5.17 PENTHIOPYRAD (253)

TOXICOLOGY

Penthiopyrad is the International Organization for Standardization (ISO)–approved name for \(N\)-\[2-(1,3-dimethylbutyl)-3-thienyl]-1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (9CI) (Chemical Abstracts Service No. 183675-82-3). It is a new fungicide that belongs to the carboxamide class. Its proposed fungicidal mode of action is inhibition of succinate dehydrogenase, resulting in the inhibition of the citric acid cycle and mitochondrial electron transport pathways. Penthiopyrad has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues.

All the pivotal studies contained certificates of compliance with good laboratory practice.

Biochemical aspects

The absorption, distribution, metabolism and excretion of penthiopyrad were investigated in rats. \(^{14}\)C-labelled penthiopyrad was rapidly and extensively absorbed from the gastrointestinal tract of rats following oral dosing. The extent of absorption was approximately 80–90% of the administered dose, independent of dose and sex. Maximum concentrations of radioactivity in plasma were observed within 0.5 hour of dosing for the low-dose group (10 mg/kg body weight [bw]) and within 1.3 hours for the high-dose group (100 mg/kg bw). Maximum tissue levels occurred within 1 hour post-dosing, with the highest concentrations of radioactivity found in liver, fat, lymph nodes and kidneys of rats. Very little penthiopyrad was retained in the tissues. There were no major sex-related differences in the pattern of excretion. Faecal excretion was the primary route of elimination, and excretion was rapid, with the majority excreted by all routes 24 hours after dosing (74.8–85.0%).

Extensive metabolism occurred at numerous positions within the molecule, including thienyl ring oxidation and conjugation with glutathione, thienyl ring opening, \(N\)-demethylation and alkyl side-chain hydroxylation, followed by oxidation to carboxylic acids and glucuronidation. The most abundant metabolite in both urine and faeces was formed as the result of \(N\)-demethylation and oxidation of the methyl moiety of the alkyl side-chain. The most abundant metabolites found in bile were formed as a result of thienyl ring oxidation to 753-F-DO, followed by its conjugation with glutathione and the catabolism of this product. Other significant metabolites in bile were glucuronic acid conjugates of the intermediate demethylated and hydroxylated metabolites. Four metabolites containing the pyrazole moiety following cleavage from the thienyl moiety were excreted in both urine and faeces. The two acids, 1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxylic acid (PCA) and 3-trifluoromethyl-1H-pyrazole-4-carboxylic acid (DM-PCA), are likely formed by amide hydrolysis from 1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxamide (PAM) and DM-PAM. PAM and subsequent metabolites account for less than 1% of the administered dose. The thienyl ring appears to be completely degraded.

Toxicological data

The median lethal dose (LD\(_{50}\)) in rats treated orally and dermally with penthiopyrad was greater than 2000 mg/kg bw. The median lethal concentration (LC\(_{50}\)) in rats treated by inhalation was greater than 5.7 mg/L of air. Penthiopyrad was not irritating to the skin of rabbits, was minimally irritating to the eyes of rabbits and was not sensitizing under the conditions of the maximization test in guinea-pigs.

Following repeated dietary dosing, the liver was the main target organ in mice, rats and dogs. In several studies, increased liver weight, liver enlargement and centrilobular hepatocellular hypertrophy were observed, as well as indications of hepatotoxicity in the form of alterations in clinical chemistry (elevated serum levels of liver enzymes, cholesterol, triglycerides and protein). The pattern of liver effects changed with dose, but not with duration of dosing. Haematological changes
(decreases in red blood cells, haemoglobin and haematocrit) were observed in mice, rats and dogs at doses higher than those causing liver toxicity. The thyroid was also a target organ in mice, rats and dogs, with effects observed only at the highest doses tested. In mice, thyroid follicular cell hypertrophy was observed in both sexes at 997/1027 mg/kg bw per day in males and females, respectively, whereas in dogs, increased thyroid weights were observed in females of the 90-day study at 864 mg/kg bw per day. In longer-term studies in rats, thyroid follicular cell hypertrophy was observed in the 1-year and multigeneration reproduction studies at the highest doses tested. Adrenal cortical hypertrophy was found in both the 90-day and 1-year dog studies at the highest doses tested. Adrenal effects were not observed in mice and were found in rats only with longer-term dosing (i.e. the reproductive toxicity study and long-term study beginning at 6 months).

The no-observed-adverse-effect level (NOAEL) in the 90-day rat study was 40 mg/kg bw per day, based on liver effects (increased serum levels of phospholipids and gamma-glutamyltranspeptidase, absolute and relative liver weights and incidences of centrilobular hepatocellular hypertrophy, Kupffer cell proliferation and hepatocellular degeneration). The NOAEL in the 90-day mouse study was 100 mg/kg bw per day, and the overall NOAEL in the dog studies was 76.7 mg/kg bw per day, in both cases based on liver effects.

In the 18-month carcinogenicity study in mice, the NOAEL was 60 mg/kg bw per day, based on effects in the liver and thyroid at the lowest-observed-adverse-effect level (LOAEL) of 200 mg/kg bw per day. There was a marginal increase in hepatocellular adenomas and carcinomas at the highest dose tested in comparison with concurrent controls; however, the incidences were similar to historical control values, and no other histopathology of the liver was observed. The concurrent control value for adenomas was lower than the historical control range. The Meeting concluded that penthiopyrad was not carcinogenic in mice.

In the 2-year rat study, the NOAEL was 27 mg/kg bw per day, based on reduced body weight gain in females and hepatic periportal fatty degeneration in males at 83 mg/kg bw per day. Effects on the kidneys (various elements of chronic progressive nephropathy, including interstitial fibrosis and renal glomerulosclerosis) were observed in male rats of all groups, including controls. The incidence, but not the severity, of this rat-specific condition was increased to similar extents in all treatment groups. The incidence of thyroid follicular cell adenomas in males was increased at the highest dose tested compared with controls (3/50, 1/50, 6/48, 2/49 and 9/49, respectively); this incidence also slightly exceeded the historical control range. There was no increase in follicular cell carcinomas. No other histopathology of the thyroid was observed in this study; however, follicular cell hypertrophy was observed at higher doses in the 1-year and multigeneration reproduction studies in rats. The Meeting concluded that high doses of penthiopyrad caused follicular cell adenomas in the thyroid.

Hepatocellular adenomas and carcinomas and follicular cell adenomas of the thyroid are common in male mice and rats, respectively. Special studies were conducted to examine liver and thyroid effects in the mouse and rat. These studies showed that penthiopyrad increased microsomal protein and cytochrome P450 activity in the liver of both mice and rats. Changes in thyroid hormones and uridine diphosphate glucuronosyltransferase activity were not concordant with the dose response for the tumours.

Penthiopyrad was adequately tested for genotoxicity in vitro and in vivo in a range of assays. Negative results were observed in all genotoxicity studies.

The Meeting concluded that penthiopyrad was unlikely to be genotoxic.

The Meeting concluded that penthiopyrad is unlikely to pose a carcinogenic risk to humans at anticipated dietary residue levels, as it was not carcinogenic in the mouse and as thyroid follicular cell adenomas in male rats are common, their incidence is only slightly increased and, in the absence of genotoxic potential, the end-point would be anticipated to exhibit a threshold.

No effects on reproduction were noted in a multigeneration reproduction study in the rat. However, there was a decrease in body weight of the offspring during early lactation in both
generations at 278 mg/kg bw per day, the highest dose tested. Also at this dose, there was a slight, but statistically significant, delay in time to preputial separation. Furthermore, at this dose, there were decreases in thymus and spleen weights, with no histopathological correlates. Effects were observed in parental animals at the intermediate and high doses and included decreased body weight and body weight gain and increased adrenal weight with adrenal cortical hypertrophy. At the high dose only, effects on the thyroid were also observed, comprising increased thyroid weight and follicular cell hypertrophy. The NOAEL for parental toxicity was 200 ppm (equal to 11 mg/kg bw per day), based on decreased body weight gain and effects on the adrenals, whereas the NOAEL for offspring toxicity was 1000 ppm (equal to 54 mg/kg bw per day), based on reduced body weight and body weight gain, delay in preputial separation and a statistically significant decrease in absolute thymus weights at 5000 ppm (equal to 278 mg/kg bw per day). The NOAEL for reproductive toxicity was 5000 ppm (equal to 278 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study in rats, increased early resorptions and post-implantation loss and decreased live young per litter and litter weight were observed when pregnant rats were dosed at 1000 mg/kg bw per day. Reductions in body weight and feed consumption were observed in maternal animals at this dose. The NOAEL for maternal and developmental toxicity in rats was 250 mg/kg bw per day. In rabbits, there was one abortion at the high dose (225 mg/kg bw per day), which occurred in the presence of a marked reduction in feed consumption and body weight in that dam. Litter and fetal weights were also reduced at the high dose, resulting in decreased gravid uterine weight. The NOAEL for maternal and developmental toxicity in rabbits was 75 mg/kg bw per day.

The Meeting concluded that penthiopyrad was not teratogenic in rats or rabbits.

In an acute neurotoxicity study, the NOAEL was 125 mg/kg bw based on clinical signs of neurotoxicity at doses of 500 mg/kg bw (decreased motor activity and body temperature, unsteady gait, hunched posture); however, there was no histological evidence of damage to the central or peripheral nervous system. There was no evidence of neurotoxicity in the 90-day neurotoxicity study. A developmental neurotoxicity study revealed no maternal effects at 500 mg/kg bw per day, the highest dose tested. In contrast, the NOAEL for offspring toxicity was 100 mg/kg bw per day, based on decreased body weight at doses of 250 mg/kg bw per day and higher.

In a 4-week immunotoxicity study in mice, the NOAEL for immunotoxicity was 250 mg/kg bw per day, based on decreased plaque-forming cells in the spleen at 1000 mg/kg bw per day. In a 4-week immunotoxicity study in rats, no adverse effects were observed at any dose up to 700 mg/kg bw per day, the highest dose tested.

**Toxicological data on metabolites**

A variety of metabolites were also assessed for toxicity. These are minor metabolites in rats that are also found in livestock, plants and soil. The oral LD$_{50}$ in rats for the metabolite DM-PCA was greater than 2000 mg/kg bw. In a 90-day feeding study in rats, the NOAEL for DM-PCA was 4000 ppm (equal to 258 mg/kg bw per day), based on reduced body weight gain and feed consumption at 16000 ppm (equal to 1200 mg/kg bw per day), the highest dose tested. DM-PCA was not genotoxic in any of an adequate range of in vitro genotoxicity assays.

The oral LD$_{50}$ in rats for the metabolite PCA was greater than 2000 mg/kg bw. In a 14-day oral gavage study of PCA in rats, the NOAEL was 1000 mg/kg bw per day, the highest dose tested. PCA was not genotoxic in any of an adequate range of in vitro and in vivo genotoxicity assays. The metabolite PAM was more acutely toxic than the parent and PCA, with an LD$_{50}$ estimated between 300 and 2000 mg/kg bw by the oral route in rats. PAM was negative in the Ames assay but was positive without activation in a mouse lymphoma assay, in which small colony mutant frequencies were increased. It induced chromosomal aberrations in mammalian cells in the absence of activation in vitro, but this clastogenicity was not confirmed in an in vivo micronucleus assay. Overall, the weight of evidence suggests that PAM has low potential for genotoxicity in vivo.
The oral LD<sub>50</sub> in rats for the metabolite N-[5-hydroxy-5-(1,3-dimethylbutyl)-2-oxo-2,5-dihydrothiophen-4-yl]-1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxamide (753-T-DO) was > 2000 mg/kg bw. The acute oral toxicity of the metabolite N-[2-(3-hydroxy-1,3-dimethylbutyl)thiophen-3-yl]-1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxamide (753-A-OH) has not been assessed. Neither 753-T-DO nor 753-A-OH was genotoxic in three in vitro assays to assess gene mutations and chromosomal aberrations.

The metabolites were not considered to be more toxic than penthiopyrad, with the exception of PAM, which was more acutely toxic than penthiopyrad and was genotoxic in vitro, but not in vivo.

There were no reports of adverse health effects in manufacturing plant personnel or in operators and workers exposed to penthiopyrad formulations during their use. Also, there was no evidence to support any findings in relation to poisoning with penthiopyrad.

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

### Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.1 mg/kg bw on the basis of a NOAEL of 11 mg/kg bw per day in the multigeneration reproduction study in rats for decreased body weight gain in F<sub>1</sub> males and adrenal effects in F<sub>1</sub> females (increased weight and cortical hypertrophy). A safety factor of 100 was applied.

The Meeting established an acute reference dose (ARfD) of 1 mg/kg bw on the basis of a NOAEL of 125 mg/kg bw in the acute neurotoxicity study in rats for clinical signs of neurotoxicity (e.g., decreased motor activity and body temperature, hunched posture, unsteady gait). A safety factor of 100 was applied.

A toxicological monograph was prepared.

### Levels relevant to risk assessment

<table>
<thead>
<tr>
<th>Species</th>
<th>Study</th>
<th>Effect</th>
<th>NOAEL</th>
<th>LOAEL</th>
</tr>
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<tbody>
<tr>
<td>Mouse</td>
<td>Eighteen-month study of toxicity and carcinogenicity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Toxicity</td>
<td>60 mg/kg bw per day</td>
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<td>Rat</td>
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<td>Carcinogenicity</td>
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<td>Two-generation study of reproductive toxicity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Parental toxicity</td>
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<td></td>
<td>Offspring toxicity</td>
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<td>Reproductive toxicity</td>
<td>278 mg/kg bw per day&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>Developmental toxicity study&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Maternal toxicity</td>
<td>250 mg/kg bw per day</td>
<td>1000 mg/kg bw per day</td>
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<td></td>
<td></td>
<td>Embryo and fetal toxicity</td>
<td>250 mg/kg bw per day</td>
<td>1000 mg/kg bw per day</td>
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<tr>
<td></td>
<td>Acute neurotoxicity study&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Neurotoxicity</td>
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<td>500 mg/kg bw</td>
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<td>Rabbit</td>
<td>Developmental toxicity study&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Maternal toxicity</td>
<td>75 mg/kg bw per day</td>
<td>225 mg/kg bw per day</td>
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<tr>
<td></td>
<td></td>
<td>Embryo and fetal toxicity</td>
<td>75 mg/kg bw per day</td>
<td>225 mg/kg bw per day</td>
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</tbody>
</table>
Species Study Effect NOAEL LOAEL

Dog Thirteen-week and 1-year studies of toxicity a,b,c,d Toxicity 3000 ppm, equal to 76.7 mg/kg bw per day 15 000 ppm, equal to 445 mg/kg bw per day

a Dietary administration.
b Highest dose tested.
c Gavage administration.
d Two studies combined.

Estimate of acceptable daily intake for humans
0–0.1 mg/kg bw

Estimate of acute reference dose
1 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 penthiopyrad

Absorption, distribution, excretion and metabolism in mammals
Rate and extent of oral absorption Rapid; ~90%
Distribution Widely distributed; highest concentrations in liver
Rate and extent of excretion Largely complete within 24 h; primarily via faeces (70–85%, bile 30–54%) and to a lesser extent urine (8–17%)
Potential for accumulation No evidence of accumulation
Metabolism in mammals Extensive
Toxicologically significant compounds (animals, plants and the environment) Parent compound, PAM

Acute toxicity
Rat LD₅₀, oral > 2000 mg/kg bw
Rat, LD₅₀, dermal > 2000 mg/kg bw
Rat, LC₅₀, inhalation (whole-body exposure) > 5.7 mg/L
Rabbit, dermal irritation Not irritating
Rabbit, ocular irritation Minimally irritating
Guinea-pig, dermal sensitization (Magnusson and Kligman) Not sensitizing

Short-term studies of toxicity
Target/critical effect Liver (clinical chemistry changes), thyroid (increased weights, hypertrophy), adrenal (increased weights, hypertrophy)
Penthiopyrad

Lowest relevant oral NOAEL 40 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL 1000 mg/kg bw per day (28-day study in rats)
Lowest relevant inhalation NOAEL No data

**Genotoxicity**
No evidence for genotoxic potential

**Long-term studies of toxicity and carcinogenicity**
Target/critical effect Body weight, liver (periportal fatty degeneration)
Lowest relevant oral NOAEL 27 mg/kg bw per day (2-year study in rats)
Carcinogenicity Unlikely to pose a carcinogenic risk to humans at anticipated dietary exposure levels

**Reproductive toxicity**
Reproduction target/critical effect No effect on fertility at highest dose tested; decrease in body weight in pups and slight delay in sexual maturation at parentally toxic dose
Lowest relevant reproductive NOAEL 278 mg/kg bw per day (highest dose tested) for reproductive effects (rats)
11 mg/kg bw per day for systemic toxicity in parent (rats)
54 mg/kg bw per day for offspring toxicity (decreased body weight) (rats)
Developmental target/critical effect Decreased fetal weight at maternally toxic dose
Lowest relevant developmental NOAEL 75 mg/kg bw per day (rabbits)

**Neurotoxicity/delayed neurotoxicity**
Neurotoxicity target/critical effect Decreased motor activity, hunched posture, unsteady gait
Lowest relevant neurotoxicity NOAEL 125 mg/kg bw (acute neurotoxicity study, rats)

**Immunotoxicity**
Not immunotoxic (mice and rats)

**Medical data**
No data

**Summary**

<table>
<thead>
<tr>
<th>Value</th>
<th>Study</th>
<th>Safety factor</th>
</tr>
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<tr>
<td>ADI 0–0.1 mg/kg bw</td>
<td>Rat, two-generation reproduction study</td>
<td>100</td>
</tr>
<tr>
<td>ARfD 1 mg/kg bw</td>
<td>Rat, acute neurotoxicity study</td>
<td>100</td>
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