpyrad and metabolite PT-CA on respiration in bovine myocardial mitochondrial Complex I were studied.

Tolfenpyrad strongly inhibited the mitochondrial electron transfer system Complex I (IC_{50}=0.003 μg/mL). Metabolite PT-CA showed little inhibition (Ref. 81).

(2) *In vivo* inhibitory effect in the rat liver mitochondrial system - Qualitative study

① Measurements of tolfenpyrad concentrations in the liver and whole blood after a single oral administration in rats (measured within a short time after administration)

Fischer rats (groups of 3 males) were given tolfenpyrad, technical grade at 0, or 160 mg/kg bw dissolved in aqueous CMC-Na solution. Tolfenpyrad concentrations in the liver and whole blood were measured 5, 15, and 30 minutes after the administration.

Tolfenpyrad was detected in the liver and the whole blood 5 minutes after the administration, and reached the maximum (liver: 0.80 μg/g, whole blood: 0.030 μg/mL) 30 minutes after the administration. Although these measurements did not represent its concentrations in mitochondria of the individual tissues and organs, tolfenpyrad would be present in the mitochondria at amounts sufficient for inducing respiratory inhibition (Refs. 10, 82).

② Study of *in vivo*/*in vitro* action and *in vitro* action on the rat liver mitochondrial respiratory system

SD rats (groups of 2 males) were given tolfenpyrad, technical grade at 0, or 160 mg/kg bw

dissolved in aqueous CMC-Na solution by gavage. Thirty minutes after the administration, livers were collected to prepare a sucrose suspension of hepatic mitochondria.

In rats treated with tolfenpyrad, the ratio (NADH-state 3/Succinate-state 3) related to oxygen consumption was 0.27, which was apparently lower than that (0.42) in control rats. Through comparison with the control group, the degree of inhibition was calculated as 41.7%, which suggested that the compound had an inhibitory effect against *in vivo* mitochondrial respiration in rats (Refs. 10, 82).

III. Evaluation

Absorption, distribution, metabolism and excretion of tolfenpyrad were studied in rats. The whole blood concentration of the compound reached the maximum concentration at 2 hours and from 6 to 8 hours after administration in males and females, respectively, with Single Low, and at from 4 to 16 hours after administration with Single High. The tissue concentrations were relatively high in the liver, kidney, brown adipose tissue and heart at around T_{max}. The main elimination pathway was fecal excretion. Tolfenpyrad was not detected in the urine, and none of the metabolites recovered more than 1.0% of the treated radioactivity. Tolfenpyrad and major metabolites PT-CA, Sul-OH-PT-CA and OH-PT-CA were detected in the feces. A small amount of tolfenpyrad was detected in the bile, and metabolites PT-CA-TA, PT-CA-GA, PT-CA, Sul-OH-PT-CA and CO-PT were detected as major metabolites. The major metabolic pathways of tolfenpyrad in rats would be the oxidation of the methyl group on the tolyloxy ring and the following oxidation of the ethyl group on the pyrazole ring and conjugation.

Plant metabolism of tolfenpyrad was studied in eggplants, cabbages and peaches. Tolfenpyrad and metabolites PT-CA, OH-PT, T-CA, T-AM, CA-T-CA, PCA, OH-T-CA and OH-PAM were detected.

Half-lives of tolfenpyrad in soils ranged from 3 to 5 days and from 127 to 179 days under aerobic and anaerobic conditions, respectively, and the major degradate was PT-CA. No degradate was found in sterilized soil.

Tolfenpyrad was stable against hydrolysis but was photolytically degraded with a half-life of from 35.0 to 35.2 hours, which was calculated as from 11.3 to 11.4 days under natural sunlight in spring in Tokyo (35 degrees north latitude).

The residual levels of tolfenpyrad and six metabolites (PT-CA, OH-PT and T-CA (studied using cucumbers, tomatoes, eggplants, cabbages and Chinese cabbages), OH-PA, OH-T-CA and CA-T-CA (studied using eggplants)) were examined in vegetables, fruits and tea plants. The highest residual value of the compound (23.3 mg/kg) was detected from tea leaves (unrefined), which were collected 7 days after a single application of 300-450 g a.i./ha of the compound, but the values decreased to 7.17, 0.83 and 0.18 mg/kg by Days 14, 21 and 30, respectively. Metabolite PT-CA was detected only in cucumbers at 0.03 mg/kg or less. Metabolites other than PT-CA were not detected under all tested conditions.

Residual values of tolfenpyrad and various degradates in soils were studied (both in the vessel and the field) using volcanic ash, light clay soil and alluvium clay loam. The half-life for tolfenpyrad alone was from 3 to 34 days, and the total of half-lives of tolfenpyrad and degradates PT-CA and PCA was from 3 to 47 days.

The LD_{50}s of tolfenpyrad were 86 mg/kg bw (olive oil) in males and 75 mg/kg bw (olive oil) in females in rats, and 80-100 mg/kg bw (olive oil) in males and 50-80 mg/kg bw (olive oil) in females in mice by oral dose, >2000 mg/kg bw in males and >3000 mg/kg bw in females in rats by dermal treatment, and 2.21 mg/L in males and 1.50 mg/L in females in rats by inhalation.

The LD_{50}s of metabolite PT-CA were 27.4 (aqueous CMC-Na solution) and 15.4 mg/kg bw (aqueous CMC-Na solution) in male and female rats, respectively, by oral dose. The LD_{50} of metabolite OH-PT was 30-60 mg/kg bw (olive oil) in male and female rats by oral dose.

The NOAELs of tolfenpyrad were 15.9 mg/kg bw/day in mice, <0.91 mg/kg bw/day in rats and 1 mg/kg bw/day in dogs in subchronic toxicity tests. The compound showed no neurotoxicity.

The NOAELs were 0.56 mg/kg bw/day in rats, 2.2 mg/kg bw/day in mice and 1 mg/kg bw/day in dogs in chronic toxicity and carcinogenicity tests. In the subchronic toxicity test in rats, relative weights of the liver and kidney increased at the minimum dose, so the NOAEL could only be settled as <0.91 mg/kg. However, the NOAEL could be settled in rats in the combined study of chronic toxicity and carcinogenicity, which was performed over a longer period of time. Therefore, there should be no problem in using the NOAEL determined in the combined study of chronic toxicity and carcinogenicity in rats for settling the ADI. Tolfenpyrad showed no carcinogenicity in rats and mice.

In the two-generation reproductive toxicity study in rats, the NOAEL was 0.75 mg/kg bw/day.

The developmental toxicity studies were performed in rats and rabbits, and the NOAELs were 1 mg/kg bw/day for dams and 3 mg/kg bw/day for fetuses in rats, and 1 mg/kg bw/day for dams and 6 mg/kg bw/day for fetuses in rabbits. Tolfenpyrad showed no teratogenic potential in either rats or rabbits.

Tolfenpyrad was not genotoxic in bacteria with and without an exogenous metabolic activation system. Tolfenpyrad induced chromosomal aberrations in cultured Chinese hamster cells (CHL), but only numerical aberrations (i.e., induction of polyploids) and no structural aberration. The chemical did not induce micronuclei in mice tested up to the maximum tolerated dose. The committee concluded that tolfenpyrad was not have any serious genotoxocity *in vivo*.

The metabolites PT-CA, OH-PT, T-CA, T-AM, CA-T-CA, OH-T-CA, OH-PAM and PCA were tested in the microbial reverse mutation test and were not mutagenic. The metabolites PT-CA and OH-PT did not induce chromosomal aberration in cultured Chinese hamster cells (CHL) or micronucleus in rats.

The NOAELs in the toxicological tests evaluated are shown in Table 12.

Table 12. The NOAELs determined in several toxicity evaluating tests

Species	Evaluating test	NOAEL (mg/kg bw/day)	Note

Mouse	90 days subchronic toxicity	Male: 15.9 Female: 20.2	
	78 weeks carcinogenicity	Male: 2.2 Female: 2.8	No carcinogenicity
Rat	90 days subchronic toxicity	Male: <0.91 Female: <1.01	
	90 days subchronic neurotoxicity	Male: 2.7 Female: 3.2	No neurotoxicity
	Combined study of chronic toxicity and carcinogenicity for 104 weeks	Male: 0.56 Female: 0.69	No carcinogenicity
	Two-generation reproductive toxicity	Parent animals and offspring: 0.75	
	Developmental toxicity	Dam: 1 Fetus: 3	No teratogenicity
Rabbit	Developmental toxicity	Dam: 1 Fetus: 6	No teratogenicity
Dog	90 days subchronic toxicity	Male and female: 1	
	1 year chronic toxicity	Male and female: 1	

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

ADI:	0.0056 mg/kg bw/day
(Referred data for ADI)	Combined study of chronic toxicity and carcinogenicity
Laboratory animal tested:	Rat
Duration:	104 weeks
Administration route:	mixed feeds
NOAEL:	0.56 mg/kg bw/day
Safety factor:	100
Residue definition for exposure assessment:	
	Tolfenpyrad (parent chemical only)

(Appendix 1: Abbreviations for metabolites/degradates)

Abbreviation	Chemical name
CA-T-AM	4-(4-carbamoylphenoxy)benzoic acid
CA-T-CA	4,4'-oxydibenzoic acid
CA-T-NH_2	4-[4-(aminomethyl)phenoxy]benzoic acid
CO-PT	3-acetyl-4-chloro-1-methyl-
CO-PT-CA	4-[4-[(3-acetyl-4-chloro-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid
DM-PT	4-chloro-3-ethyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide
OH-PT	4-chloro-(1-hydroxyethyl)-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide
OH-PAM	4-chloro-3-(1-hydroxyethyl)-1-methylpyrazole-5-carboxamide
OH-PT-CA	4-[4-[[4-chloro-3-(1-hydroxyethyl)-1-methylpyrazol-5-yl]carbonylaminomehtyl]phenoxy] benzoic acid
OH-PT-OH	4-chloro-3-(1-hydroxyethyl)-*N*-[4-[4-(hydroxymethyl)phenoxy]benzyl]-1-methylpyrazole-5-carboxamide
OH-T-CA	4-[4-(hydroxymethyl)phenoxy]benzoic acid
OH-T-OH	bis[4-(hydroxymethyl)phenyl]ether
PCA	4-chloro-3-ethyl-1-methylpyrazole-5-carboxylic acid
PT-CA	4-[4-[(4-chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid
PT-CA-GA	Glucuronic acid conjugate of PT-CA
PT-CA-Me	4-[4-[(4-chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic methyl ester
PT-CA-TA	2-[4-[(4-chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]phenylcarbonylamino]ethane-1-sulfonic acid
PT-CHO	4-chloro-3-ethyl-*N*-[4-(4-formylphenoxy)benzyl]-1-methylpyrazole-5-carboxamide
PT-OH	4-chloro-3-ethyl-*N*-[4-[4-(hydroxymethyl)phenoxy]benzyl]-1-methylpyrazole-5-carboxamide
PT(A)-4OH	4-chloro-3-ethyl-*N*-(4-hydroxybenzyl-1-methylpyrazole-5carboxamide
T-AM	4-(*p*-tolyloxy)benzamide
T-CA	4-(*p*-tolyloxy)benzoic acid
Sul-OH-PT-CA	4-[4-[[4-chloro-1-methyl-3-(1-sulfoxyethyl)pyrazole-5-yl]]]carbonylaminomethyl]phenoxy]benzoic acid

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

Abbreviation	Name
ADI	Acceptable daily intake
A/G	Albumin/globulin
ALP	Alkaline phosphatase
GPT	Glutamic pyruvic transaminase
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
NOAEL	No observed adverse effect level
RBC	Red blood cell
TAR	Total applied radioactivity
TG	Triglyceride
TRR	Total radioactivity residue
WBC	White blood cell
γ-GTP	γ-Glutamyl transpeptidase

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