5.34 TOLFENPYRAD (269)

TOXICOLOGY

Tolfenpyrad, a pyrazole insecticide, is the ISO-approved name for 4-chloro-3-ethyl-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide (IUPAC), which has the CAS number 129558-76-5. Tolfenpyrad has broad insecticidal activity against a variety of pests on egg, larval, nymphal and adult stages and is used on a variety of crops. The pesticidal mode of action is thought to be the inhibition of complex I of the respiratory electron transport chain in the mitochondria.

Tolfenpyrad has not previously been evaluated by JMPR and is being reviewed by the present Meeting at the request of CCPR.

All critical studies contained statements of compliance with GLP unless otherwise specified.

Biochemical aspects

After administration of a single oral dose of radiolabelled tolfenpyrad to rats, maximum concentrations in blood and plasma were reached 2–8 hours after a low dose (1 mg/kg bw) and 4–12 hours after a high dose (20 mg/kg bw). Excretion of radioactivity in urine (2–3%) and bile (51–70%) and residual radioactivity in the carcass (5–11%) 48 hours after dosing indicate that absorption was at least 58% of the dose. Plasma half-lives were 11–28 hours. Radioactivity was widely distributed to the tissues, higher concentrations being found in liver, kidney, bone marrow and brown fat. Seven days after dosing, 88–93% and 2–3% of the radioactivity were excreted in faeces and urine, respectively. In faeces, 4–15% of the radioactivity represented tolfenpyrad, and 24–49% of the radioactivity represented the metabolite PT-CA (see Table 1 for names of metabolites). In plasma, liver and kidney, 91–100% of the radioactivity represented PT-CA, indicating extensive metabolism of tolfenpyrad. In bile, 50–67% of the administered dose was excreted within 48 hours, the major part as PT-CA-TA, PT-CA-glucuronide and PT-CA, whereas low levels of Sul-OH-PT-CA and CO-PT and other, unidentified metabolites were also detected. These data indicate extensive conjugation of PT-CA in the liver and subsequent excretion into the bile. Only 0.1–0.4% of the administered dose was present in bile as unchanged tolfenpyrad. In bile duct–cannulated rats, only 3–8% of the administered dose was excreted into faeces, predominantly as tolfenpyrad (up to 6%) and PT-CA (up to 1%). PT-CA is deconjugated following its biliary excretion and excreted in faeces. In urine, no intact tolfenpyrad was detected. Various individual metabolites (including OH-PAM and CA-T-CA) were present in urine at less than 0.5% of the administered dose, with the exception of PT-CA and PT-CA-TA (these metabolites could not be further separated), which were present at up to 1.9% of the administered dose. Observed differences in metabolite levels between sexes, doses and position of radiolabel were minor. Following repeated dosing of [^{14}C]tolfenpyrad, plasma concentrations of radioactivity stabilized after two or three administrations at 1.5–3 times the plasma concentration found after the first dose. Tissue distribution, excretion and metabolism were similar following the single low and high doses and following single and repeated dosing.

List of abbreviations and chemical names of metabolites used in the report

Abbreviation	Chemical name
OH-PT	4-Chloro-3-(1-hydroxyethyl)-1-methyl-*N*-[4-(*p*-tolyloxy) benzyl]pyrazole-5-carboxamide
CO-PT	3-Acetyl-4-chloro-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide
PT-CA	4-[4-[(4-Chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid
OH-PT-CA	4-[4-[(4-Chloro-3-(1-hydroxyethyl)-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid
T-CA	4-(*p*-Tolyloxy)benzoic acid

Abbreviation	Chemical name
CA-T-CA	4,4′-Oxydibenzoic acid
PAM	4-Chloro-3-ethy1-1-methylpyrazole-5-carboxamide
OH-PAM	4-Chloro-3-(1-hydroxyethyl)-1-methylpyrazole-5-carboxamide
Sul-OH-PT-CA	4-[4-[(4-Chloro-1-methyl-3-(1-sulfoxyethyl)pyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid
PT-CA-TA	2-[4-[4-[(4-Chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]phenylcarbonylamino]ethane-1-sulfonic acid
PT(A)-4OH	4-Chloro-3-ethyl-*N*-(4-hydroxybenzyl)-1-methylpyrazole-5-carboxamide
T-AM	4-(4-Tolyloxy)benzamide
PCA	4-Chloro-3-ethyl-1-methylpyrazole-5-carboxylic acid
OH-T-CA	4-[4-(Hydroxymethyl)phenoxy] benzoic acid

Toxicological data

The oral LD_{50} values for tolfenpyrad dissolved in aqueous carboxymethylcellulose were greater than or equal to 113 mg/kg bw in two rat studies, and the oral LD_{50} for tolfenpyrad dissolved in olive oil was greater than or equal to 75 mg/kg bw in one rat study. The LD_{50} for dermal toxicity was greater than 2000 mg/kg bw in rats. The acute inhalation LC_{50} was 1.5–2.21 mg/L. Tolfenpyrad was not irritating to the skin and slightly irritating to the eye of rabbits. Tolfenpyrad was not a skin sensitizer in a Magnusson and Kligman test in guinea-pigs.

In repeated-dose toxicity studies with tolfenpyrad, multiple adverse effects were observed. In a 90-day toxicity study in mice using dietary concentrations of 0, 15, 100 and 300 ppm (equal to 0, 2.4, 15.9 and 46.2 mg/kg bw per day for males and 0, 3.0, 20.2 and 57.9 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 15.9 mg/kg bw per day), based on increased AST activity, increased relative heart weight in males and increased relative liver weight in both sexes at 300 ppm (equal to 46.2 mg/kg bw per day). In a 90-day toxicity study in rats using dietary concentrations of 0, 15, 80 and 160 ppm (equal to 0, 0.906, 4.78 and 9.33 mg/kg bw per day for males and 0, 1.01, 5.17 and 9.32 mg/kg bw per day for females, respectively), the NOAEL was 15 ppm (equal to 0.906 mg/kg bw per day), based on changes in clinical chemistry, a reduced white blood cell count, dark brown change of the liver, hypertrophy of the proximal renal tubular epithelium and the acinar cells in the mandibular glands in females and an increase in mast cells in the mesenteric lymph nodes, diffuse hypertrophy of hepatocytes and hypertrophy of the pancreatic acinar cells in both sexes at 80 ppm (equal to 4.78 mg/kg bw per day).

In a 28-day as well as a 90-day capsule study in dogs using doses of 0, 1, 5 and 10 mg/kg bw per day, the NOAEL was 1 mg/kg bw per day, based on the increased incidence of vomiting at 5 mg/kg bw per day. In a second 90-day capsule study in dogs with administration of tolfenpyrad at doses of 0, 10, 30 and 100 mg/kg bw per day, mild toxicity (i.e. vomiting, soft and mucous faeces) was observed at 10 mg/kg bw per day, the lowest dose tested. Severe toxicity including mortality was observed at doses of 30 and 100 mg/kg bw per day. In a 1-year capsule study in dogs using doses of 0, 1, 5 and 20/10 mg/kg bw per day, the NOAEL was 1 mg/kg bw per day, based on increased incidences of vomiting, soft or mucous stool and salivation and increased ALT level at 5 mg/kg bw per day. In all the studies in dogs, vomiting and soft stool were observed as early as the 1st day of dosing. Therefore, the overall NOAEL for these effects was 1 mg/kg bw per day, with an overall LOAEL of 5 mg/kg bw per day.

In a 78-week toxicity study in mice using dietary concentrations of 0, 15, 150 and 500/400/300 ppm (equal to 0, 2.2, 20.8 and 60.9 mg/kg bw per day for males and 0, 2.8, 27.1 and

75.9 mg/kg bw per day for females, respectively), the NOAEL was 15 ppm (equal to 2.2 mg/kg bw per day), based on decreased body weight gain and feed consumption and changes in organ weights observed in males and females at 150 ppm (equal to 20.8 mg/kg bw per day). No increased incidences of tumours were observed at doses up to 500/400/300 ppm (equal to 60.9 mg/kg bw per day), the highest concentration tested.

In a 2-year toxicity and carcinogenicity study in rats using dietary concentrations of 0, 15, 40 and 80 ppm (equal to 0, 0.561, 1.5 and 3.1 mg/kg bw per day for males and 0, 0.686, 1.9 and 3.8 mg/kg bw per day for females, respectively), the NOAEL was 15 ppm (equal to 0.561 mg/kg bw per day), based on reduced feed intake, increased severity of basophilic foci of altered hepatocytes, sinus histiocytosis of the mesenteric lymph nodes and hypertrophy of the proximal renal tubule epithelia in females at 40 ppm (equal to 1.5 mg/kg bw per day). There was no compound-related increase in the incidence of tumours.

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

Tolfenpyrad was tested for genotoxicity in an adequate range of in vitro and in vivo assays. These assays provided no evidence of genotoxic potential.

The Meeting concluded that tolfenpyrad is unlikely to be genotoxic.

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

A standard two-generation reproductive toxicity study in rats was performed in which dietary concentrations were adjusted to maintain the desired dose levels of 0, 0.75, 1.5 and 3 mg/kg bw per day. This was followed by a modified non-GLP two-generation reproductive toxicity study focusing on the effects of tolfenpyrad on immune function, in which the dietary concentrations were adjusted to maintain target dose levels of 0, 0.75 and 3 mg/kg bw per day. The overall NOAEL for parental toxicity from these two studies was 1.5 mg/kg bw per day, based on moribundity, decreased body weight gain and decreased feed consumption at 3 mg/kg bw per day observed in the first study. The overall NOAEL for offspring toxicity was 0.75 mg/kg bw per day, based on a reduction in absolute and relative thymus weights in males and females of the F_2 generation at 1.5 mg/kg bw per day, observed in the first study, and on reduced body weight gain during lactation and a reduced number of F_1 pups at PND 4, black change in the peritoneal cavity after birth in F_1 and F_2 pups, lower thymus and spleen weights soon after birth in F_1 pups and in F_2 male pups, reduced thymus and spleen cellularity, and changes in immune cell ratios in the spleen in F_2 male pups at 3 mg/kg bw per day, observed in the second study. The Meeting concluded that the small reductions in absolute and relative thymus weights observed at 0.75 mg/kg bw per day, in the absence of other relevant effects, were not toxicologically significant. In the second study, humoral immunity and cellular immune function were normal in adult F_1 and F_2 rats. The overall NOAEL for reproductive toxicity was 1.5 mg/kg bw per day, based on a range of effects occurring late in gestation resulting in a reduced number of live offspring, observed in the first but not in the second study, at 3 mg/kg bw per day.

In a developmental toxicity study in rats using doses of 0, 1, 3 and 4.5 mg/kg bw per day, the NOAEL for maternal toxicity was 1 mg/kg bw per day, based on reduced body weight gain and feed consumption at 3 mg/kg bw per day, observed during the first days of treatment. The NOAEL for embryo and fetal toxicity was 3 mg/kg bw per day, based on decreased fetal weight, increased incidence of skeletal variations and delayed ossification observed at 4.5 mg/kg bw per day. No evidence of a teratogenic effect was observed.

In a developmental toxicity study in rabbits using doses of 0, 1, 3 and 6 mg/kg bw per day, the NOAEL for maternal toxicity was 1 mg/kg bw per day, based on one mortality observed at 3 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 6 mg/kg bw per day, the highest dose tested. No evidence of a teratogenic effect was observed.

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

In an acute oral (gavage) neurotoxicity study in rats using doses of 0, 20, 40 and 60 mg/kg bw in males and 0, 10, 20 and 40 mg/kg bw in females, the LOAEL was 10 mg/kg bw, the lowest dose tested, based on reductions in body weight and feed consumption observed in females on the day of dosing. Clinical signs were observed at 40 mg/kg bw in females and at 60 mg/kg bw in males. No neurotoxicity was observed.

In a 90-day neurotoxicity study in rats using dietary concentrations of 0, 15, 40 and 80 ppm (equal to 0, 1.0, 2.7 and 5.4 mg/kg bw per day for males and 0, 1.2, 3.2 and 6.0 mg/kg bw per day for females, respectively), the NOAEL was 40 ppm (equal to 3.2 mg/kg bw per day), based on reductions in body weight gain and feed consumption in females at 80 ppm (equal to 6.0 mg/kg bw per day). No clinical, functional or histological signs of neurotoxicity were observed at doses up to 80 ppm (equal to 5.4 mg/kg bw per day), the highest dose tested.

The Meeting concluded that tolfenpyrad is not neurotoxic.

In the modified two-generation reproductive toxicity study focusing on effects of tolfenpyrad on immune function (see above), tolfenpyrad caused changes in the immune system in rat pups but did not affect normal humoral immunity or cellular immune function in adult rats.

Toxicological data on metabolites and/or degradates

Studies of acute oral toxicity were performed with the tolfenpyrad metabolites OH-PT, PT-CA, PT(A)-4OH, T-CA, T-AM, CA-T-CA, OH-T-CA, PAM, OH-PAM and PCA dissolved in aqueous carboxymethylcellulose. In general, the metabolites had low acute toxicity, except for OH-PT ($LD_{50} \geq 35.5$ mg/kg bw) and PT-CA ($LD_{50} \geq 15.4$ mg/kg bw), which were slightly more toxic than tolfenpyrad. All these metabolites showed negative results in tests for reverse mutation induction in bacteria (Note: PT(A)-4OH and PAM were not tested). OH-PT and PT-CA were also tested in a chromosomal aberration test in vitro and a micronucleus test in vivo. No genotoxicity was observed. In a 4-week dietary study in rats, the toxicity of tolfenpyrad at dietary concentrations of 0, 10, 30 and 100 ppm was compared with the toxicities of PT-CA and OH-PT at dietary concentrations of 0, 3, 10, 30 and 100 ppm. The NOAEL for tolfenpyrad was 30 ppm (equal to 2.5 mg/kg bw per day), based on a mild reduction in feed consumption in males and females, a mild reduction in body weight gain observed in males and increased incidences and severity of hypertrophy of acinar cells in pancreas in females at 100 ppm (equal to 8 mg/kg bw per day). The NOAEL for PT-CA was 100 ppm (equal to 8.1 mg/kg bw per day), the highest dose tested, and the NOAEL for OH-PT was 100 ppm (equal to 8.4 mg/kg bw per day), the highest dose tested. In view of the lower LD_{50} values for the metabolites PT-CA and OH-PT compared with tolfenpyrad, the Meeting considered these two compounds to be toxicologically relevant.

In the absence of any toxicological information on the livestock metabolite OH-PT-CA, but on consideration of the similarity of its structure to that of tolfenpyrad, it was assumed that OH-PT-CA was of similar toxic potency to tolfenpyrad. No data were available on the toxicity of the livestock metabolites, PT-CA conjugates and OH-PT-CA conjugates. However, as these are likely to be hydrolysed to PT-CA and OH-PT-CA, respectively, the Meeting concluded that their toxicities should be considered equivalent to those of PT-CA and OH-PT-CA.

Human data

No information on adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to tolfenpyrad was available.

The Meeting concluded that the existing database on tolfenpyrad is sufficient to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI for tolfenpyrad of 0–0.006 mg/kg bw on the basis of a NOAEL of 0.561 mg/kg bw per day in a 2-year rat study with tolfenpyrad, for reduced feed intake, increased severity of basophilic foci of altered hepatocytes, sinus histiocytosis of the mesenteric lymph nodes and hypertrophy of the proximal tubule epithelia in females at 1.5 mg/kg bw per day, using a safety factor of 100.

The Meeting established an ARfD of 0.01 mg/kg bw for tolfenpyrad based on a NOAEL of 1 mg/kg bw per day for reduced body weight and feed consumption observed during the first days of treatment in a developmental toxicity study with tolfenpyrad in rats at 3 mg/kg bw and an overall NOAEL of 1 mg/kg bw per day for vomiting and soft stool observed on the 1st day of treatment in 28-day, 90-day and 1-year studies with tolfenpyrad in dogs at 5 mg/kg bw per day. A safety factor of 100 was applied. The ARfD provides a margin of exposure of 1000 over the LOAEL in the acute neurotoxicity study in rats. The Meeting considered it unlikely that the acute effects observed in rats and dogs are the result of the unpalatability of tolfenpyrad, as the effects were observed after gavage or capsule administration. The Meeting also considered it unlikely that the acute effects were secondary to local gastrointestinal irritation, as no such effects were reported in any of the studies.

The Meeting considered that the ADI and ARfD are also applicable to the metabolites PT-CA and OH-PT, which showed similar toxicity to tolfenpyrad in LD_{50} studies but lower toxicity in a 4-week dietary study. In addition, the Meeting considered the ADI and ARfD applicable to all the livestock metabolites: OH-PT-CA, PT-CA conjugates and OH-PT-CA conjugates. The Meeting noted that in the absence of data on the effects of these metabolites in long-term and developmental toxicity studies in rats and capsule studies in dogs (i.e. studies that formed the basis of the ADI and ARfD), it would not be possible to establish the relative potency of the metabolites to tolfenpyrad in order to refine the dietary exposure assessment.

A toxicological monograph was prepared.

Levels relevant for risk assessment of tolfenpyrad

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	15 ppm, equal to 2.2 mg/kg bw per day	150 ppm, equal to 20.8 mg/kg bw per day
		Carcinogenicity	500/400/300 ppm, equal to 60.9 mg/kg bw per day[b]	—
Rat	Thirteen-week study of toxicity[a]	Toxicity	15 ppm, equal to 0.906 mg/kg bw per day	80 ppm, equal to 4.78 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	15 ppm, equal to 0.561 mg/kg bw per day	40 ppm, equal to 1.5 mg/kg bw per day
		Carcinogenicity	80 ppm, equal to 3.1 mg/kg bw per day[b]	—
	Two-generation studies of reproductive toxicity[a,c,d]	Parental toxicity	1.5 mg/kg bw per day	3 mg/kg bw per day
		Offspring toxicity	0.75 mg/kg bw per day	1.5 mg/kg bw per day
		Reproductive toxicity	1.5 mg/kg bw per day	3 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	1 mg/kg bw per day	3 mg/kg bw per day
		Embryo and fetal toxicity	3 mg/kg bw per day	4.5 mg/kg bw per day
	Acute neurotoxicity	Neurotoxicity	40 mg/kg bw[b]	—

Species	Study	Effect	NOAEL	LOAEL
	study[e]	Toxicity	—	10 mg/kg bw[f]
	Ninety-day neurotoxicity study[a]	Neurotoxicity	80 ppm, equal to 5.4 mg/kg bw per day	—
Rabbit	Developmental toxicity study[e]	Maternal toxicity	1 mg/kg bw per day	3 mg/kg bw per day
		Embryo and fetal toxicity	6 mg/kg bw per day[b]	—
Dog	Four-week, 13-week and 1-year studies of toxicity[c,g,h]	Toxicity	1 mg/kg bw per day	5 mg/kg bw per day

[a] Dietary administration.

[b] Highest dose tested.

[c] Two or more studies combined.

[d] Dietary concentrations of tolfenpyrad were adjusted over the course of the study in order to obtain the required daily doses (mg/kg bw per day) for the different dose groups and sexes. Therefore, ppm values are not presented.

[e] Gavage administration.

[f] Lowest dose tested.

[g] Identical NOAELs and LOAELs were observed in all three dog studies. In all studies, vomiting and soft stool were observed on the 1st day of testing.

[h] Capsule administration.

Estimate of acceptable daily intake

0–0.006 mg/kg bw

Estimate of acute reference dose

0.01 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid (T_{max} 2–8 h); ≥ 58% (rats)
Dermal absorption	No data available (probably relatively low in view of data from LD_{50} and short-term studies with oral and dermal dosing)
Distribution	Widely distributed (rats)
Potential for accumulation	Low
Rate and extent of excretion	88–93% in faeces and 2–3% in urine; plasma half-lives at 1 and 20 mg/kg bw: 11–28 h
Metabolism in animals	Extensive, rapidly metabolized in the liver and subsequently

	conjugated and then excreted into bile
Toxicologically significant compounds in animals, plants and the environment	Tolfenpyrad, PT-CA, PT-CA conjugates, OH-PT, OH-PT-CA, OH-PT-CA conjugates
Acute toxicity	
Rat, LD_{50}, oral	Dissolved in aqueous carboxymethylcellulose: ≥ 113 mg/kg bw
	Dissolved in olive oil: ≥ 75 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	≥ 1.5 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slightly irritating
Guinea pig, dermal sensitization	Not sensitizing (Magnusson and Kligman)
Short-term studies of toxicity	
Target/critical effect	Many end-points affected
Lowest relevant oral NOAEL	0.906 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	200 mg/kg bw per day, the highest dose tested (rat)
Lowest relevant inhalatory NOAEC	2 mg/m^3 (rat)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Feed intake, liver, kidneys, mesenteric lymph nodes (rats)
Lowest relevant NOAEL	0.561 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Target/critical effect	Reduced number of live offspring at parentally toxic doses
Lowest relevant parental NOAEL	1.5 mg/kg bw per day
Lowest relevant offspring NOAEL	0.75 mg/kg bw per day
Lowest relevant reproductive NOAEL	1.5 mg/kg bw per day
Developmental toxicity	
Target/critical effect	Decreased fetal weight, increased skeletal variations at maternally toxic doses
Lowest relevant maternal NOAEL	1 mg/kg bw per day (rat, rabbit)
Lowest relevant embryo/fetal NOAEL	3 mg/kg bw per day (rat)
Neurotoxicity	
Acute and subchronic neurotoxicity	Not neurotoxic
Other toxicological studies	
Immunotoxicity	Increased thymus and spleen weights and changed immune cell ratios in pups but not in adults
Studies with PT-CA	
Acute toxicity	LD_{50} ≥ 15.4 mg/kg bw

4-week dietary toxicity	8.1 mg/kg bw per day, the highest dose tested
Genotoxicity	Not genotoxic
Studies with OH-PT	
Acute toxicity	$LD_{50} \geq 35.5$ mg/kg bw
4-week dietary toxicity	8.4 mg/kg bw per day, the highest dose tested
Genotoxicity	Not genotoxic
Medical data	
	No data

Summary

	Value	Study	Safety factor
ADI	0–0.006 mg/kg bw	Two-year study of toxicity in rats	100
ARfD	0.01 mg/kg bw	Developmental toxicity study in rats; 28-day, 90-day and 1-year toxicity studies in dogs	100

RESIDUE AND ANALYTICAL ASPECTS

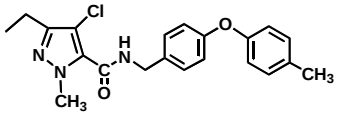
The toxicological and residue evaluation of tolfenpyrad was scheduled for the 2013 JMPR by the Forty-fourth Session of the CCPR.

Tolfenpyrad is a broad spectrum insecticide and a miticide, with contact activity against target pests on eggs, larvae, nymphs, and adults. It also has anti-feeding activity on larvae of lepidopteran insects. It belongs to the pyrazole class of insecticides. It has activity against several economically important insect pests of vegetables, fruits, nuts, vines and row crops.

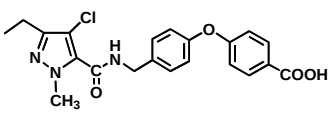
The Meeting received information from the manufacturer on identity, the animal and plant metabolism, environmental fate analytical methods, storage stability, effect of processing, animal feeding studies, and results of supervised trials on almonds, cantaloupe,, cauliflower, cherries, cucumbers, cotton seed, grapes (table), grapefruits, lemons, oranges, peaches, pears, pecans, peppers, plums, potatoes, summer squash, tea and tomatoes.

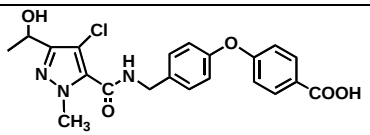
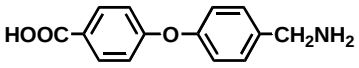
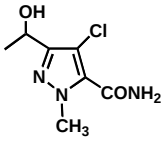
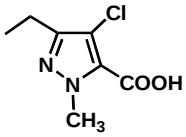
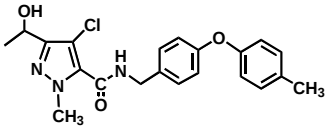
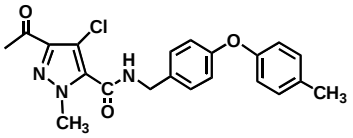
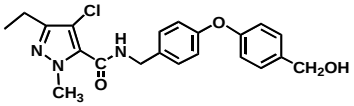
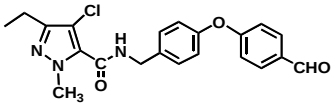
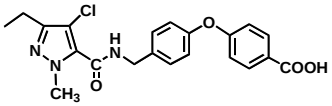
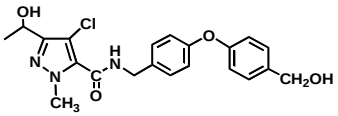
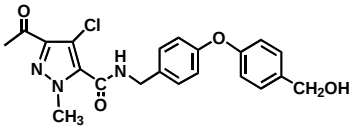
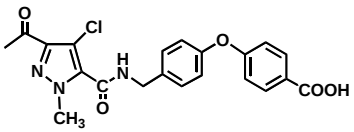
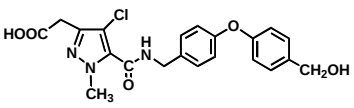
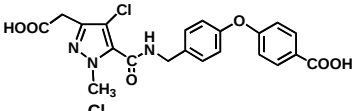
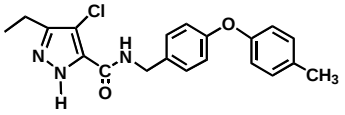
Chemical name and structure

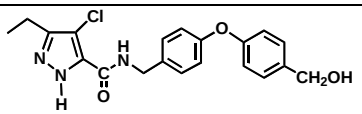
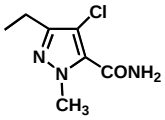
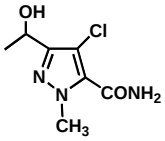
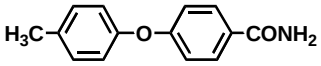
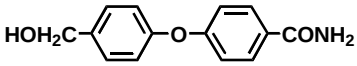
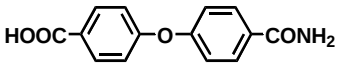
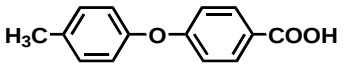
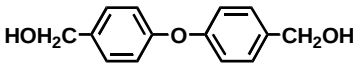
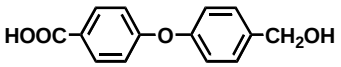
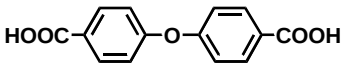
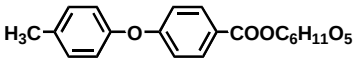
4-chloro-3-ethyl-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide



Chemical names and structures of metabolites referred to in the appraisal by codes:

Code Name	Chemical name	Structure	Matrices
PT-CA	4-[4-[(4-chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid		rat, plant, soil animal commodities

Code Name	Chemical name	Structure	Matrices
OH-PT-CA	4-[4-[[4-chloro-3-(1-hydroxyethyl) -1-methylpyrazol-5-yl]carbonylamino-methyl]phenoxy] benzoic acid		rat, plant, animal
CA-T-NH_2	4-[4-(aminomethyl)phenoxy]benzoic acid		Photodecompositio n, animal
OH-PAM	4-chloro-3-(1-hydroxyethyl)-1-methylpyrazole-5-carboxamide		rat, plant, soil, eggs
PCA	4-chloro-3-ethyl-1-methylpyrazole-5-carboxylic acid		Plant, soil, eggs
OH-PT	4-chloro-3-(1-hydroxyethyl)-1-methyl-*N*-[4-(p-tolyloxy)benzyl] pyrazole-5-carboxamide		rat, plant
CO-PT	3-acetyl-4-chloro-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl] pyrazole-5-carboxamide		plant
PT-OH	4-chloro-3-ethyl-*N*-[4-[4-(hydroxymethyl)phenoxy]benzyl]-1-methylpyrazole-5-carboxamide		rat, plant, soil, photodecomposition
PT-CHO	4-chloro-3-ethyl-*N*-[4-(4-formylphenoxy)benzyl]-1-methylpyrazole-5-carboxamide		rat, plant, soil, photodecomposition
PT-CA	4-[4-[(4-chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid		rat, plant, soil animal commodities
OH-PT-OH	4-chloro-3-(1-hydroxyethyl)-*N*-[4-[4-(hydroxymethyl)phenoxy]benzyl]-1-methylpyrazole-5-carboxamide		rat, plant, animal commodities
CO-PT-OH	3-acetyl-4-chloro-*N*-[4-[4-(hydroxymethyl)phenoxy]benzyl]-1-methylpyrazole-5-carboxamide		plant
CO-PT-CA	4-[4-[(3-acetyl-4-chloro-1-methylpyrazol-5-yl) carbonyl-aminomethyl]phenoxy]benzoic acid		plant
CA-PT-OH	[4-chloro-5-[N-[4-(4-hydroxymethyl) phenoxy]benzylcarbamoyl]-1-methylpyrazol-3-yl]acetic acid		rat, plant
CA-PT-CA	4-[4-[3-(carboxymethyl-4-chloro-1-methylpyrazol-5-yl]carbonyl-aminomethyl]phenoxy]benzoic acid		rat, plant
DM-PT	4-chloro-3-ethyl-*N*-[4-(*p*-tolyloxy) benzyl]pyrazole-5-carboxamide		plant

Code Name	Chemical name	Structure	Matrices
DM-PT-OH	4-chloro-3-ethyl-*N*-[4-[4-(hydroxymethyl)phenyl]benzyl] pyrazole-5-carboxami		plant
PAM	4-chloro-3-ethyl-1-methylpyrazole-5-carboxamide		rat, plant, soil
OH-PAM	4-chloro-3-(1-hydroxyethyl)-1-methylpyrazole-5-carboxamide		rat, plant, soil, eggs
T-AM	[4-(*p*-tolyloxy)benzamide		plant
OH-T-AM	4-[4-(hydroxymethyl)phenoxy] benzamide		plant
CA-T-AM	4-(4-carbamoylphenoxy)benzoic acid		plant
T-CA	4-(*p*-tolyloxy)benzoic acid		rat, plant
OH-T-OH	*bis*[4-(hydroxymethyl)phenyl]ether		pant
OH-T-CA	4-[4-(hydroxymethyl)phenoxy] benzoic acid		rat, plant
CA-T-CA	4,4'-oxydibenzoic acid		rat, plant
T-CA-Glu	glucose conjugate of 4-(*p*-tolyloxy) benzoic acid		plant

Animal metabolism

The Meeting received reports of animal metabolism studies in lactating goats and laying hens. The studies were conducted with [^{14}C] tolfenpyrad labelled on the pyrazole and tolyl rings.

Metabolism in laboratory animals is summarized under toxicology.

Lactating goats

[Pyrazole-^{14}C]-tolfenpyrad and [tolyl-^{14}C]-tolfenpyrad were administered orally (12.3–12.5 mg/kg feed/day) to two lactating goats once daily for five consecutive days. Milk was collected twice daily and excreta were collected once daily. The total recovery of radiolabel was 76.1% and 96.3% of the administered dose (AD) for the pyrazole- and tolyl-labels, respectively. Most of the administered dose was recovered in the excreta (46.8–49.8%) and gastrointestinal tracts (19.2–32.6%) at sacrifice.

Administration of [tolyl-^{14}C]-tolfenpyrad resulted in consistently higher residues in tissues than the administration of [pyrazole-^{14}C]-tolfenpyrad. The total radioactive residues (TRR) expressed

as tolfenpyrad mg equivalents/kg were in liver (25 mg/kg, 12%AD) and kidney (6.93 mg/kg, 0.7%AD) and substantially lower in the fat (0.36 mg/kg, 0.4%AD), milk (0.17 mg/kg, 19% AD) and muscle (0.14 mg/kg, 0.1–0.2%AD). No free metabolite unique to only one of the radiolabels was found.

The parent tolfenpyrad was present up to 4.1% TRR (< 0.01 mg/kg) in milk, 10% TRR (0.01 mg/kg) in muscle, 17.3%TRR (0.06 mg/kg) in fat, and was not detected in liver and kidney.

Following the administration of pyrazole- and tolyl- labelled tolfenpyrad, the predominant residues were PT-CA in free and its conjugated form. The free and conjugated PT-CA, respectively, amounted up to 7.6% and 48% of TRR (0.01–0.08 mg/kg) in milk, 68% TRR (0.09 mg/kg) (its conjugate was not detected (nd) in muscle from the tolyl label), 52% and 9.0% of TRR (13–1.74 mg/kg) in liver, 63% and 3.5% of TRR (4.33–0.21 mg/kg) in kidney and nd–34.1% of TRR(nd–0.09 mg/kg) in fat. PT-CA is rapidly conjugated in the liver. But in the gastrointestinal tract it is deconjugated.

OH-PT-CA was present in free form 17% TRR (0.03 mg/kg), in milk, 8.9%TRR (< 0.01 mg/kg,) in muscle, 27% TRR (6.69 mg/kg,) in liver, 19.3% TRR (1.34 mg/kg)in kidney, and in conjugated from 1.2%TRR (0.21 mg/kg) in liver and 0.3%TRR in kidney (0.02 mg/kg,). It was not detected in fat.

In addition, CA-T-NH_2 could be released by hydrolysis from milk (19.4%TRR) and fat (5.3%TRR).

In summary, tolfenpyrad is oxidized at the tolyl-methyl group to PT-CA. Further oxidation at the pyrazole ethyl group of PT-CA produces OH-PT-CA. Both PT-CA and OH-PT-CA occur as free metabolites in milk, liver, kidney, and muscle. PT-CA and its hydrolysis metabolites (PCA and CA-T-NH_2) are converted into nonpolar lipids in milk and fat. Saponification of the lipid conjugates releases PT-CA, PCA, and CA-T-NH_2.

Laying hens

[Pyrazole-^{14}C]-tolfenpyrad and [tolyl-^{14}C]-tolfenpyrad were administered orally (in gelatine capsules) to two separate groups of hens once daily for seven consecutive days. The administered daily dose was 12.6–13.5 mg/kg feed/day. Eggs were collected twice daily and excreta were collected once daily. Hens were sacrificed approximately 22–23 hours after the last dose administration. Liver, muscle, fat and gastrointestinal tracts with contents were collected for analysis.

The total recovery of radiolabel was 85.4% and 91.4% of the administered dose (AD) for the pyrazole- and tolyl-labels, respectively. Of the total dose 2.3–2.4% remained in the gastrointestinal tract one day after the last dose. The total identified residues were 66–78% in eggs, 85–89% in muscle, 84–96% in liver and 62–73% in fat. In tissues, the total radioactive residue (TRR) was highest in liver (up to 1.94 mg/kg; 0.8% AD) and lower in eggs (0.3% AD), fat (0.1% AD) and muscle (0.1% AD).

The parent tolfenpyrad was only present at 0.06 mg/kg concentration in fat (15% TRR) and < 0.01 mg/kg concentration in eggs (2.4%TRR), muscle (1.8% TR) and liver (0.2% TRR).

Following the administration of pyrazole- and tolyl-labelled tolfenpyrad, the predominant residues were PT-CA in free and its conjugated form. The free PT-CA amounted up to 40.5% TRR (0.07 mg/kg) in eggs, 85%TRR (0.1 mg/kg) in muscle and conjugated PT-CA, respectively, amounted up to 40.5–29% TRR (0.07–0.04 mg/kg) in eggs, 79% TRR (1.3 mg/kg) in liver, and 15% TRR ((0.07 mg/kg) in fat. The conjugated PT-CA was present in eggs, liver and fat at 29, 11.4, and 34.5% of TRR, respectively.

OH-PAM was present in free form at 12.6% TRR (0.02 mg/kg) in eggs, and 12.4% TRR (0.02 mg/kg) in muscle. It was not detected in liver and fat.

OH-PT-CA was detected at 2.7% TRR (< 0.01 mg/kg) in muscle, 5.2% TRR (0.09 mg/kg) in liver. It was not detected in eggs and fat.

In summary, the initial metabolite of tolfenpyrad is PT-CA occurring as a major residue in eggs, liver, kidney, muscle, and fat. Further oxidation at the pyrazole-ethyl group of PT-CA produces OH-PT-CA that occurs in liver and muscle. OH-PAM occurs as a free metabolite in eggs and muscle. Tolfenpyrad is a trace residue in eggs, liver, and muscle but was more abundant in fat. PT-CA and its hydrolysis product (PCA) are incorporated into non-polar lipid conjugates occurring in eggs and fat.

Plant metabolism

Cabbage

[Tolyl-^{14}C]-tolfenpyrad was applied to individual cabbage plants in a spray chamber. One application was made at a rate corresponding to 750 g ai/ha. Samples were taken at day 0 (immediately after the spray dried) and at 7, 14 and 28 days.

Twenty eight days after application 99.7% of the TRR (8.39 mg tolfenpyrad equivalent/kg) was on the outer leaves, 78.7% in the organo-soluble fraction and 15.9% in the water-soluble fraction, and only 0.3% of the TRR (0.03 mg equiv./kg) was in the heads, distributed between the water soluble (0.2%) and organo soluble fraction (0.1%).

Tolfenpyrad was found in the outer leaves at levels of 12.6 mg/kg (89% of TRR) immediately after application decreasing to 4.6 mg/kg (55.0% of TRR) after 28 days. In samples taken 28 days after application, OH-PT, OH-T-CA, OH-T-OH, and CA-T-AM were present at 0.54 mg/kg (6.4% of TRR, 0.33 mg/kg (3.9% of TRR), 0.31 mg/kg (3.7% of TRR), and 0.20 mg/kg (2.4% of TRR), respectively. Other metabolites were present at lower proportions. In cabbage head without outer leaves neither the parent compound nor any of the identified metabolite were detected (< 0.01 mg/kg).

In a second cabbage study, [pyrazole-^{14}C]-tolfenpyrad was applied once to individual cabbage in a spray chamber at a rate corresponding to 750 g ai/ha. Cabbage samples were collected at 28 days after application. At that time 97.2% of the AD (9.22 mg/kg) was distributed on the outer leaves and 2.8% (0.23 mg/kg) in the heads.

Tolfenpyrad was found in the outer leaves at levels of 4.7 mg/kg (49.8% of TRR). The identified metabolites, expresses as TRR, were 7.9% OH-PT (0.75 mg/kg), 3.4% OH-PT-OH and 2.9% OH-PT-CA. Other metabolites were detected at levels of ≤ 0.20 mg/kg (≤ 2.1% of AD). In the head, levels of metabolites did not exceed 0.1% of TRR.

Peach

[Tolyl-^{14}C]-tolfenpyrad was applied to individual peach plants in a spray chamber. One branch and one fruit were treated on each plant. One application was made at a rate corresponding to 750 g ai/ha.

Immediately after application, 83.5% of the AD was distributed on the leaves with 11.8% on the stem and 4.7% on the fruit. There was no significant change in distribution 56 days after application; TRRs remained were 83.1%, 7.5% and 9.3%, respectively.

In the fruit, parent tolfenpyrad was found at 3.0 mg/kg (100% of AD) immediately after application decreasing to 0.79 mg/kg (77% of TRR) by day 56. The majority of residues (8.4% TRR) were in the peel while only 0.4% TRR in the pulp. The metabolites did not exceed 2.8% TRR throughout the study period, except the glucose conjugate of T-CA at 6.1%TRR.

In the second peach study [pyrazole-^{14}C]-tolfenpyrad was applied to individual peach plants in a spray chamber. One branch and one fruit were treated on each plant. One application was made at a rate corresponding to 750 g ai/ha. Peach fruits were collected 53 days after application. At that

time, 86.1% of the AD was distributed on the leaves, 7.3% on the stem and 6.6% (0.77 mg/kg) on the fruit concentrated mainly in the peel (11 mg/kg) with low concentration (0.12 mg/kg) in the pulp.

In the fruit, parent tolfenpyrad was found at levels of 0.53 mg/kg (65% of TRR) 53 days after application. The only identified metabolite, OH-PAM, was found in the pulp at 0.03 mg/kg (4.0% of TRR).

In the leaves, parent tolfenpyrad was found at levels of 21.1 mg/kg (32.7% of TRR) after 56 days. Free PT-CA was the main metabolite found at 10.0 mg/kg (15.5% of TRR) with a contribution of glucose-conjugated PT-CA at 0.94 mg/kg (1.5% of TRR) followed by other metabolites present at less than 10% TRR.

The studies indicated that the translocation of unchanged tolfenpyrad was very limited. The predominant part of the TRR was located in the peel (86.4–94.6%) of the fruit residue.

Radish

[Tolyl-U-^{14}C]-tolfenpyrad or [pyrazole-^{14}C]-tolfenpyrad were applied to radish located outdoors. Each plot received two applications, 14 days apart, at a nominal rate of 230 g ai/ha. Radish plants were sampled 1 day after the second application and separated into root and foliage samples.

Labelled tolfenpyrad distributed into the roots (0.44–0.59 mg/kg, 4.9–5.2% of AD) and the foliage (7.0–11 mg/kg, 94.8–95.1% of TRR).

The major residues in radish roots were tolfenpyrad (0.24 mg/kg, 54.0% of TRR), PT-CA (0.11 mg/kg, 21.5% of TRR). Other metabolites amounted to less than 10%TRR except conjugated OH-PAM and PAM which were the major metabolite found in radish roots at 32.2% and 26.7% TRR respectively.

The major residue in foliage was tolfenpyrad (9.31 mg/kg, 85.0% of TRR) with a much lesser amount of metabolites amounting to less than 4% of TRR.

In summary, the metabolic pathways of tolfenpyrad in three different crops were considered comparable. In each case, unchanged parent accounted for a very significant proportion of the residue. All three crops contain the identified metabolites OH-PT (not observed but assumed in radish as an intermediate to OH-PAM), OH-PAM, PAM and PT-CA.

Environmental fate

The Meeting received information on photolysis on soil, aerobic degradation in soil, aqueous photolysis and confined and field rotational crop studies.

Soil photolysis

A photolysis study of [^{14}C] tolfenpyrad was conducted in sandy loam soil exposed to artificial light (290 nm) for 13 days. In the light exposed samples, unextracted radiocarbon increased slowly reaching an average of 3.2% (pyrazole label) and 10.7% (tolyl label) of applied radioactivity (AR) by day 313. Only minimal radiocarbon was recovered (0.3%) in traps of organic volatiles for light exposed samples, and none in dark control samples. Photoproducts consisted mainly of PT-CHO, PAM and OH-PAM present at a maximum of 6.6%, 11.3% and 3.5% AD, respectively in both labels. Tolfenpyrad showed negligible degradation in dark control samples with an average of 90% still present as parent in both labels at the end of the study. The PT-CA was the major degradate (2.0% in pyrazole labelled material and 4.7% in tolyloxy labelled material). The calculated half-life of tolfenpyrad was 444 days from tolyl label and 624 days from pyrazole label. The results indicate that photolysis is a very minor route of the degradation of tolfenpyrad

Aerobic soil metabolism

An aerobic soil metabolism study was conducted on a California sandy loam soil using [^{14}C] tolfenpyrad. The treated samples were incubated in the dark at 25 °C for periods up to 365 days. Tolfenpyrad degraded rapidly in soil under aerobic conditions and represented an average of 30.0% AR by day 21, declining to an average of 1.6% at the end of the incubation period. The primary degradates observed in the study were CO_2, PT-Cam PCA and soil bound residues. The soil bound residues were completely mineralised within one year. The calculated DT_{50} and DT_{90} values were maximum 14 and 78 days, respectively, indicating that tolfenpyrad is not persistent.

Confined rotational crop study

Tolfenpyrad radiolabelled in two positions (pyrazole- and tolyl-rings) was applied to test plots at a target application rate of 350 g ai/ha. Lettuce, radish and wheat were planted at intervals of 30, 120 and 365 days after single bare soil application. Samples were taken at appropriate harvest times and analysed for residues.

Metabolites at ≥ 0.01 mg/kg were OH-PAM (free and conjugated), OH-PCA (free and conjugated), and PAM (radish foliage and root only). A number of other metabolites were detected in combined extracts, but each was < 0.01 mg/kg or < 10% of TRR. Conjugates of OH-PAM and OH-PCA were liberated by acid hydrolysis.

Following pyrazole labelled tolfenpyrad application the detected metabolites in lettuce amounted up to 0.02 mg tolfenpyrad equivalent. The free and conjugated OH-PAM, OH-PCA and PAM were the major metabolites, each was less than 26% TRR No other single metabolite represented more than 2.5% TRR (< 0.01 mg/kg).

In radish foliage the detected metabolites (at 0.02–0.03 mg tolfenpyrad equivalent/kg) were the OH–PAM conjugates (20%TRR) the OH-PCA conjugates (24% TRR) as well as free OH-PAM and PAM with lesser amounts of OH-PCA and PT-CA.

In radish roots, the only detected metabolites were the OH-PCA conjugates (27% TRR) as well as free OH-PAM (8.5%TRR) and PAM (24.5%TRR).

In wheat grain, none of the metabolites were detected (< 0.01 mg/kg).

In wheat forage the predominant metabolites were the OH-PAM conjugates (≤ 0.16 mg eq/kg, 29.7% TR at 30 days PBI) the OH-PCA conjugates (0.17 mg eq/kg) as well as free OH-PCA (0.09) with lesser amounts of OH-PAM, PAM and PCA. No other single metabolite represented more than 5.2% TRR (0.01 mg/kg).

In wheat hay and straw the maximum concentrations were for OH-PAM conjugates (0.4 mg eq/kg, 32%TR) and OH-PCA conjugates (0.17 mg eq/kg). With exception of OH-PAM conjugates in wheat hay, all identified metabolites show a decrease with increasing aging of the soil.

Following the treatment with tolyl- labelled compound no relevant concentration of any individual compounds were found in lettuce, radish, wheat forage, hay, straw and grain at any plant-back interval. Only trace amounts of PT-CA were found in radish (120-day) and wheat hay (30-day) samples. In summary, tolfenpyrad is a minor residue (< 0.01 mg/kg) in confined rotational crops (lettuce, radish, and wheat). Most radiolabelled residues derived from cleavage of the amide bond, resulting in pyrazole and diphenyl ether fragments.

Field rotational crop studies

Two field trials were carried on mustard greens as the primary crop treated at about maximum seasonal rate of 0.598 kg ai/ha. The primary crop was removed from the trials at normal harvest with a PHI of one day after last application. Rotational crops (radish, lettuce and sorghum) were planted at intervals of 14, 28–30 and 58–60 days after last application.

At normal harvest of the rotational crops, no residues of tolfenpyrad, OH-PAM, OH-PCA and PAM were found above the LOQ in radish roots, lettuce and sorghum forage, grain and stover. Residues of OH-PAM and OH-PCA at the LOQ (0.01 mg/kg) were found at rotational intervals of 14 and 30 days after last application only in radish tops from one trial site.

Methods of analysis

The HPLC methods for determining the parent compound and OH-PT metabolite residues in plant matrices are based on three repeated extractions with methanol, followed by various solid phase extraction clean-up(s). The cleaned samples either concentrated or diluted to known volume before determination with HPLC-MS/MS. The LOQ for both compounds in all matrices is 0.01 mg/kg, except tea (0.05 mg/kg). The specificity of the detection was assured with two mass transitions. Average recoveries were all within the acceptable range of 70–120%, with relative standard deviations (RSD) below 20%.

For the rotational crop study the method used was validated for the determination of tolfenpyrad, OH-PAM, PAM and OH-PCA. Recovery data were generated from three samples fortified at the LOQ and three samples fortified at 10 × LOQ for each matrix. The mean percentage recoveries at 0.01 mg/kg and 0.1 mg/ kg were generally between 70–110% with RSD < 20%. There were some deviations especially when the extracts were hydrolysed. The mean recoveries and RSD values were in some cases outside the nominal ranges, but the differences were not significant taking into account the limited number of tests.

The HPLC-MS/MS methods were developed for determination of tolfenpyrad and its metabolites PT-CA, OH-PT-CA and PCA in animal commodities with an LOQ of 0.01 mg/kg. The milk samples were extracted with methanol, the tissues with methanol/water (5/1). The extracts were partitioned into ethyl acetate after adding either citric acid (milk and fat) or sodium chloride (muscle, liver and kidney). After evaporation to dryness the extracts were taken up in methanol or hexane and partly subjected to SPE clean-up. The other part of the milk, liver and fat extracts was hydrolysed. The final extracts were analysed by HPLC/MS/MS. The specificity of the detection was assured by two mass transitions for tolfenpyrad and three transitions for the metabolites. Repeatability data was generated from three samples fortified at the LOQ and three samples fortified at 10 × LOQ for each matrix. The mean percentage recoveries at each fortification level were within 70–110, except PT-CA in liver with hydrolysis (65% at 0.01 mg/kg), fat with hydrolysis (60% at 0.1 mg/kg) and OH-PT-CA in fat with hydrolysis (64% at 0.01 and 69% at 0.1 mg/kg). In spite of some deviations, the methods applied in the studies are considered suitable for the intended purpose.

Stability of residues in stored analytical samples

In plant matrices, freezer storage stability (at about -20 ± 5 °C) of tolfenpyrad and OH-PT has been demonstrated in tomatoes, apples, lettuce, grapes, oranges, almonds (nutmeat and hulls), cottonseed oil and potato flakes (18 months), peaches (4 months), prunes (dried) (5 months), cucumbers (5.5 months), cauliflower (6 months), and tea (12 months).

Freezer storage stability of tolfenpyrad, OH-PAM, OH-PCA and PAM has also been demonstrated in radish (roots) (112 days), lettuce (150 days) and sorghum forage (114 days), stover (87 days) and grain (109 days). This covers high acid, high water, high starch and high oil content crops.

When stored < 0 ºC tolfenpyrad, PT-CA and OH-PT-CA were stable in bovine muscle (85 days), kidney (85 days), fat (99 days) and milk (177 days). The average OH-PT-CA residue remained in liver was 39% after 111 days storage, but the procedural recoveries (41%) were similar. PCA was stable in fat and milk.

Definition of the residue

In goat, the parent tolfenpyrad was not detected in liver, and kidney, but it was found at < 0.01 mg/kg in milk (2.9–4.1% of TRR) and muscle (10% TRR) and at 0.04–0.06 mg/kg in fat (13.6–17.3% TRR).

The major radioactive residue derived from the administration of pyrazole and tolyl labelled tolfenpyrad was PT-CA being present up to 0.03 mg/kg (16.9% TRR) in milk, 0.09 mg/kg (63.% TRR) in muscle, 13.1 mg/kg (51.8% TRR) in liver, 4.33 mg/kg (62.6% TRR) in kidney, and 0.06 mg/kg (16.3% TRR) in fat.

The concentration and % proportion of TRR of OH-TP-PCA derived from administration of pyrazole- or tolyl-labelled tolfenpyrad was up to 0.03 mg/kg (16.9%) in milk, < 0.01 mg/kg (8.9%) in muscle, 6.8 mg/kg (26.9%) in liver, 1.3 mg/kg (19.3%) in kidney, and it was not detected in fat. The other metabolites identified were present at substantially lower concentrations.

Dairy cattle feeding study revealed that in milk, the only detected residue (> 0.01 mg/kg) is PT-CA which can be recovered after hydrolysis with maximum concentration of 0.27 mg/kg. In cream derived from 2.5 and 25 ppm dose groups the parent tolfenpyrad was present in about 0.01–0.02 mg/kg, respectively, and PT-CA were present at approximately 25 times higher concentration in conjugated form than in free form. In muscle, fat, liver and kidney the PT-CA is the major residue. Hydrolysis of samples revealed that only free PT-CA is present in the liver.

In the study with laying hens administered with labelled tolfenpyrad the parent tolfenpyrad was found at low concentrations < 0.01 mg/kg, (1.2–1.8% TRR) in eggs, muscle and liver, but it was present at 0.06 mg/kg (14–15%TRR) in fat.

The PT-CA occurs as a major residue up to 0.07 mg/kg (41%TRR) in eggs, 0.10 mg/kg (85%TRR) in muscle, 1.4 mg/kg (79%TRR) in liver, and 0.07 mg/kg 15% TRR) in fat. PT-CA is converted to OH-PT-CA, which was found up to 0.09 mg/kg (5.2% TRR) in liver and < 0.01 mg/kg (2.7%TRR) muscle.

Analytical methods are available for the simultaneous determination of tolfenpyrad, and free PT-CA, OH-PT-CA and PCA in one step and the conjugates can be released in a separate step after alkaline hydrolysis. However, the latter procedure could be carried out with sometimes low and varying recovery and it is not considered suitable for routine analyses.

Taking into account the relative proportions and concentration of the parent tolfenpyrad and its metabolites, and the availability suitable analytical method, the sum of tolfenpyrad and the free PT-CA are considered suitable marker compounds for enforcement purposes. For dietary risk assessment the free and the conjugated PT-CA, OH-PT-CA should be considered, because they have a toxic potency similar to PT-CA and OH-PT-CA.

PT-CA, the major residue component is present in higher concentration in muscle than in fat. OH-PT-CA was not present in fat. The Meeting concluded that the residue is not fat soluble.

The parent tolfenpyrad was present in outer leaves of cabbage at 4.71 mg/kg 28 days after treatment while the concentrations of all the identified metabolites were below 0.3 mg/kg

The concentrations of parent tolfenpyrad were 3.95 mg/kg (89.4%TRR) and 0.37 mg/kg (70% TRR) in peach fruits 14 and 28 days after treatment. In the same samples and sampling time T-AM, PT-OH, PT-CA were present at 0.12 and< 0.02 mg/kg, 0.06 and nd mg/kg, and 0.06 and nd mg/kg, respectively.

Radish leaves and roots on day 1 after the 2nd application contained 5.7 mg/kg and 0.24 mg/kg parent residue respectively, while any of the identified metabolites were present at less than 10% and 20% of the parent compound, respectively.

No residue is expected above 0.01 mg/kg in any rotational crops.

The Meeting noted that the parent tolfenpyrad is the major residue in plant commodities and it is a good marker for compliance with GAP.

The Meeting recommended the following residue definitions for tolfenpyrad:

Definition of the residue for compliance with the MRL and estimation of dietary intake for plant commodities: *tolfenpyrad*.

Definition of the residue for compliance with the MRL and estimation of dietary intake for animal commodities: *sum of tolfenpyrad, and free and conjugated PT-CA (4-[4-[(4-chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid and OH-PT-CA (4-[4-[[4-chloro-3-(1-hydroxyethyl) -1-methylpyrazol-5-yl]carbonylaminomethyl]phenoxy] benzoic acid) (released with alkaline hydrolysis) expressed as tolfenpyrad*.

The residue is not fat soluble.

Results of supervised residue trials on crops

The Meeting received supervised trials for a number of commodities from the USA for which there were no authorised uses. Trials were received from Japan where tolfenpyrad is authorised for use on tea. The residues obtained from supervised trials not supported by GAP are summarized in the JMPR Monograph but were not used for the estimation of STMR, HR and maximum residue levels.

Tea

Four residue trials were conducted on green tea in 1997–98 in Japan. One foliar application of tolfenpyrad was made with spray solutions of 0.0015 kg/hL following the design of reverse decline trials. The treated plots of each trial were harvested at a PHI of 7, 14, 21 and 30 days after treatment and the samples were analysed twice at intervals of about 1 year.

The GAP in Japan permits one foliar application of 15% EC formulation in 1000 times dilution using 2000–4000 L/ha water. The PHI is 14 days.

The average residues obtained in replicate samples taken at 14 days were: 3.77, 4.29, 7.05 and 12.1 mg/kg.

The Meeting estimated a maximum residue level of 30 mg/kg, an STMR value of 5.65 mg/kg and 13.8 mg/kg of STMR and HR values for green tea, respectively

Fate of residues during processing

Following one application of tolfenpyrad at rates of 0.30 kg ai/ha or 0.45 kg ai/ha, mean residues of tolfenpyrad were substantially reduced in tea infusion to the levels of 0.20 mg/kg, 0.06–0.08 mg/kg, 0.01 mg/kg and < 0.01 mg/kg, respectively, at PHIs of 7, 14, 21 and 30 days.

In a second study tolfenpyrad was applied at a rate of 0.6 kg ai/ha. Tolfenpyrad residues were substantially reduced in tea infusion to the levels of 1.12–2.21 mg/kg, 0.14–0.49 mg/kg and < 0.01 mg/kg, respectively, at PHIs of 7, 14 and 28 days. The two sets of trials gave about four times different average processing factors, therefore the larger factor (0.043) is used for dietary intake assessment.

The Meeting estimated for green tea infusion an STMR value of 0.24 mg/kg.

Residues in animal commodities

Farm animal dietary burden

As there are no registered uses on animal feed, the animal burden cannot be calculated.

Farm animal feeding studies

In a dairy cattle feeding study, tolfenpyrad was administered orally by gelatine capsules for 28 consecutive days to 3 groups of 3 cows at dose levels equivalent to 2.5 (2×), 7.5 (6×) and 25 (20×) ppm in feed. Residues of tolfenpyrad and its metabolites PT-CA, OH-PT-CA or PCA were determined in milk and tissues. Neither the parent compound nor any of the metabolites were detected in any samples derived from control animals.

Residues in milk

No quantifiable residues of tolfenpyrad, PT-CA, OH-PT-CA or PCA were detected in milk from cows treated with tolfenpyrad at the 2× and 6× dose levels, except PT-CA (0.02 mg/kg and 0.08 mg/kg, respectively) released by hydrolysis. In milk samples of the 6× dose group, no quantifiable free or conjugated residues were detected for tolfenpyrad, OH-PT-CA or PCA, whereas free or conjugated residues of PT-CA were present with maximums of 0.01 mg/kg and 0.27 mg/kg. In general the free metabolite corresponds to approximately 5–10% of the conjugated metabolite.

In milk samples of the 20× dose group, no free PCA and only trace levels of tolfenpyrad and OH-PT-CA averaging below LOQ were detected. Free PT-CA close to the LOQ was found after day 13. Conjugated PT-CA was found at significantly higher concentrations, reaching a plateau near 0.25 mg/kg by day 16. During two weeks of depuration no metabolites were detected in milk with the exception of a residue of 0.07 mg/kg PT-CA in the 31 days milk sample of the 20× dose group.

Residues in cream and skim milk

In cream samples from the 2× dose group, only conjugated PT-CA residues were detected averaging 0.02 mg/kg and 0.01 mg/kg for days 13 and 28, respectively. No quantifiable residues were found in skimmed milk samples from the 2× dose group. In cream samples from the 20× dose group, comparable levels were found for free and conjugated tolfenpyrad corresponding to approximately 0.02 mg/kg for both 13 and 28 days. Conjugated PT-CA in cream was approximately 25 times higher than free PT-CA being present at a level of about 0.02 mg/kg. In skimmed milk only PT-CA was found in samples of the 20× dose group at levels of 0.01 mg/kg for free and 0.04–0.05 mg/kg for the conjugated form.

Residues in muscle

Tolfenpyrad and OH-PT-CA residues were not present in quantifiable concentrations in the samples of every dose groups. The average PT-CA residues were present at 0.01 mg/kg, 0.02 mg/kg and 0.05–0.09 mg/kg in samples of dose groups of 2.5 ppm, 7.5 ppm and 25 ppm.

Residues in liver

No parent tolfenpyrad residues were found in any treated liver sample. Residues of PT-CA were found in all dose levels in approximate proportion to the level of dosing. Free PT-CA residues after 28 days of dosing averaged for 0.65 mg/kg for the 2× dose level, 2.0 mg/kg for the 6× dose level, and 4.8 mg/kg for the 20× dose level. Conjugated residues of PT-CA were at a similar level, suggesting that only free PT-CA is present in the liver. After fourteen days of depuration, PT-CA residues (0.03 mg/kg) were reduced by a factor > 100 compared to the 28 days level (4.8 mg/kg). Also OH–PT-CA was found at a lesser extent than PT-CA. Residues of free OH-PT-CA averaged at 0.03 mg/kg, 0.07 mg/kg and 0.27 mg/kg for the 2×, 6× and 20× dose levels, respectively. Conjugated residues were less than or equal to residues of free OH-PT-CA, suggesting that only free metabolite is present in liver.

Residues in kidney

No parent tolfenpyrad residues were found in kidney samples of the 2× and 6× dose groups. In the 20× dose group tolfenpyrad was present at the LOQ (0.01 mg/kg). Residues of PT-CA were found in all dose levels in approximate proportion to the level of dosing. PT-CA residues after 28 days of dosing were averaged for 0.13 mg/kg for the 2× dose level, 0.49 mg/kg for the 6× dose level, and 1.3 mg/kg for the 20× dose level. Residues of OH-PT-CA were less than the LOQ (< 0.01 mg/kg) for the 2× dose level and averaged at 0.02 mg/kg and 0.09 mg/kg for the 6× and 20× dose level, respectively. After fourteen days of depuration, residues of tolfenpyrad were below the LOD, and residues of PT-CA and OH-PT-CA were below the LOQ (< 0.01 mg/kg).

Residues in fat

For the 2× dose level, no residues of tolfenpyrad, OH-PT-CA or PCA were found. Residues of free tolfenpyrad in the 6× and 20× dose levels averaged for 0.01 mg/kg and 0.065 mg/kg, respectively. Residues of free PT-CA in fat were below the LOQ (< 0.01 mg/kg) for the 2× dose level and averaged at 0.01 mg/kg and 0.04 mg/kg for the 6× dose level and the 20× dose level, respectively. Residues of OH-PT-CA and PCA were only detected in the 20× dose group samples at levels below the LOQ (< 0.01 mg/kg). Residues after sample hydrolysis were in a similar order indicating that no conjugated residues were present in fat. The depuration period showed a steady decline in residues with no determinable residues present by day 14 after last dosing.

Considering the residues in samples derived from the highest (20×) dose group, the free and conjugated PT-CA released with hydrolysis were the major residues in muscle (0.09 mg/kg), liver (6.9 mg/kg) kidney (1.8 mg/kg), and fat (0.04 mg/kg)

Parent tolfenpyrad was only present in milk cream and fat in the highest dose group.

PT-CA concentration rapidly decreased during depuration.

Animal commodity maximum residue levels

Without calculated animal burden no residue levels can be calculated for animal commodities.

RECOMMENDATIONS

On the basis of the data from supervised trials, the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits and for IEDI assessment.

Definition of the residue for compliance with the MRL and estimation of dietary intake for plant commodities: *tolfenpyrad*.

Definition of the residue for compliance with the MRL and estimation of dietary intake for animal commodities: sum of tolfenpyrad, and free and conjugated PT-CA (4-[4-[(4-chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid and OH-PT-CA (4-[4-[[4-chloro-3-(1-hydroxyethyl) -1-methylpyrazol-5-yl]carbonylaminomethyl]phenoxy] benzoic acid) (released with alkaline hydrolysis) expressed as tolfenpyrad.

The residue is not fat soluble.

DIETARY RISK ASSESSMENT

Long-term intake

The evaluation of tolfenpyrad resulted in recommendations for STMR-P value for green tea infusion which was used for the calculation. The results are shown in Annex 3. The International Estimated Daily Intake for the 13 GEMS/Food diet based on estimated STMR value was up to 0–11% of maximum ADI of 0.006 mg/kg bw. The Meeting concluded that the long-term intake of residues of tolfenpyrad from green tea is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-term Intake (IESTI) for tolfenpyrad was calculated for green tea infusion for which STMR-P value was estimated. The results are shown in Annex 4. The IESTI was 50 to 100% of the ARfD (0.01 mg/kg bw) for the general population.

Meeting concluded that the short-term intake of residues of tolfenpyrad resulting from its use on green tea is unlikely to present a public health concern.