

5.19 PROTHIOCONAZOLE (232)

TOXICOLOGY

Prothioconazole is the ISO approved common name for the substance for which the IUPAC nomenclature is 2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione (CAS No. 178928-70-6). It is a systemic triazolinthione fungicide, the targets for which are most of the economically important diseases caused by *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes* in cereals, oilseed rape and peanuts. Its mode of action is interference with the synthesis of ergosterol in the target fungi by inhibition of CYP51, which catalyses demethylation at C14 of lanosterol or 24-methylene dihydrolanosterol, leading to morphological and functional changes in the fungal cell membrane.

The residue definition for risk assessment in plant commodities is the metabolite prothioconazole-desthio, while in animal commodities it is the sum of prothioconazole-desthio, prothioconazole-desthio-3-hydroxy (M14) and prothioconazole-desthio-4-hydroxy (M15), and their conjugates expressed as prothioconazole-desthio. For the active ingredient prothioconazole and its metabolite prothioconazole-desthio, complete data sets were submitted. Prothioconazole-desthio is considered to be the toxicologically relevant compound. While no independent studies of toxicity with M14 and M15 were available, both metabolites and their glucuronide conjugates were identified and quantified in studies with prothioconazole and prothioconazole-desthio in rats; the toxicology of M14 and M15 can thus be considered to be included in the databases provided for these compounds.

Prothioconazole was reviewed for the first time by the present Meeting at the request of the CCPR.

All critical studies complied with GLP.

Biochemical aspects

In rats given [¹⁴C]prothioconazole labelled in either the triazole or phenyl rings as a single oral dose at either 2 or 150 mg/kg bw, the radiolabel was rapidly and extensively (> 90%) absorbed from the gastrointestinal tract, the t_{\max} calculated from plasma concentrations being 0.1–0.7 h for males and females. There were no significant differences related to sex, higher or lower dose or multiple doses.

The highest concentrations of radioactivity were found in the gastrointestinal tract and liver, as demonstrated by dissection and liquid scintillation counting and confirmed by whole-body autoradiography. Radioactivity concentrations in the liver were markedly higher in male rats than in females. Relatively high concentrations were also found in the thyroid. Distribution was rapid and was followed by extensive loss of radioactivity from tissues and organs. The highest concentrations of prothioconazole equivalents were recorded in the liver, followed by kidney, fat, thyroid and adrenal gland.

Excretion was initially extensive and relatively rapid, mainly via the faeces, about > 70% being eliminated within 24 h, although the subsequent rate of excretion was low. Extensive biliary excretion (90%) was shown in bile-duct cannulated rats; evidence for enterohepatic recirculation was also seen in these rats.

Study of metabolism using both phenyl- and triazole-ring-labelled molecules indicated that the prothioconazole structural skeleton remained largely intact, although prothioconazole was extensively metabolized. The major types of metabolic reactions identified were conjugation with glucuronic acid, oxidative hydroxylation of the phenyl moiety and desulfuration. The principle metabolites found in the excreta were prothioconazole-*S*-glucuronide, prothioconazole-desthio and prothioconazole itself. Many of the 18 metabolites identified were derived from the desthio metabolite (i.e., in which the triazole sulfur had been eliminated). The desthio metabolite was found

almost exclusively in the faeces and represented between 3.5% and 17.7% of the administered dose. The systemic proportion of radiolabel as prothioconazole-desthio was very low; not more than about 0.07% of the administered dose was found in the urine. The *S*- or *O*-glucuronide conjugates were the principle systemic metabolites and were found in amounts of up to 7.7% of the administered dose in rat urine. These conjugates were also overall the most abundant, occurring at about 46% of the administered dose in bile, followed by the parent compound, prothioconazole (about 1–22%), and prothioconazole-desthio (about 0.4–18%).

Toxicological data

The acute toxicity of prothioconazole is low, the oral LD₅₀ being > 6200 mg/kg bw in rats. At this dose, there were no deaths and clinical signs were limited to decreased motility and diarrhoea 1–6 h after dosing. The dermal LD₅₀ in rats was > 2000 mg/kg bw and the inhalation LC₅₀, also in rats, was > 4.9 mg/L for a 4-h exposure. Prothioconazole is not irritating to rabbit skin and eyes and is not sensitizing either in the Buehler skin patch test in guinea-pigs or in the local lymph node assay in mice.

Initial studies with repeated doses showed that prothioconazole could be unstable when formulated with diet, hence most studies were performed using dosing by gavage. A 4-week study in rats given prothioconazole by different dosing routes established that plasma concentrations in rats dosed by gavage at 1000 mg/kg bw per day were 3–6-fold those in rats given diets containing prothioconazole at 10 000 ppm, equivalent to 1000 mg/kg bw per day, and this was consistent with the observation of more marked effects in rats dosed by gavage.

The liver was consistently identified as a target organ in short-term studies in rats, mice and dogs, although there were some species differences in the hepatic effects observed. Increased liver weights and increased activities of several liver enzymes were observed in mice, rats (particularly females) and dogs. Microscopic lesions were also observed in the liver, including an increase in pigmented material in dogs, centrilobular fatty change and focal necrosis in mice and cytoplasmic changes and centrilobular hepatocellular hypertrophy in rats and mice. Some of these effects were consistent with induction of hepatic enzymes. None of the effects recorded in the liver persisted after 4- and 8-week recovery periods in rats and dogs, respectively.

The kidney was the primary target organ in dogs and was also identified as a target organ in rats, but not in mice. The effects on the kidneys consisted of increased weights and changes in histology, namely increased incidence and severity of basophilic tubules and tubular dilatation in rats, and interstitial fibrosis and inflammation in dogs. These findings did not persist after a recovery period in rats, but there was only partial recovery in dogs. In rats, these kidney changes correlated with greatly increased water intakes, indicating disturbance of kidney function and systemic water homeostasis.

The following NOAELs were derived from short-term studies in which prothioconazole was administered orally:

- In a 14 week study in mice dosed by gavage, the NOAEL was 25 mg/kg bw per day on the basis of increased liver weights and various histological changes in the liver at 100 mg/kg bw per day;
- In studies of up to 14 weeks in rats dosed by gavage, the NOAEL was 100 mg/kg bw per day on the basis of increased water consumption, decreased urine output, increased liver weights in females, and histological changes in the liver and kidney at 500 mg/kg bw per day;
- In 13-week and 1-year studies in dogs dosed by gavage, the overall NOAEL was 25 mg/kg bw per day on the basis of minimal histological changes in the kidneys at 40 mg/kg bw per day.

In long-term studies in rats and mice dosed by gavage, the primary target organs were the liver and kidney. There was no evidence for any carcinogenic potential in rats or mice. The hepatic effects observed in rats were increased incidences of eosinophilic or clear-cell foci. The other liver effects observed in rats and mice (increased weights, centrilobular hypertrophy with cytoplasmic changes) were consistent with induction of hepatic enzymes. There was slight alteration in the concentrations of plasma thyroid hormones in rats, but there was no associated thyroid histopathology.

The kidney effects in rats were increased organ weight and increased severity of chronic progressive nephropathy accompanied by markedly increased water consumption, effects on urine analysis, crystalline material in the urine sediment and transitional-cell hyperplasia in the urinary bladder. In mice, responses comprised decreased organ weight, tubular degeneration and regeneration and subcapsular tubular degeneration with interstitial fibrosis. The kidney effects were more marked in rats than in mice, and treatment of rats for more than 1 year was associated with prolonged and increasingly severe functional deficit in the kidneys. Males were consistently more markedly affected than females. In rats, the kidney dysfunction and resulting dehydration caused mortality at doses of between 500 and 1000 mg/kg bw per day.

In a long-term study in rats dosed by gavage for 2 years, the NOAEL was 5 mg/kg bw per day on the basis of gross and microscopic changes in the liver and kidneys at 50 mg/kg bw per day. In a long-term study in mice dosed by gavage for 18 months, the NOAEL was 10 mg/kg bw per day on the basis of reduced body weights and gross and microscopic changes in the liver and kidneys at 70 mg/kg bw per day.

Prothioconazole was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. Although genotoxicity was not observed in tests for gene mutation in vitro, there was equivocal evidence for DNA damage and confirmed evidence for the induction of chromosomal aberrations in vitro; however, these observations were not confirmed in the relevant assays conducted in vivo.

The Meeting concluded that prothioconazole is unlikely to be genotoxic.

On the basis of the absence of carcinogenicity in rodents and the absence of genotoxicity in vivo, the Meeting concluded that prothioconazole is unlikely to pose a carcinogenic risk to humans.

In a multigeneration study in rats, effects were observed on the liver and kidneys at higher doses in parental animals. Some of these observations were consistent with the findings of short-term and long-term studies of toxicity. The NOAEL for systemic toxicity in the parental rats was 9.7 mg/kg bw per day on the basis of reduced body weight and effects on organ weights at 95.6 mg/kg bw per day. In the offspring, the NOAEL was 95.6 mg/kg bw per day on the basis of reduced pup-weight gain, reduced spleen weight and delayed preputial separation at 726 mg/kg bw per day. The NOAEL for reproductive effects was 95.6 mg/kg bw per day on the basis of disruption to the oestrus cycle, reduced number of implantation sites and litter size, increased time to insemination and increased duration of gestation at 726 mg/kg bw per day. Although several of these observations were not statistically significantly different, they were consistent features of the group receiving the highest dose in contrast to the other groups, but they did not result in effects on mating, fertility or gestation indices.

The highest dose of 726 mg/kg bw per day caused parental toxicity that was probably related to kidney dysfunction and resulting dehydration, which in other studies in rats given repeated doses were a cause of mortality at doses of between 500 and 1000 mg/kg bw per day. Effects on developing pups also were restricted to the group receiving a dose of 726 mg/kg bw per day.

The Meeting concluded that prothioconazole was toxic to the reproductive system and to developing offspring at a dose that was accompanied by toxicity in parental rats.

In a study of developmental toxicity in which rats were given prothioconazole by gavage on days 6–19 of gestation, the NOAEL for maternal toxicity was 80 mg/kg bw per day on the basis of

reduced body-weight gain and increased water consumption and urination at 500 mg/kg bw per day. Examination of the fetuses revealed an increased incidence of microphthalmia and of rudimentary supernumerary ribs, together with retarded fetal development, at 1000 mg/kg bw per day. Marked maternal toxicity was also recorded at this dose. Although the developmental effects, which included a statistically significant increased incidence in microphthalmia (on a fetal basis) occurred at a dose of 1000 mg/kg bw per day, microphthalmia was also observed in the groups at 500 and 80 mg/kg bw per day, but not in the controls. Rudimentary supernumerary ribs, which occur spontaneously in untreated rats of this strain, were significantly increased in a dose-related manner at all doses, including 80 mg/kg bw per day, the lowest dose tested. The Meeting noted there were indications that the incidence of this variation in the group receiving the vehicle only may have been particularly low in this experiment; however, incidences in all groups treated with prothioconazole were higher than the upper limit of the range for historical controls over the relevant period.

In order to further investigate the occurrence of microphthalmia, a different rat substrain was selected for which the available database on historical controls revealed a virtually-zero background incidence of this malformation. Since the strain was nevertheless sensitive to direct, specific oculo-teratogenic effects, it was well suited for investigation of the specificity of microphthalmia formation caused by prothioconazole. In this second study of developmental toxicity in rats, prothioconazole did not cause microphthalmia or other specific malformations at any dose up to and including 750 mg/kg bw per day. These results would seem to support the hypothesis that the increase in microphthalmia seen in the original study of developmental toxicity was a non-specific enhancement of a common spontaneous effect; however, the mechanism by which microphthalmia was induced has not been investigated or described. There was an increase in the incidence of rudimentary (comma shaped) supernumerary 14th ribs that was significant on a fetal basis at 750 mg/kg bw per day, but not on a litter basis, and was not increased at 80 mg/kg bw per day or at the lower dose. The NOAEL for maternal toxicity was 80 mg/kg bw per day on the basis of reduced body-weight gain, increased water consumption, reduced food consumption and clinical chemical indications for functional impairment of liver and kidney function at 750 mg/kg bw per day. The NOAEL for developmental toxicity was 80 mg/kg bw per day on the basis of a statistically significant increase in the incidence of rudimentary supernumerary 14th ribs at 726 mg/kg bw per day.

In a study of developmental toxicity in which rabbits were given prothioconazole by gavage on days 6–27 of gestation, the NOAEL for maternal toxicity was 80 mg/kg bw per day on the basis of mortality, body-weight loss or reduced body-weight gain and reduced food consumption at 350 mg/kg bw per day. The NOAEL for developmental toxicity was 80 mg/kg bw per day on the basis of abortions, total litter losses, reduced fetal weights and retarded ossification at 350 mg/kg bw, where there was clear evidence of severe maternal toxicity.

In a study of neurotoxicity in rats given a single dose of prothioconazole by gavage, the NOAEL was 218 mg/kg bw per day on the basis of transient clinical signs at 877 mg/kg bw per day. There were no neurohistopathological changes in nerve tissue and no persistent signs of neurobehavioural toxicity.

In a 90-day study of neurotoxicity in rats given prothioconazole by gavage, the NOAEL was 100 mg/kg bw per day on the basis of clinical signs, reduced body weights and reduced motor and locomotor activity at 1000 mg/kg bw per day. The reduced motor and locomotor activity is likely to be secondary to the systemic toxicity evident in these animals rather than clear neurobehavioural toxicity. There were no neurohistopathological changes in nerve tissue or muscle.

The Meeting concluded that prothioconazole is unlikely to cause neurotoxicity in humans.

There were no indications of immunotoxicity in general studies of toxicity in dogs, rats and mice.

Some aspects of the toxicology of certain metabolites of prothioconazole found in wheat (mainly straw), but not necessarily in rats—exceptions being prothioconazole-triazolinone (M03) and prothioconazole-desthio—were investigated.

Triazole (1,2,4-triazole) and its metabolites, triazole alanine and triazole acetic acid, are metabolites of difenoconazole, the toxicology of which was summarized by JMPR 2007 and by the present Meeting. No triazole-free metabolites were found using phenyl-labelled prothioconazole. The other metabolites summarized here were prothioconazole-desthio, prothioconazole-sulfonic acid (M02), prothioconazole-desthio-*alpha*-hydroxy (M18), prothioconazole-desthio-*alpha*-acetoxy (M19), prothioconazole-benzylpropyldiol (M09), prothioconazole-triazolinone (M03). M03 is also found in rat urine in which it represents up to 2% of the administered parent compound. The data submitted on these substances indicate that, except for prothioconazole-desthio and M02, they are not toxicologically relevant metabolites. A single-dose study of oral toxicity indicated that the LD₅₀ of prothioconazole-sulfonic acid is > 200 mg/kg bw and < 2000 mg/kg bw. The LD₅₀ values for prothioconazole-desthio-*alpha*-hydroxy (M18), prothioconazole-desthio-*alpha*-acetoxy (M19) and prothioconazole-benzylpropyldiol are all > 2000 mg/kg bw. Prothioconazole-sulfonic acid has been tested in a 90-day dietary study of toxicity in rats. The NOAEL was 500 ppm, equal to 34 mg/kg bw per day, on the basis of histomorphological alterations in the urinary bladder at 2000 ppm, equal to 136 mg/kg bw per day. No other repeat-dose studies of toxicity have been conducted with these metabolites.

In a study of developmental toxicity in rats given prothioconazole-sulfonic acid by gavage on days 6–20 of gestation, the NOAEL for maternal toxicity was 150 mg/kg bw per day on the basis of increased mortality, reduced food consumption and reduced body-weight gain at 750 mg/kg bw per day. The NOAEL for developmental toxicity was 150 mg/kg bw per day on the basis of increased incidence of total implantation loss and the occurrence of reduced fetal weight gain and reduced ossification at 750 mg/kg bw per day. Prothioconazole-sulfonic acid did not show any teratogenic potential.

None of the metabolites was active in tests for mutagenicity with strains of *Salmonella typhimurium*.

The most toxicologically significant of the prothioconazole metabolites is prothioconazole-desthio. A largely complete toxicology dossier was available for this compound.

Prothioconazole-desthio was rapidly and almost completely absorbed from the gastrointestinal tract of rats, with a plasma t_{max} of about 1.5 h, but maximum plasma concentrations were low. The plasma t_{max} of prothioconazole-desthio in pregnant rats treated by gavage was similar to that in male rats. The mean concentration of radioactivity in the body minus the gastrointestinal tract was > 3.5% of the administered dose, indicating that there was little distribution to the peripheral tissues; the highest concentrations (about 3% of the administered dose) were found in the liver. Excretion occurred predominantly via the bile, and the elimination half-life and mean residence time were prolonged due to intensive enterohepatic re-circulation. No potential for bioaccumulation was expected. The bile metabolites identified indicated that metabolism proceeded via oxidation only of the phenyl moiety, with subsequent glucuronidation and methylation of the oxidation products. These oxidation reactions yielded metabolites without their former aromatic character; nevertheless, the cyclopropyl and triazole ring structures of prothioconazole-desthio remained intact.

The acute toxicity of prothioconazole-desthio is low, the oral LD₅₀ being approximately 2200 mg/kg bw in rats and mice. In both species, deaths were delayed, by 4–13 days in rats and 1–4 days in mice. In rats and mice, no clinical signs were observed at 100 mg/kg bw. The observations recorded for mice given higher doses were apathy, piloerection, laboured breathing, staggering gait and increased urination in males at 500 mg/kg bw and in females at 1000 mg/kg bw. Spastic gait and reduced mobility were also noted in females at > 100 mg/kg bw, but these signs were noted in males only at doses of > 2000 mg/kg bw. Atony, weak reflexes, emaciation, pallor, narrowed palpebral fissures (separation between the upper and lower eyelids), red crusted eyelids, bloody snout, prone position and leg extension occurred in both sexes at high doses. Some clinical signs were evident shortly after treatment but others showed a delayed onset. All signs had resolved by day 13 in male rats and by day 18 in females. In mice, the clinical signs of response were motility and respiratory disturbances, piloerection, staggering gait, narrowed palpebral fissures, lacrimation, a spasmodic

state, temporary rolling over, prostration or lying on the side. These were mainly observed at up to moderate intensity, developed shortly after treatment in some cases, and persisted at maximum levels up to the eleventh day of the study in the male mice or up to the seventh day in the females. The dermal LD₅₀ in rats was > 5000 mg/kg bw and the inhalation LC₅₀, also in rats, was > 5.08 mg/L for an exposure of 4 h. Prothioconazole-desthio is not irritating to rabbit skin and eyes and is not sensitizing in the Buehler skin patch test in guinea-pigs.

In short-term studies of toxicity, a common target organ in rat, mouse and dog was the liver and effects on this organ formed the basis for the NOAEL in the short-term studies in rats. Effects in the liver (not always adverse and not always at critical doses for those effects that were adverse) included increased organ weight, induction of CYP isoenzymes, hepatocellular hypertrophy, increased hepatocytic fatty vacuolation, single-cell or focal necrosis, hydropic degeneration and increased ploidy. The NOAELs in dietary studies were 2.2 mg/kg bw per day in a 13-week study in rats and 10 mg/kg bw per day in a 30-week study in dogs. No NOAEL was identified in mice, but it was certainly greater than 12 mg/kg bw per day.

Long-term dietary studies in rats and mice of prothioconazole-desthio confirmed that the primary target organ was the liver. The liver effects were increased weights, hypertrophy, cytoplasmic change and a shift in fat storage from the periportal (usual) to the centrilobular region of the liver in rats and increased incidences of periportal fat accumulation in the liver of mice. Mild alteration in plasma thyroid hormone concentrations in rats was possibly a consequence of induction of hepatic enzymes, but there was no accompanying notable histopathology in the thyroids. In addition, in rats, there were increased incidences of adrenal cortical vacuolization in males at either of the two higher doses. There was no evidence for the carcinogenicity of prothioconazole-desthio in rats or mice. The NOAEL in a 2-year dietary study in rats was 20 ppm, equal to 1.1 mg/kg bw per day, on the basis of microscopic changes in the liver and ovary at 140 ppm, equal to 8.0 mg/kg bw per day. The NOAEL in an 18-month dietary study in mice was 12.5 ppm, equal to 3.1 mg/kg bw per day, on the basis of microscopic changes in the liver at 50 ppm, equal to 12.8 mg/kg bw per day.

Prothioconazole-desthio was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. Genotoxicity was not observed in any of these assays.

The Meeting concluded that prothioconazole-desthio is unlikely to be genotoxic.

On the basis of the absence of carcinogenicity in rodents and the absence of genotoxicity, the Meeting concluded that prothioconazole-desthio is unlikely to pose a carcinogenic risk to humans.

The reproductive toxicity of prothioconazole-desthio was investigated in a one-generation pilot study and a two-generation study of reproduction in rats. One study for potential developmental toxicity after oral dosing was conducted in rats and one in rabbits. In addition, prothioconazole-desthio was tested for developmental neurotoxicity in one study in rats. Reproductive effects of prothioconazole-desthio in rats comprised reduced litter size, reduced pup viability, pre-weaning growth retardation and an increased incidence of cleft palate. In the main two-generation study, a number of females in the parental and F₁ generations exhibited dystocia (difficulty in giving birth). In both the pilot and main study, the NOAELs for parental toxicity were similar to, or lower than, the NOAELs for reproductive and neonatal effects. The NOAEL for systemic toxicity in the parental rats was 40 ppm, equal to 2.7 mg/kg bw per day, on the basis of hepatocellular vacuolation in males at 160 ppm, equal to 10.4 mg/kg bw per day. In the offspring, the NOAEL was 160 ppm, equal to 10 mg/kg bw per day, on the basis of decreased neonatal viability, reduced pup weight gain, and cleft palate at 640 ppm, equal to 41 mg/kg bw per day. The NOAEL for reproductive effects was 40 ppm, equal to 10 mg/kg bw per day, on the basis of dystocia at 640 ppm, equal to 41 mg/kg bw per day.

In a study of developmental toxicity in rats given prothioconazole-desthio by gavage on days 6–15 of gestation, the NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of reduced body weight gain, reduced food consumption and increased liver weight and histological changes in the liver at 100 mg/kg bw per day. There was no NOAEL for developmental toxicity in this study, in which there were increased incidences of fetuses with supernumerary ribs at all doses, including

10 mg/kg bw per day, the lowest dose tested. In a follow-up to this study, another study of developmental toxicity was conducted in rats given prothioconazole-desthio over a lower dose range by gavage on days 6–15 of gestation. The NOAEL for developmental toxicity was 1 mg/kg bw per day on the basis of increased incidence of supernumerary rudimentary ribs at 3 mg/kg bw per day.

In a study of developmental toxicity in rabbits given prothioconazole-desthio by gavage on days 6–18 of gestation, the NOAEL for maternal toxicity was 2 mg/kg bw per day on the basis of histological changes in the liver at 10 mg/kg bw per day. The NOAEL for developmental toxicity was 2 mg/kg bw per day on the basis of increased incidence of fetuses with any abnormality (primarily arthrogyrosis and cleft palate) at 10 mg/kg bw.

In a study of developmental neurotoxicity in rats given prothioconazole-desthio from day 6 of gestation until day 21 of lactation, the NOAEL was 500 ppm, equal to 43.3 mg/kg bw per day, the highest dose tested, on the basis of the absence of effects on neurobehavioural, learning and memory parameters, on brain weight, brain morphometry and on neuropathology parameters at this dose.

In summary, on the basis of the results of the submitted studies of toxicity, the acute oral toxicity of both prothioconazole and its desthio metabolite was low and neither compound showed any mutagenic or carcinogenic potential. The NOAELs for the short-term and long-term studies as well as the studies of reproductive toxicity and developmental toxicity were clearly lower for prothioconazole-desthio than for prothioconazole. In the studies of developmental toxicity, increased incidences of cleft palate in rats and rabbits were observed with prothioconazole-desthio at doses of 100 and 50 mg/kg bw per day, respectively, with no cleft palate induction at 30 and 10 mg/kg bw per day, respectively. Cleft palate was not observed in studies of developmental toxicity with the parent compound, prothioconazole, but was observed in a study of reproductive toxicity in rats given prothioconazole at a dose of 41 mg/kg bw per day.

No adverse effects have been identified in workers involved in the development, production or formulation of prothioconazole. No further information on medical surveillance or poisoning incidents was available.

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

Toxicological evaluation

Prothioconazole

An ADI of 0–0.05 mg/kg bw was established for prothioconazole based on the NOAEL of 5 mg/kg bw per day, identified on the basis of gross and microscopic changes in the liver and kidneys in a 2-year study of toxicity and carcinogenicity in rats treated by gavage, and a safety factor of 100.

An ARfD of 0.8 mg/kg bw was established for women of childbearing age based on a NOAEL of 80 mg/kg bw per day, identified on the basis of a marginally increased incidence of supernumerary rudimentary ribs that might be attributable to a single exposure at 750 mg/kg bw per day in a study of developmental toxicity in rats, and with a safety factor of 100. The Meeting concluded that the establishment of an ARfD for the general population was not necessary on the basis of its low acute toxicity, the lack of evidence for any acute neurotoxicity and absence of any other toxicologically relevant effect that might be attributable to a single dose.

Prothioconazole-desthio

Since the residue definition for risk assessment in all commodities is expressed as prothioconazole-desthio and this metabolite is of higher toxicity than the parent, ARfD values and an ADI were also established for prothioconazole-desthio.

An ADI of 0–0.01 mg/kg bw was established for prothioconazole-desthio based on the NOAEL of 1.1 mg/kg bw per day, identified on the basis of microscopic changes in the liver and ovaries in a 2-year dietary study of toxicity and carcinogenicity in rats, and with a safety factor of 100.

An ARfD of 0.01 mg/kg bw was established for women of childbearing age based on a NOAEL of 1 mg/kg bw per day, identified on the basis of increased incidence of supernumerary rudimentary ribs that might be attributable to a single exposure at 3 mg/kg bw per day in a study of developmental toxicity in rats, and with a safety factor of 100. Although the increased incidence at 3 mg/kg bw per day was only significant on the basis of the number of fetuses, this was the lower limit of a clear dose-related response curve.

The Meeting also established an ARfD of 1 mg/kg bw for the general population based on a NOAEL of 100 mg/kg bw, identified on the basis of clinical signs in studies of toxicity in mice and rats given single doses, and a safety factor of 100.

A toxicological monograph was prepared.

Levels relevant to risk assessment for prothioconazole

Species	Study ^a	Effect	NOAEL	LOAEL
Mouse	18-month study of toxicity and carcinogenicity	Toxicity	10 mg/kg bw per day	70 mg/kg bw per day
		Carcinogenicity	500 ^b mg/kg bw per day	—
Rat	Two-year studies of toxicity and carcinogenicity	Toxicity	5 mg/kg bw per day	50 mg/kg bw per day
		Carcinogenicity	750 ^b mg/kg bw per day	—
	Two-generation study of reproductive toxicity	Reproductive toxicity	95.6 mg/kg bw per day	726 mg/kg bw per day
		Parental toxicity	9.7 mg/kg bw per day	95.6 mg/kg bw per day
		Offspring toxicity	95.6 mg/kg bw per day	726 mg/kg bw per day
	Developmental toxicity	Maternal toxicity	80 mg/kg bw per day	750 mg/kg bw per day
Embryo and fetal toxicity		80 mg/kg bw per day	750 mg/kg bw per day	
Rabbit	Developmental toxicity	Maternal toxicity	80 mg/kg bw per day	350 mg/kg bw per day
		Embryo and fetal toxicity	80 mg/kg bw per day	350 mg/kg bw per day

^a In all cases, prothioconazole was administered by gavage.

^b Highest dose tested.

Levels relevant to risk assessment for prothioconazole-desthio

Species	Study	Effect	NOAEL	LOAEL
Mouse	Single dose LD ₅₀ study	Toxicity	100 mg/kg bw	500 mg/kg bw
	18-month study of toxicity and carcinogenicity	Toxicity	12.5 ppm equal to 3.1 mg/kg bw per day	50 ppm equal to 12.8 mg/kg bw per day
Carcinogenicity		200 ppm equal to	—	

			51.7 mg/kg bw per day ^b	
Rat	Single-dose LD ₅₀ study	Toxicity	100 mg/kg bw	500 mg/kg bw
	Two-year studies of toxicity and carcinogenicity	Toxicity	20 ppm equal to 1.1 mg/kg bw per day	140 ppm equal to 8.0 mg/kg bw per day
		Carcinogenicity	980 ppm equal to 57.6 ^b mg/kg bw per day	—
	Two-generation study of reproductive toxicity	Reproductive toxicity	160 ppm equal to 10.0 mg/kg bw per day	640 ppm equal to 41.2 mg/kg bw per day
		Parental toxicity	40 ppm equal to 2.7 mg/kg bw per day	160 ppm equal to 10.4 mg/kg bw per day
		Offspring toxicity	160 ppm equal to 10.0 mg/kg bw per day	640 ppm equal to 41.2 mg/kg bw per day
	Developmental toxicity	Maternal toxicity	30 mg/kg bw per day ^b	—
Embryo and fetal toxicity		1 mg/kg bw per day	3 mg/kg bw per day	
Rabbit	Developmental toxicity	Maternal toxicity	2 mg/kg bw per day	10 mg/kg bw per day
		Embryo and fetal toxicity	2 mg/kg bw per day	10 mg/kg bw per day
Dog	30-week study of toxicity	Toxicity	10.1 mg/kg bw per day	69.9 mg/kg bw per day

^b Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.05 mg/kg bw (for prothioconazole)

0–0.01 mg/kg bw (for prothioconazole-desthio)

Estimates of acute reference doses

0.8 mg/kg bw for women of childbearing age (for prothioconazole)

Unnecessary for the general population (for prothioconazole)

0.01 mg/kg bw for women of childbearing age (for prothioconazole-desthio)

1 mg/kg bw for the general population (for prothioconazole-desthio).

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 prothioconazole and prothioconazole-desthio

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of oral absorption

Rapid and extensively, > 90%

Distribution

Distributed throughout the body; higher concentrations

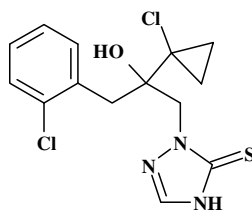
	in liver and gastrointestinal tract	
Potential for accumulation	No evidence	
Rate and extent of excretion	High, > 70% within 24 h, but subsequently low rate	
Metabolism in animals	18 metabolites identified	
Toxicologically significant compounds (animals, plants and environment)	Parent, prothioconazole-desthio and M02	
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<i>Acute toxicity</i>	<i>Prothioconazole</i>	<i>Prothioconazole-desthio</i>
Rat, LD ₅₀ , oral	> 6200 mg/kg bw	> 2500 mg/kg bw
Rat, LC ₅₀ , inhalation	> 4.9 mg/L ^a (4 h)	> 5.08 mg/L ^a (4 h)
Rat, LD ₅₀ , dermal	> 2000 mg/kg bw ^a	> 5000 mg/kg bw ^a
Rabbit, dermal irritation	Not irritating	Not irritating
Rabbit, ocular irritation	Not irritating	Not irritating
Dermal sensitization	Not sensitizing (Buehler skin patch test in guinea-pigs; lymph node assay in mice)	Not sensitizing (Buehler skin patch test in guinea-pigs)
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<i>Short-term studies of toxicity</i>		
Target/critical effect	Liver, kidney	Liver
Lowest relevant oral NOAEL	25 mg/kg bw per day (3-month study in dogs,	2.2 mg/kg bw per day (13-week study in rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day ^a (4-week study in rats)	No data
Lowest relevant inhalation NOAEC	No data	No data
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<i>Genotoxicity</i>		
	Not genotoxic in vivo, but mixed results in vitro	Not genotoxic
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<i>Long-term studies of toxicity and carcinogenicity</i>		
Target/critical effect	Liver, kidney	Liver, kidney
Lowest relevant NOAEL	5 mg/kg bw per day (2-year study in rats)	1 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic	Not carcinogenic
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<i>Reproductive toxicity</i>		
Reproductive target/critical effect	Disruption of oestrus cycle; reduced implantation sites and litter size; increased time to insemination; increased gestation time; all associated with severe maternal toxicity	Decreased neonatal viability, reduced pup-weight gain, and cleft palate at maternal toxic doses
Lowest relevant reproductive NOAEL	100 mg/kg bw per day	10 mg/kg bw per day
Developmental target/critical effect	Not teratogenic; abortions, total litter loss, reduced fetal body weight, delayed	Teratogenic (cleft palate, arthrogryposis); abnormal fetuses, increased incidence of supernumerary

		ossifications, rudimentary supernumerary ribs	rudimentary ribs
Lowest relevant developmental NOAEL		80 mg/kg bw per day (rat, rabbit)	1 mg/kg bw per day (rat, rabbit)
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Neurotoxicity/delayed neurotoxicity			
		No signs of neurotoxicity	No signs of neurotoxicity
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<i>Other toxicological studies</i>			
		Induction of liver xenobiotic metabolizing enzymes	Clinical signs of toxicity in single-dose studies (LD ₅₀) in rats and mice
		Several metabolites in addition to prothioconazole- desthio and MO2 have been investigated, but are not considered to be toxicologically significant	
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<i>Medical data</i>			
		No reports of toxicity in workers exposed during manufacture or use	
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Summary			
<i>Prothioconazole</i>	<i>Value</i>	<i>Study</i>	<i>Safety factor</i>
ADI	0–0.05 mg/kg bw	Dog, 1-year study of toxicity; and rat, 2-year study of toxicity and carcinogenicity	100
ARfD	0.8 mg/kg bw for women of childbearing age	Rat, study of developmental toxicity	100
<i>Prothioconazole- desthio</i>	<i>Value</i>	<i>Study</i>	<i>Safety factor</i>
ADI	0–0.01 mg/kg bw	Rat, 2-year study of toxicity and carcinogenicity	100
ARfD	0.01 mg/kg bw for women of childbearing age	Rat, study of developmental toxicity	100
	1 mg/kg bw for the general population	Rat and mouse, LD ₅₀ studies	100

^a Only dose tested.

RESIDUE AND ANALYTICAL ASPECTS

Prothioconazole was considered for the first time by the present meeting. It is a systemic fungicide with a triazolinthione structure. The manufacturing process is not enantiomer-selective. All technical quality prothioconazole is produced as a 50:50 racemate.



IUPAC: 2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione

CAS: 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione.

The code and descriptive names of metabolites mentioned in the appraisal are:

M01	JAU 6476-S-methyl
M02	JAU 6476-sulfonic acid
M03	JAU 6476-triazolinone
M04	JAU 6476-desthio
M05	JAU 6476-N-glucuronide
M06	JAU 6476-S-glucuronide
M08	JAU 6476-4-hydroxy
M09	JAU 6476-benzylpropyldiol
M11	JAU 6476-disulfide
M12	JAU 6476-thiazocine
M13	1,2,4-triazole
M14	JAU 6476-desthio-3-hydroxy
M15	JAU 6476-desthio-4-hydroxy
M16	JAU 6476-desthio-5-hydroxy
M17	JAU 6476-desthio-6-hydroxy
M18	JAU 6476-desthio- α -hydroxy
M19	JAU 6476-desthio- α -acetoxy
M20	2-chlorobenzoic acid
M21	JAU 6476-desthio-3-hydroxy-glucoside
M22	JAU6476-desthio-4-hydroxy-glucoside
M23	JAU 6476-desthio-6-hydroxy-glucoside
M24	JAU 6476-desthio-hydroxy-dienyl-cysteine
M28	JAU 6476-desthio-hydroxy-methoxy
M29	Triazolylacetic acid (TAA)
M30	Triazolylhydroxypropionic acid (THP)
M31	Triazolylalanine (TA)
M32	JAU 6476-desthio-3,4-dihydroxy-diene
M33	JAU 6476-desthio-3,4-dihydroxy
M34	JAU 6476-desthio-dihydroxy
M36	JAU 6476-desthio-4,5-dihydroxy-diene
M38	JAU 6476-desthio-dihydroxy-diene
M40	JAU 6476-dihydroxy-diene
M44	JAU 6476-desthio-phenyl-cysteine
M45	JAU 6476-triazolyl-ethanol

M46	JAU 6476-triazolyl-ethanol-glucoside
M52	JAU 6476-desthio-3,4-dihydroxy-dienyl-glucuronide
M54	JAU 6476-desthio-3-hydroxy-glucoside-malonic acid
M55	JAU 6476-desthio-4-hydroxy-glucoside-malonic acid
M56	JAU 6476-desthio-6-hydroxy-glucoside-malonic acid
M59	JAU 6476-hydroxy-sulfonic acid glucoside
M60	JAU 6476-hydroxy-disulfonic acid glucoside
M62	JAU 6476-triazolyl-sulfonic acid-ethanol-glucoside
M71	JAU 6476-desthio-glucuronide
M73	JAU 6476-desthio-dihydroxy-dienyl-glucuronide
M74	JAU 6476-desthio-4-hydroxy-glucuronide
M75	JAU 6476-desthio-hydroxy-glucuronide
M80	Thiocyanate
M82	JAU 6476-desthio-hydroxy-methoxy-sulfate
M84	JAU 6476-desthio-hydroxy-sulfate
M85	JAU 6476-desthio-4,5-dihydroxy-dienyl-glucuronide
M87	JAU 6476-desthio-3-hydroxy-glucuronide

The Meeting received comprehensive information for the evaluation of prothioconazole as a new compound in accordance with the data requirements specified in the *FAO Manual*⁴⁰.

The metabolism of prothioconazole, in plants and animals was investigated using [phenyl-UL-¹⁴C]prothioconazole referred to as phenyl-label, and [3,5-triazole-¹⁴C] labelled parent compound referred to as triazole-label. In addition, studies were conducted using [phenyl-UL-¹⁴C]- and [3,5-triazole-¹⁴C] labelled prothioconazole-desthio (*MO4*) which is derived from the parent compound by losing the thione group.

Animal metabolism

Information was provided on the metabolism of prothioconazole in rats, lactating goats and laying hens.

When rats were orally dosed the prothioconazole was rapidly absorbed and excreted mainly via the bile. The major types of metabolic reactions identified were conjugation with glucuronic acid, oxidative hydroxylation of the phenyl moiety and desulfuration. Many of the metabolites were derived from prothioconazole-desthio.

Prothioconazole-S-glucuronide, prothioconazole-desthio and prothioconazole were the principal components, in addition to 10 minor metabolites identified in excreta. The studies with prothioconazole showed that metabolite prothioconazole-desthio, which is the major metabolite of prothioconazole in wheat, amounted to about 18% of the administered dose occurred in the faeces, and a maximum 0.07% in the urine. A study with the metabolite prothioconazole-desthio, which is the major metabolite of prothioconazole in wheat, revealed that about 68% to 74% of the administered dose occurred in the faeces, and a maximum of 10% to 11% in the urine.

The goat metabolism studies followed the same design. In each trial one goat received 10 mg/kg body weight/day dose on three consecutive days in intervals of 24 h. The dose level corresponded to about 195 ppm test substance in the feed. The animals were sacrificed 5 h after administration of the final dose.

⁴⁰ FAO. 2002. Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed. Appendix IX. Maximum proportion of agricultural commodities in animal feed. *FAO Plant Production and Protection Paper*, 170.

Following the administration of phenyl-, triazole-labelled prothioconazole or phenyl labelled prothioconazole-desthio, the goats excreted about 67, 59 and 74% administered dose, respectively, within five hours after the last dose. About 0.02–0.05% of the total dose was found in the milk, and about 0.7–1.9% of the total dose was in the organs and tissues.

The TRR values, derived from the administration of labelled parent compound, expressed in mg as equivalents/kg sample material, were 6.1–6.2 mg/kg in liver, 4.5–6.8 mg/kg in kidney, 0.17–0.21 mg/kg in omental fat, 0.11–0.162 mg/kg perirenal fat, 0.11–0.15 mg/kg in subcutaneous fat and 0.08–0.14 mg/kg in muscle.

The metabolic profiles for milk and the edible tissues and organs showed that parent prothioconazole was a major compound in all tissues and organs (> 10% of TRR), but only of minor importance in milk (< 1–3% of TRR or ≤ 0.005 mg/kg). Other metabolites detected in all matrices were prothioconazole-S-glucuronide, prothioconazole-desthio and a number of the hydroxy moiety containing metabolites (*M08*, *M15*, *M32*, *M38*, *M40*,), their glucuronides (*M10*, *M49*, *M52*, *M69*, *M72*, *M73*, *M74*) and sulfate conjugates (*M82*, *M83*, *M84*). Following the administration of triazole labelled prothioconazole the only label specific metabolite identified was thiocyanate: 41.1% of TRR, 0.061 mg/kg in milk, 30% in muscle, 12% in fat, 9% in kidney and 2% in liver. Thiocyanate is well known as the main detoxification product after cyanide exposure, and it is a natural constituent of milk.

The administration of phenyl-labelled prothioconazole-desthio resulted in somewhat higher level of total residues. The presence of triazole derivatives or free 1,2,4-triazole at concentrations above 0.01 mg/kg was excluded in each matrix under investigation.

In order to simplify the metabolic pattern, the organs and milk of goat was subjected to acid hydrolysis. The large number of metabolites present in the extracts (*M32*, *M36*, *M52*, *M71*, *M73*, *M74*, *M75*, *M84*, *M85*, *M86*, *M87*, *M91*) was reduced to prothioconazole-desthio, *M14*, *M15*, *M33*, *M35* and *M71*.

When prothioconazole-desthio was administered to goat the proportion of identified/characterized metabolites in the TRR after the acid hydrolysis was higher in milk, muscle and kidney than without hydrolysis, and it was practically equal in liver (58.4% and 59.9%) and fat (75% and 74%)

Six laying hens were orally dosed with phenyl or triazole radiolabelled prothioconazole at a dose level of 10 mg/kg body weight. The hens received the doses on three consecutive days at intervals of 24 h. The animals were sacrificed 5 h after the administration of the final dose.

The edible tissues and organs (liver, fat and muscle), pooled excreta and eggs collected daily were analysed by HPLC and TLC. Extractability was high for all tissues and ranged from 77% to 98% of the TRR. The identification rates ranged from 42% and 84%.

About 78% of the phenyl labelled prothioconazole dose administered was already excreted five hours after the last dose. Very low amounts of the total dose were found in eggs during the experimental phase (about 0.01%) and in organs/tissues investigated after sacrifice of the animals (about 0.9%). In the study with the triazole label about 66% of the administered dose was excreted five hours after the last dose. Also very low amounts of the total dose were found in eggs during the experimental phase (about 0.01%) and in organs/tissues investigated after sacrifice of the animals (about 0.8%).

The TRR values, derived from the phenyl- and triazole labelled prothioconazole and expressed in mg as equivalents/kg sample material were, respectively, 4.0–3.5 mg/kg in liver, 0.036–0.05 mg/kg in eggs, 0.45–0.29 mg/kg in subcutaneous fat, 0.089–0.12 mg/kg in muscle.

The parent compound was the major residue component in liver (31% of TRR, 1.1 mg/kg), fat (30% of TRR, 0.14 mg/kg) and muscle (11% of TRR, 0.01 mg/kg). Metabolites exceeding 10% of TRR were prothioconazole-desthio (29.0% of TRR, 0.13 mg/kg) and prothioconazole -S-methyl (20% of TRR, 0.088 mg/kg) in fat, and prothioconazole-S-glucuronide in muscle (15.5% of TRR,

0.014 mg/kg), and liver (*M06*, 15% of TRR, 0.53 mg/kg). The other metabolites occurring in smaller proportion were *M05*, *M08*, *M10*, *M15*, *M80* and the label specific metabolites (*M45* and 1,2,4-triazole). All other metabolites identified were either glucuronic acid conjugates derived directly from prothioconazole or from the hydroxylated parent compound or sulfate and glucuronic acid conjugates (*M52*, *M82*, *M83*, *M84*) (in sum 14% of TRR, 0.47 mg/kg).

In eggs the major residue components were prothioconazole-S-glucuronide (24% of TRR, 0.012 mg/kg), prothioconazole-desthio (20% of TRR, 0.007 mg/kg), *M45* (15.6% of TRR, 0.008 mg/kg), 1,2,4-triazole (11% of TRR, 0.006 mg/kg), and thiocyanate (9.8% of TRR, 0.005 mg/kg). Prothioconazole (3.6% of TRR, 0.002 mg/kg), *M15* and *M01* were also detected ranging from 1.9% to 3.3% of the TRR.

In summary, the metabolic profile was similar in goats and hens and their edible tissues investigated in the studies with phenyl- and triazole-labelled prothioconazole. The parent compound was one of the major residues in most matrices of goat and hen with the exception of egg in which prothioconazole-desthio was predominant.

The majority of metabolites were derived from the intact parent molecule, retaining the triazolinthione structure, which was detected in all studies with prothioconazole, independent of the radiolabel used. A label specific metabolite common for hen and goat was thiocyanate. This metabolite was detected in all sample materials under investigation. It was a major metabolite in milk and muscle of goat and was detected at about 10% of the TRR in eggs of hens. Two additional label specific metabolites were identified exclusively in laying hen: free 1,2,4-triazole and prothioconazole-triazolyl-ethanol, which were detected in all matrices, including eggs, but they were not present in milk, organs or edible tissues of goat.

The key metabolite in all matrices was prothioconazole-S-glucuronide. Due to the conjugation with glucuronic acid, the sulfur was protected against cleavage. Thus, the metabolic route via prothioconazole-desthio was impeded. Prothioconazole-desthio and all its derivatives accounted in each sample matrix, except in fat and eggs, for less than 20% of the TRR. The major metabolic routes include molecules containing the intact parent compound.

Plant metabolism

The behaviour and metabolism of prothioconazole after spray application in wheat, peanut and sugar beets was investigated using phenyl- and triazole-labelled parent compound. Additionally, the metabolism of phenyl-labelled prothioconazole after seed treatment of wheat and the metabolism of prothioconazole-desthio following spray application were studied.

When phenyl- and triazole-labelled prothioconazole was used for foliar treatment of wheat approximately at the recommended rate (0.2 kg/ha), the total radioactive residue (TRR) levels in forage, hay, straw and grain were 10 and 8.0 mg/kg, 8.9 and 11 mg/kg, 27 and 8 mg/kg and 0.08 and 5 mg/kg (ai equivalents), respectively.

When the seeds were treated at 1× rate the total radioactive residue (TRR) levels were very low and amounted to 0.02 mg/kg in forage, 0.02 mg/kg in hay, 0.03 mg/kg in straw and 0.008 mg/kg (as equivalents) in grain, respectively. Following the 5× treatment the TRR were 0.07 mg/kg in forage, 0.09 mg/kg in hay, 0.28 mg/kg in straw and < 0.01 mg/kg in grain.

Following foliar application with phenyl and triazole labelled parent compounds, the identified metabolites accounted respectively for 73% and 66% of the TRR in forage, 65% and 75% of the TRR in hay, 66% and 61% of the TRR in straw and 34% and 94% of the TRR in grain.

Prothioconazole was extensively metabolized in wheat. Prothioconazole-desthio was found as the main metabolite in all crop parts: forage, (35.4% of the TRR, 3.7 mg/kg), hay (18.5% of the TRR, 1.64 mg/kg), straw (22% of the TRR, 6.0 mg/kg) and grain (16% of the TRR, 0.014 mg/kg).

The hydroxylated metabolites of prothioconazole-desthio (*M14*, *M15*, *M17*) and the corresponding glucosides were present in forage, hay and straw, but wheat grain contained only *M14*, *M15*.

In addition, the parent compound and the following metabolites were identified in wheat forage, hay and straw: *M02*, *M03*, *M11* and *M18*.

Following the foliar application of prothioconazole-desthio, TRR in forage was 10 mg/kg (day 0) and 11 mg/kg (day 14). The TRRs in straw, and grain were 29 mg/kg and 2.9 mg/kg, respectively.

Identified metabolites in the tested crop parts accounted for 90–94% of the TRR in forage, 84% of the TRR in straw and 94% of the TRR in grain.

The prothioconazole-desthio was slowly metabolized in wheat. It was the dominant constituent of the residue in forage (77% of TRR) and straw (72% of TRR) at harvest. However, it was only detected in small amounts in grain (0.07 mg/kg), where the residue was mainly made up by triazolylacetic acid (0.91 mg/kg) and triazolylalanine (1.72 mg/kg). Free 1,2,4-triazole was not detected in any of the crop parts.

The behaviour and metabolism of phenyl- and triazole-labelled prothioconazole were investigated after 3 spray applications with EC 250 formulation to peanuts at a rate of 297 g as/ha/application.

The total radioactive residue (TRR) levels in peanut hay were 107.5 mg/kg and 47.4 mg/kg (parent equivalents) for the phenyl- and triazole-labelled parent compound, respectively. The TRR in nutmeat was 0.29 mg/kg and 1.4 mg/kg (parent equivalents) for the phenyl- and triazole-label, respectively. Identified metabolites accounted for 74% and 77% of the TRR in peanut hay and 65% and 83% of the TRR in nutmeat for the phenyl- and triazole-label, respectively.

Following the treatments with phenyl-labelled parent compound, the major metabolites in peanut hay included prothioconazole-desthio (28% of TRR, 30 mg/kg) and its derivatives (*M14/M15*) amounting to 7.3% of TRR, 7.8 mg/kg and 2.0% of TRR, 2.2 mg/kg, respectively. In addition to the parent compound two other metabolites of prothioconazole were identified as prothioconazole-sulfonic acid and *M03* (2.1% of TRR, 2.3 mg/kg and 1.6% of TRR, 1.7 mg/kg, respectively). None of these compounds were detected in nutmeat. Furthermore, metabolites derived from prothioconazole-desthio and *M02* but lacking the aromaticity of the phenyl ring were detected in the hay and in the case of prothioconazole-desthio derivatives in nutmeat too. But the main portion of radioactivity (48% of TRR in the MSPD extracts) of the nutmeat was characterized as natural occurring oil, and was determined as fatty acids.

When triazole labelled parent compound was applied the metabolites identified in peanut hay included prothioconazole-desthio (as main metabolite, 24% of the TRR, 11 mg/kg) and its hydroxylated derivatives (*M14*, *M15* amounting to 6.6% of TRR, 3.1 mg/kg and 3% of TRR, 1.4 mg/kg, respectively). Two other metabolites of prothioconazole were identified as *M02* and *M03* (2.7% of TRR, 1.3 mg/kg and 3.6% of TRR, 1.7 mg/kg, respectively). Furthermore, metabolites derived from prothioconazole-desthio and *M02* but lacking the aromaticity of the phenyl ring were detected in the hay. With the exception of prothioconazole-desthio, none of these metabolites were detected in nutmeat.

The major metabolites in nutmeat, are conjugates of 1,2,4-triazole (*M31*, 48% of TRR, 0.67 mg/kg and *M30*, 24% of the TRR, 0.34 mg/kg). However, free 1,2,4-triazole was not detected in peanuts. A small portion of the radioactivity of nutmeat (3.0% of the TRR) was characterized as fatty acids in naturally occurring oil. The detection of radiolabelled fatty acids in nutmeat is assumed to be a consequence of the mineralisation of phenyl-labelled prothioconazole to $^{14}\text{CO}_2$ in the soil which is subsequently taken up by the plant and incorporated into natural products.

Four foliar spray applications of prothioconazole were made to sugar beet plants at an average rate of 288 and 289 g as/ha/application for a total rate of 1152 and 1157 g ai/ha of the phenyl- or triazole-labelled parent compound, respectively.

The TRR levels in sugar beet tops were 4.3 mg/kg and 5.2 mg/kg (expressed as mg as equivalents/kg) for the phenyl- and triazole-labelled parent compound, respectively. The TRR in sugar beet roots was 0.12 mg/kg and 0.13 mg/kg for the phenyl- and triazole-label, respectively. Identified metabolites accounted for 65% and 69% of the TRR in sugar beet tops and 60% and 61% of the TRR in the roots for the phenyl- and triazole-label, respectively. Additionally, 33% and 29% of the TRR was characterized in the tops and 32% and 33% in the roots for the phenyl- and triazole-label, respectively.

When the phenyl labelled compound was used the major metabolite identified in sugar beet tops were prothioconazole-desthio (28% of TRR, 1.2 mg/kg) and isomers of its hydroxyl-glucosides (*M21/M22/M23*), *M24* and *M59*. In the sugar beet roots only prothioconazole-desthio (58% of the TRR, 0.068mg/kg) and *M03* were identified. In addition to the parent compound, the following metabolites were identified in sugar beet tops: *M03*, *M24*, *M59* and *M60*. In the sugar beet roots only prothioconazole-desthio (25% of the TRR, 0.033 mg/kg) and *M03* was identified.

In the case of treatment with triazole labelled compound, the metabolites identified in sugar beet tops were prothioconazole-desthio (19% of the TRR, 0.99 mg/kg), and the corresponding hydroxy-glucoside isomers (*M21/M22/M23*).

Prothioconazole was extensively metabolized in sugar beets to numerous components; only a small quantity of unchanged prothioconazole was detected (5–7% of TRR from triazole and phenyl labelled studies). The major metabolite was prothioconazole-desthio arising from oxidation of the sulfur of the triazolinthione ring to form the corresponding sulfonic acid with subsequent elimination of the sulfonic acid group. Hydroxylation of the phenyl ring and/or benzylic carbon to form multiple monohydroxy isomers was observed with subsequent conjugation with glucose or further reaction to produce *M24*. The triazole moiety was released leading to triazolylalanine and triazolylhydroxypropionic acid. These metabolites may also have been formed as a result of 1,2,4-triazole uptake from the soil followed by immediate conjugation. Free 1,2,4-triazole was not detected suggesting an immediate conjugation of the released triazole. Additional triazole-label specific metabolites were formed by elimination of the chlorophenyl moiety (*M45*, *M46* and *M62*). The metabolic pathway is similar to that seen in peanuts and spring wheat conducted with phenyl- or triazole-labelled prothioconazole.

In summary, irrespective of the crop or application mode (foliar or soil), the major metabolites found in all crops were prothioconazole-desthio and, specific to the triazole-label studies, the metabolites triazolylalanine, triazolylhydroxypropionic acid and triazolylacetic acid. Based on the results of these studies it was postulated that 1,2,4-triazole (*M13*) was taken up from the soil and transformed directly in the plants to these metabolites. No free 1,2,4-triazole was detected in any matrix, either in the target plant metabolism studies or in the confined rotational crops study.

Environmental fate

Prothioconazole is a very weak acid and its water solubility is low at pH 4 and increases with increasing pH. It is readily soluble at pH 9. Its log K_{ow} increases from 2.0 at pH 9 to 4.2 at pH 4. Its vapour pressure and volatility are low.

Prothioconazole was found to be stable at pH 7 and 9 while only very low degradation was observed at pH 4. Hydrolysis is of minor importance for its degradation in the environment.

The photodegradation of prothioconazole was studied in sterile aqueous buffer solution at pH 7 and 25 °C using [phenyl-UL-¹⁴C] and [3,5-Triazole-¹⁴C]prothioconazole. Under the experimental conditions prothioconazole was completely photodegraded. Experimental half-lives were determined to be 48 h (mean of two labels). Prothioconazole-desthio was identified as main degradation product

at a maximum level of 56% of the applied radioactivity. Two further major metabolites were identified as prothioconazole-thiazocine at 15% and 1,2,4-triazole at 12%. Recovery at the latest sampling intervals ranged from 104% to 107% of the applied radioactivity.

The experimental data indicate that the solar radiation contributes to the primary degradation and elimination of prothioconazole in aquatic systems of the environment.

Aerobic degradation of prothioconazole was studied in several soils under laboratory conditions in the dark applying the test substance at about 600 g ai/ha treatment, equivalent to the maximum recommended field application rate for one growing season.

The amount of radioactivity, expressed in percent of applied radioactivity, bound to soil increased during the test period and reached a maximum and then decreased until the end of the test period. In the course of the studies, the amounts of radioactivity which could be extracted decreased. At all sampling intervals, no volatile organic compounds were found (< 0.1% of the applied radioactivity). Prothioconazole was rapidly degraded in soil under aerobic conditions to CO₂, the final degradation product. Parallel to mineralisation, bound residues were formed. The calculated DT₅₀ values of prothioconazole determined in the laboratory soil degradation studies were in the range of 0.07 to 1.3 days. The DT₅₀ values of the two major metabolites prothioconazole-S-methyl and prothioconazole-desthio determined in the laboratory trials were in the range of 5.9 to 46 days and 7.0 to 34 days, respectively.

A total of eight metabolites were identified or characterized in the soil extracts along with the parent compound and ¹⁴CO₂. The major metabolites (> 10% of the applied radioactivity) were *M01* and prothioconazole-desthio, which were both degradable under aerobic conditions and thoroughly metabolized to carbon dioxide. Prothioconazole -sulfonic acid, *M03*, *M13*, *M14*, *M15*, *M16*, *M17* and *M20* were found as minor metabolites.

Eight field trials were conducted at different sites in northern and southern Europe. The DT₅₀ values for prothioconazole ranged from 1.3 to 2.8 days (mean: 1.7 days). The corresponding DT₉₀ values were in the range of 4.4 to 9.3 days (mean: 5.8 days). The dissipation times for prothioconazole-desthio ranged from 16 to 72 days (mean: 42 days), the corresponding DT₉₀ values ranged from 54 to 240 days (mean: 140 days). Prothioconazole-S-methyl concentrations never exceeded the LOQ of 6 µg/kg, corresponding to less than 3% of the initial concentration of the active substance. No residues of prothioconazole or its metabolites were detected at a depth below 10 cm in the soil, with the exception of the day 89 in one trial, where residues of prothioconazole-desthio were detected between the LOD and the LOQ in the 10–20 cm layer.

Crop rotation studies

Two confined rotational crop studies were conducted in wheat, Swiss chard and turnips using phenyl- and triazole-labelled parent compound. The phenyl-labelled prothioconazole was applied once at a rate of 578 g as/ha, while four applications were made to the soil with the triazole-labelled prothioconazole at an average rate of 204 g as/ha/application. The triazole labelled compound gave higher residue concentrations ranging from 0.25 to 0.57 mg/kg in wheat forage, from 2.0 to 2.6 mg/kg in wheat hay, 1.4 to 1.7 mg/kg in wheat straw and from 3.8 to 5.9 mg/kg in wheat grain. The highest residues were observed either in the 2nd or 3rd rotations except wheat straw showing the highest residue in 1st rotation.

In the study using the triazole-labelled prothioconazole the major metabolites found in all matrices were triazolylalanine, triazolylhydroxypropionic acid and triazolylacetic acid. No free 1,2,4-triazole was detected in any matrix. Minor metabolites detected in most matrices were prothioconazole-desthio (except wheat grain), *M18*, *M45*, and *M46*. The concentrations of minor metabolites common to both labels were lower than both identification triggers (<< 10% of TRR and < 0.05 mg/kg).

The parent compound was present if detected at all at < 0.005 mg/kg, prothioconazole-desthio was detected in all parts of the wheat plants and amounted to 0.045 mg/kg in wheat straw in the 1st rotation. Conjugation played an important role in the degradation of prothioconazole.

Field rotational crop trials were conducted at three locations in the USA (Georgia, Indiana and Kansas) to measure the magnitude of prothioconazole residues in field crops at 1-, 4-, 8-, and 12-month plant-back intervals (PBIs) following the use of the 480 SC formulation on a target crop.

Each trial contained a control and a treated plot. Two foliar spray applications with the 480 SC were made 14 (\pm 2) days apart to bare soil in the treated plot. The total application rate was about double (800 g/ha) that of the highest label rate for prothioconazole in USA. The total prothioconazole derived residue was less than the LOQ of 0.05 mg/kg (0.02 mg/kg for grain only) in all crop RACs at the 1-month PBI. No further analyses were conducted.

It can therefore be stated that no residues above respective LOQs of 0.02 and 0.05 mg/kg would be expected in rotational crops, for human consumption, following the use of prothioconazole at maximum application rates.

Methods of analysis

The meeting received a number of validated analytical methods for the determination of residues in plant, animal tissue, milk and soils.

The residue components detected and the basic principles of the methods are summarized below.

Prothioconazole-desthio was determined with the extended DFG method S 19 in combination with GPC cleanup and GC/MS detection. The LOQs were for plant commodities were 0.02 mg/kg (tomato, orange, wheat grain, and rape seed), 0.05 mg/kg (wheat forage and straw), and for commodities of animal origin 0.01 mg/kg (milk) and 0.02 mg/kg (meat, egg and fat).

Parent prothioconazole, prothioconazole-desthio and prothioconazole sulfonic acid were extracted from plant materials with a mixture of methanol (MeOH), 30% hydrogen peroxide (H₂O₂), and aqueous sodium bicarbonate (NaHCO₃) at 65 °C. This extraction procedure converts prothioconazole to a mixture of prothioconazole-sulfonic acid and prothioconazole-desthio. The unchanged prothioconazole-desthio residues are extracted directly. After cleaning the extracts the residues were determined with HPLC/MS/MS. The method was validated in barley hay, straw, and grain, canola grain, mustard greens, peanut hay and nutmeat, rice straw and grain, turnip tops and roots, wheat forage, hay, straw, and grain, and wheat bran, flour, germ, middlings and shorts. The LOQ ranged between 0.02 and 0.05 mg/kg depending on the sample matrix. The method requires specific instrument setup, and stable isotopes as internal standards consequently it is not readily applicable for enforcement purposes.

Other methods are also available for the prothioconazole sulfonic acid and prothioconazole-desthio, or prothioconazole and prothioconazole-desthio in various plant matrices with LOQs ranging from 0.01 to 0.05 mg/kg depending on the sample material.

Residues of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio and prothioconazole-4-hydroxy-desthio, and conjugates that can be converted to one of these compounds via acid hydrolysis in matrices of animal origin can be determined by HPLC-MS/MS (Method 00655 plus modifications). Homogenized sample materials of meat, liver and kidney were extracted twice with a mixture of acetonitrile/water, and centrifuged, each. The supernatant was evaporated to the aqueous remainder, then diluted with water, acidified and refluxed for 2 h. Milk samples were hydrolysed and purified directly after dilution with water. This hydrolysis step was performed to convert non-aromatic precursor compounds and glucuronic acid bound analogues into prothioconazole-desthio-3-hydroxy and prothioconazole-desthio-4-hydroxy. Quantification was carried out with HPLC-MS/MS. The LOQ for milk was 0.004 mg/kg and for meat, liver, kidney and fat 0.01 mg/kg.

Comparison of chromatographic profiles before and after hydrolysis clearly shows that groups of minor unidentified metabolites have disappeared. In their place the compounds with a common moiety, i.e., prothioconazole-desthio, *M14*, *M15*, *M33* and *M35* are emerging or increasing. Since the major part of the radioactivity (58–84%) is recovered, and the proportion of identified/characterized residues is higher after acid hydrolysis than without hydrolysis in milk, kidney and muscle and similar in liver and fat, it can be concluded that a significant proportion of the unidentified compounds are converted to the common moiety products.

Prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy residues in various bovine matrices can also be determined by LC-MS/MS (Method JA006-A04-02). This method is based on extraction with acetonitrile/water containing 250 mg/mL L-cysteine HCl (4:1 v/v), and hydrolysis of the extracts with 5N HCl under reflux for 2 hours. Prothioconazole, prothioconazole-desthio, prothioconazole-4-hydroxy are quantified individually. The LOQ for each of the three analytes are: 0.004 mg/kg for milk; 0.010 mg/kg for skim milk, cream, and muscle; 0.010 mg/kg for liver; 0.010 mg/kg for kidney; and 0.050 mg/kg for fat.

Stability of pesticide residues in stored analytical samples

Freezer storage stability of metabolite prothioconazole-desthio, the main residue component in plants was examined in wheat, canola (seed, pod, straw), spinach (leaves), sugar beet (root, leaf with root collar), tomato (fruit), and field pea (field pea dried) at about minus 18 °C or below. The results demonstrate that, under freezer conditions, residues of prothioconazole-desthio were stable over a storage period of up to 36 months in wheat and at least 24 months for the other crops.

The storage stability periods are longer than the longest period of time for which samples from European field residue trials presented in this dossier were stored prior to analysis (cereals and oil seed rape).

The prothioconazole and prothioconazole-desthio residues were found to be stable (< 30% decomposition) in wheat hay, wheat straw, canola seeds, mustard greens, turnip root and tomato fruit during 36–42 months of freezer storage.

Residue definition

Prothioconazole is extensively metabolized and the most important pathways for metabolism are common to wheat, peanut and sugar beet. The nature of the residue found in wheat after foliar spray application, seed treatment and as a rotational crop was similar.

The majority of the metabolites are simply multiple structural isomers of monohydroxylated prothioconazole-desthio (*M14-M17*) and their conjugates [glucosides (*M21-M23*) and malonyl-glucosides (*M54-M56*)], and prothioconazole-dihydroxy-olefin and its conjugates. Oxidative hydroxylation led to isomers of prothioconazole-dihydroxy-diene and their conjugates. Although the sum of these compounds and their conjugates were as high as 42% of the TRR in an individual crop matrix, these conjugated and/or hydroxylated metabolites represented individually < 10% of the TRR in the plant matrices.

The proportion of parent prothioconazole was low in wheat grain, wheat forage, wheat hay, straw, peanut hay, peanut nutmeat, sugar beet tops, sugar beet roots, sugar beet forage and in rotational crops if detected at all.

Irrespective of the crop or application mode, the major metabolites found in all crops were prothioconazole-desthio and triazolylalanine, triazolyl-hydroxy-propionic acid and triazolylacetic acid. The major plant metabolite, prothioconazole-desthio was slowly metabolized in wheat. It was the dominating constituent of the residue in forage and straw at harvest. However, it was only detected in small amounts in grain where the residue was mainly made up by triazolylacetic acid and triazolylalanine. No free 1,2,4-triazole was detected in any matrix either in the target plant metabolism studies or in the confined rotational crops study.

The metabolic profiles for milk and the edible tissues and organs showed that parent prothioconazole was a major compound in all tissues and organs but only of minor importance in milk. Compounds detected in all matrices in the study with phenyl-labelled prothioconazole-desthio were prothioconazole-desthio (except for milk), *M32*, *M74*, *M52*, and sulfate conjugates of prothioconazole-desthio-hydroxy, prothioconazole-desthio-dihydroxy, prothioconazole-desthio-hydroxy-methoxy in laying hens.

Following the administration of triazole labelled prothioconazole the only label specific metabolite identified was thiocyanate. Triazole derivatives or free 1,2,4-triazole were not found at concentrations above 0.01 mg/kg in any goat matrix. Free triazole did not exceed a residue level of 0.04 mg/kg in laying hen matrices.

The most abundant metabolite was prothioconazole-S-glucuronide. Prothioconazole-desthio was also present in all sample materials, but in much lower concentrations than prothioconazole-S-glucuronide. An exception was fat in hen and goat and eggs in hen, in which prothioconazole-desthio was predominant. In eggs and all edible tissues of hen metabolite prothioconazole-S-methyl was additionally identified. Animal feeding studies showed that the residues are not concentrated in fat of meat or milk cream. As the total residue composed of several hydroxy derivatives and their conjugates, the Meeting concluded that the residues of prothioconazole are not fat soluble.

There are analytical procedures for the determination of prothioconazole residues in various combinations. A GC/MS multi residue method has been validated for the determination of prothioconazole-desthio. An LC-MS/MS total residue method converts prothioconazole, its metabolites and their conjugates to a mixture of prothioconazole sulfonic acid and prothioconazole-desthio. Another method is suitable for the determination of prothioconazole-desthio, prothioconazole-3-hydroxy-desthio and prothioconazole-4-hydroxy-desthio and conjugates that can be converted to one of these compounds via acid hydrolysis in/on matrices of animal origin by HPLC-MS/MS. The major part of the TRR (58–84%) is recovered with this method.

Supervised trials indicated that residues measured as the sum of prothioconazole sulfonic acid and prothioconazole-desthio were higher than the prothioconazole-desthio alone.

The Meeting noted that 1,2,4-triazole, triazolyl-acetic acid and triazolyl-alanine may be derived from several sources. Field trials performed in USA indicated that the sum of the conjugates of triazolyl-alanine and triazolyl-acetic acid amounted to a maximum of 0.92 mg/kg and 1.76 mg/kg in barley and wheat grain, 0.66 mg/kg in canola seed and 3.39 in peanut meat. However, free 1,2,4 triazole was not detected in any of the samples above the LOQ. These findings agree with the information obtained from metabolism studies. As these compounds may be present in food commodities from different sources they are not suitable for enforcement purposes. The relatively low level of conjugated residues in food commodities and the low toxicity of triazolyl-acetic acid and triazolyl-alanine (max ADI of 1 mg/kg) do not justify their inclusion for dietary risk assessment.

Taking into consideration the toxicological significance of the metabolites, the major residues in plant (including the fact that animal feed commodities are almost free of the parent prothioconazole) and animal commodities and the practicality of enforcing the residue limits, the Meeting recommends the following residue definition: for both enforcement and dietary risk assessment:

for plant commodities for enforcement and dietary risk assessment: *prothioconazole-desthio*,

for animal commodities for enforcement: *prothioconazole-desthio*; and for dietary risk assessment: *the sum of prothioconazole-desthio, prothioconazole-desthio-3-hydroxy, prothioconazole-desthio-4-hydroxy and their conjugates expressed as prothioconazole-desthio*.

Results of supervised residue trials on crops

The meeting received supervised trial data for prothioconazole uses on barley, wheat, triticale (one seed dressing trial), dried beans and peas, oil seed rape and canola, sugar beet, soya bean and peanut.

Prothioconazole was applied as a foliar spray using an EC (emulsifiable concentrate), SC (suspension concentrate) formulation, and for seed dressing of cereals using a FS (flowable concentrate) formulation. The both use patterns are permit for use on cereals.

The trials were performed in Brazil, Canada, Europe and USA. Labels and English translations, where necessary, were provided from 20 countries.

The trials were performed in compliance with GLP and good documentation was provided.

In the Brazilian and European trials the main plant metabolite prothioconazole-desthio was determined. In the Canadian and US trials the residues of parent prothioconazole and its metabolites were converted to a mixture of prothioconazole-desthio and prothioconazole-sulfonic acid (both expressed as parent molar equivalents) and summed with unchanged prothioconazole-desthio (expressed as parent molar equivalents) to give a total prothioconazole derived residue. The methods applied were validated by recovery experiments prior to and concurrent with the residue analyses. The performance of the methods concurred with the current quality requirements.

In trials from Brazil, Canada and USA only the total residue was reported, though the individual residue components were measured separately. Thus the presentation of the results did not comply with the *FAO Manual* specifying that individual residue data should be reported separately (Analysis of samples, page 25) and could not be used for estimation of residue levels.

Pulses (dried beans and peas)

Supervised trials on dried peas (13) and dried beans (10) were carried out with 3 foliar applications of a SC 480 formulation at a target rate of 200 g/ha/application in Canada (9) and USA (14) corresponding to US GAP.

Only total residues were reported.

Sugar beet

The Meeting received reports of 12 residue trials on sugar beets from the USA complying with the US GAP.

Only total residues were reported.

Cereal grains

A total of 123 trials were carried out on cereals (wheat, triticale and barley) with the SC 480, EC250 and FS200 formulations in Canada, Europe and the USA. Only total residues were reported from the USA and Canadian trials.

In Germany, Ireland, and the UK one seed dressing and up to 3 foliar applications for wheat and rye, and a maximum of 2 applications for barley and oat can be made at a rate of 200 g ai/ha. The PHI is 35 days in Germany, and the last treatment should be made at growing stage of BBCH 69–71 in the UK.

The maximum registered application rates for barley, oat, triticale and wheat seed treatments are 100 mg ai/kg of seed in the UK, 75 mg/kg in France and 25–50 mg/kg in Germany. The registered rates in other European countries are within this range or lower in few cases.

A total of 17 trials on barley were reported from Germany, France, Italy, Spain, Sweden and the UK, where three applications were made at each site: one seed dressing with a nominal rate of 150 mg ai/kg seed and two foliar applications with 200 g ai/ha. The seed dressing rate was 1.5 times higher than the recommended label rate.

The prothioconazole-desthio residues in barley grain at 35–57 days, matching the PHI or growing stage specified on the label, were: < 0.01 (10), 0.01, 0.01, 0.02 (4) mg/kg.

In 19 European trials on wheat, the application rate for seed dressing was approximately double the GAP rate and 3 foliar applications were made instead of two in South European trials. Nevertheless, no prothioconazole-desthio residue could be detected (< 0.01 mg/kg) in any of the 16 grain samples taken at 35 days post application, or 16 samples taken between 42–64 days after last application, except in one trial conducted in the UK where 0.32 mg/kg residue was found.

Taking into account the total residues derived from similar application rates (0.6 mg/kg in barley and 0.061 mg/kg in wheat) the Meeting concluded that the 0.32 mg/kg residue value was atypical and was not considered.

Based on the similar residue data on barley and wheat available from European trials and the similar use patterns for cereals, the Meeting estimated an STMR value of 0.01 mg/kg and a maximum residue level of 0.05 mg/kg for barley, oat, rye, triticale and wheat.

Oils seeds

A total of 34 trials on rape/canola were carried out with either an EC250 or SC 480 formulations. The trials were performed in Canada (16), France (7), Germany (2), the UK (2), Sweden (1) and the USA (6).

In the 22 Canadian and USA trials only the total residue was reported.

In France, Germany, the UK and several other countries in Europe the application rate is within 125–175 g/ha and the PHI ranges between 35 and 56 days.

The European trials were performed with 2 applications at 175 g ai/ha nominal rate and samples were collected 56 to 67 days after the second treatment, which correspond to the GAP of the UK.

The prothioconazole-desthio residues derived from trials evaluated against the UK GAP were: < 0.01 (7), 0.01 (3) and 0.02 mg/kg.

The Meeting estimated a maximum residue level of 0.05 mg/kg and STMR of 0.01 mg/kg for rape seed.

Peanut

The GAP in the USA permits 4 applications at a rate of 200 g ai/ha (800 g ai/ha/season) at 14 days intervals and 14-day PHI. The hay and by-products cannot be fed to animals.

In 12 trials from the USA, performed according to GAP, the total residues in nutmeat were below the LOQ (< 0.02 mg/kg) in all samples.

The Meeting noted that the metabolism studies indicated very low residues in nutmeat and no total residue was detected in any of the samples, and decided to use the total residue data for estimating a maximum residue level of 0.02* mg/kg and an STMR of 0.01 mg/kg.

Soya bean

The GAP of the USA specifies 3 applications at a maximum rate of 105 g/ha and 21 days PHI. No information was available on permitted Brazilian uses. Nineteen US trials did not comply with GAP, and only the total residue was reported.

Primary feed commodities

The basic information on registered uses is provided under food commodities. Only the relevant residue data are summarized below.

Total residues in soya bean forage and hay and sugar beet tops derived from supervised trials were recorded, but not evaluated by the Meeting. The total residues in peanut hay were not considered as it is not allowed to be used as an animal feed in the USA where the trials were conducted.

Cereal forage and straw

The Meeting noted that forage samples were taken up to 28 days after last application. However, several countries labels do not contain any restriction on grazing. As the 7-day sampling interval was considered the shortest under practical conditions, residues measured at 7 days were used for estimation of animal dietary burdens.

In North European trials the prothioconazole-desthio residues in wheat forage at 7 days post-application were: 0.11, 0.32, 0.57, 0.65, 0.78, 0.89, 0.92, 1.0, 1.1, and 1.8 mg/kg.

In barley forage at the 7 day sampling the residues were: 0.6, 0.85, 1.0, 1.2, 1.7(2), 2.0 and 2.6 mg/kg.

The Meeting noted that the residues in barley and wheat forage were in the same range, and based on the combined data (0.11, 0.32, 0.57, 0.60, 0.65, 0.78, 0.85, 0.89, 0.92, 1.0, 1.0, 1.1, 1.2, 1.7, 1.7, 1.8, 2 and 2.6 mg/kg) estimated a STMR of 0.96 mg/kg and a highest residue of 2.6 mg/kg for barley, oat, rye, triticale and wheat forage.

In wheat straw (between 35 and 64 days after last treatment) the residues were: 0.08, 0.09, 0.11, 0.14, 0.15, 0.19, 0.20, 0.25, 0.27, 0.31, 0.42, 0.47, 0.52, 0.53, 0.72 (3), 0.77 and 1.0, mg/kg.

In barley straw (between 35–57 days) the residues were: 0.08, 0.1, 0.1, 0.13, 0.13, 0.14, 0.14, 0.16, 0.19, 0.24, 0.3, 0.38, 0.53, 0.75, 1.1, 1.1 and 1.2 mg/kg.

The Meeting considered the barley and wheat straw residue data were from the same population and based on the combined residue data (0.08, 0.08, 0.09, 0.1, 0.1, 0.11, 0.13, 0.13, 0.14, 0.14, 0.14, 0.15, 0.16, 0.19, 0.19, 0.20, 0.24, 0.25, 0.27, 0.3, 0.31, 0.38, 0.42, 0.47, 0.52, 0.53, 0.53, 0.72(3), 0.75, 0.77, 1.0, 1.1, 1.1, and 1.2 mg/kg) estimated on dry weight basis a STMR of 0.30 mg/kg (median value of 0.26 uncorrected for moisture content) and highest residue of 1.36 and a maximum residue level of 2 mg/kg, for barley, oat, ray, triticale and wheat straw.

Rape forage

Samples of green forage were only taken at day 0. The residues present in day 0 samples do not represent the practical situation and cannot be used for estimation of animal burden.

Fate of residues during processing

A hydrolysis study with [Phenyl-UL-¹⁴C]prothioconazole in buffered drinking water was conducted under conditions representative for core processing procedures in order to determine their possible influence on the nature of the residues. The samples were incubated at 90 °C at pH 4 for 20 minutes (pasteurisation), 100 °C at pH 5 for 60 minutes (baking, brewing and boiling) and 120 °C at pH 6 for 20 minutes (sterilisation).

HPLC analyses of incubated samples demonstrated that prothioconazole degraded slightly ($\leq 11\%$) to prothioconazole-desthio at 120 °C at pH 6.

A field trial was conducted to measure the magnitude of prothioconazole residues in/on wheat grain, aspirated grain fractions, bran, flour, germ, middling, and shorts following two foliar spray applications of prothioconazole 480 SC to wheat at five-fold exaggeration of the maximum recommended label use rate.

Mature wheat grain was harvested 47 days after the last treatment, and processed with a procedure which simulated commercial processing practices.

The residues of prothioconazole and prothioconazole-desthio were measured as prothioconazole-desthio and prothioconazole sulfonic acid. The individual residues were summed to give a total prothioconazole derived residue. The LOQ for total residue was 0.02 mg/kg for wheat grain, bran, flour, germ, middling, and shorts, and 0.25 mg/kg for aspirated grain fractions.

The total residues of prothioconazole in grain at harvest were 0.05 mg/kg. The corresponding residues in aspirated grain fraction were 12.5 mg/kg.

The residues in the processed products were up to 0.12 mg/kg in bran, < 0.02 mg/kg in flour, 0.10 mg/kg in germs, 0.03 mg/kg in middling and 0.05 mg/kg in shorts. No control interferences were detected. The calculated processing factors were 250 for aspirated grain fraction, 2.4 for wheat bran, 0.4 for flour, 2 for germ, 0.6 for middling and 1 for shorts.

A field trial was conducted to measure the magnitude of prothioconazole residues in canola seed, canola meal and canola refined oil following two foliar spray applications with 480 SC at 1.0 kg ai/ha, which corresponded to a five-fold (5×) exaggeration of the maximum recommended label use rate. Mature canola plants were cut 47 days after the second treatment, dried on the field for 5 days, then processed using procedures which simulated commercial processing practices.

The residues of prothioconazole and prothioconazole-desthio were measured as prothioconazole-desthio and prothioconazole sulfonic acid. The LOQ for total prothioconazole residue was 0.02 mg/kg for canola seed, meal and refined oil. The results indicated that no concentration (<0.7×) of the total prothioconazole derived residue was seen in canola meal and refined oil.

Field trials were conducted to measure the magnitude of prothioconazole residues in peanut nutmeats, peanut meal, peanut refined oil, dry roasted peanuts and peanut butter as well as in soya bean processed commodities of meal, hulls and refined oil. The total amount of prothioconazole 480 SC applied represented a five-fold (5×) exaggeration of the maximum recommended label use rate. The processed fractions were analysed for total residues. The total prothioconazole derived residue did not concentrate (<1×) either in the peanut refined oil, dry roasted peanuts and peanut butter, or in soya bean meal and refined oil.

Farm animal feeding studies

The cattle feeding studies were conducted administering prothioconazole-desthio or the parent compound via capsules to lactating dairy cows at three dose levels for 28 or 29 consecutive days. At the end of the dosing period, the cows were sacrificed within 24 h after the last capsule treatment. The liver, kidney, fat (composite omental and perirenal), and muscle (composite of loin, elbow and flank) were collected for analysis.

Milk was collected for analysis twice daily at regular intervals during the dosing period and composited for each cow. In addition, a portion of the morning milk from one cow of the highest dose level was subjected to an accumulation test in milk fat on the day before sacrifice.

Following the administration of prothioconazole-desthio at rates of 4 mg/kg feed, 25 mg/kg feed, or 100 mg/kg feed the samples were analysed for prothioconazole-3-hydroxy-desthio, prothioconazole-4-hydroxy-desthio, and prothioconazole-desthio. The LOQ were 0.01 mg/kg for muscle, liver, kidney and fat, and 0.004 mg/kg for milk.

Prothioconazole-desthio total residues, expressed as mg/kg prothioconazole-desthio equivalents, were observed in liver and kidney at all feeding levels with a linear dose relationship.

- In liver total residues ranged from 0.02 to 0.05 mg/kg at the 4 mg/kg feeding level, from 0.18 to 0.26 mg/kg at the 25 mg/kg feeding level, and from 0.61 to 1.6 mg/kg at the 100 mg/kg feeding level.

- In kidney total residues ranged from 0.01 to 0.04 mg/kg at the 4 mg/kg feeding level, from 0.11 to 0.17 mg/kg at the 25 mg/kg feeding level, and from 0.41 to 1.1 mg/kg at the 100 mg/kg feeding level. In muscle and fat, total residues were considerably lower.
- In muscle, total residues were below the LOQ (0.01 mg/kg) at the 4 mg/kg and 25 mg/kg feeding levels, and ranged from 0.01 to 0.03 mg/kg at the 100 mg/kg feeding level.
- In fat, total residues were below the LOQ (0.01 mg/kg) at the 4 mg/kg feeding level, and ranged from 0.01 to 0.02 mg/kg at the 25 mg/kg feeding level, and from 0.03 to 0.14 mg/kg at the 100 mg/kg feeding level.
- from a single population or the equivalent of a single population;

Prothioconazole-desthio total residues in milk at the highest dose level increased from < 0.004 mg/kg (day 1) to a plateau level (day 4 to day 29) of 0.006 to 0.010 mg/kg for two animals and of 0.013 to 0.021 mg/kg for one animal, while no residue could be detected at lower dose levels. Liquid-liquid-partitioning of whole milk against n-hexane showed that prothioconazole-desthio was in milk fat and the 3-hydroxy and 4-hydroxy metabolites (M14 and M15) remained in the aqueous phase. However, the total residues remained preferentially in the aqueous phase, i.e., 0.015 mg/kg with only 0.004 mg/kg in the n-hexane phase, indicating no accumulation in milk fat.

When cows were dosed with the parent compound at levels of 9.9 mg/kg feed, 29.5 mg/kg feed, and 98.4 mg/kg feed, the samples were analysed for prothioconazole, prothioconazole-desthio and prothioconazole-4-hydroxy. The LOQ for the total residue was 0.005 mg/kg in milk; 0.01 mg/kg in skim milk, milk cream, liver, kidney, and muscle; and 0.05 mg/kg in fat.

The total average prothioconazole-desthio residues (prothioconazole, prothioconazole-desthio and prothioconazole-4-hydroxy) at the dose groups of 9.9 mg/kg feed, 29.5 mg/kg feed, and 98.4 mg/kg feed were respectively: < 0.05 mg/kg in fat, 0.07, 0.21, and 0.80 mg/kg in kidney, 0.10, 0.28 and 0.8 mg/kg in liver, and < 0.01, 0.01 mg/kg in muscle.

The highest total prothioconazole residue in the milk from the highest (5×) dose group was equal to or less than 0.006 mg/kg. All milk samples from the 29.5 mg/kg (1.5×) dose group contained < 0.005 mg/kg (< LOQ) total prothioconazole residue. Minimal concentration (1.1× concentration) of prothioconazole residues occurred in cream and no concentration (<1× concentration) occurred in skim milk.

Poultry

A summary of a feeding study with laying hens was provided. In this study three groups of laying hens were dosed via capsule for 29 consecutive days with 0.26, 0.79, and 2.6 mg/kg feed/day. Following the administration of the highest dose, the total prothioconazole residues (sum of prothioconazole-desthio, prothioconazole-4-hydroxy and prothioconazole) were below the LOQ in eggs (< 0.005 mg/kg) and liver, muscle and fat (< 0.01 mg/kg) samples.

Farm animal dietary burden

The Meeting noted that the feeding study conducted with parent prothioconazole does not represent the practical residue situations where the feed items contained only low levels (< 5%) of the parent compound while the major part of the residue was the prothioconazole-desthio. Consequently, the dietary burden was calculated from the prothioconazole-desthio residues measured in feed commodities and it was compared to the residues found in animal commodities after the administration of prothioconazole-desthio.

The Meeting estimated the dietary burden in farm animals on the basis of the diets listed in Annex 6 of the 2006 JMPR Report (OECD Feedstuffs Derived from Field Crops). Calculation from highest residue, STMR for some bulk commodities and STMR-P values provides the levels in feed

suitable for estimating MRLs, while calculation from STMR and STMR-P values for feed is suitable for estimating STMR values for animal commodities.

Estimated maximum and mean dietary burdens of farm animals

Dietary burden calculations for beef cattle, dairy cattle are provided in Annex 6.

		Animal dietary burden, total prothioconazole ppm of dry matter diet		
		US-Canada	EU	Australia
Beef cattle	max	2.61	2.61	10.40^a
	mean	0.96	0.96	3.84^b
Dairy cattle	max	4.17	2.60	7.80^c
	mean	1.55	0.96	2.75

^a Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat.

^b Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat.

^c Highest dairy cattle dietary burden suitable for STMR estimates for mammalian milk.

Dietary burden calculations for poultry

		Animal dietary burden, total prothioconazole ppm of dry matter diet		
		US-Canada	EU	Australia
Broiler chicken	max	0.009	0.008	0.008
	mean	0.009	0.008	0.008
Laying	max	0.009	1.050 ^a	0.006
	mean	0.009	0.39 ^b	0.006

^a Highest maximum dietary burden suitable for MRL estimates for poultry meat and eggs.

^b Highest mean dietary burden suitable for STMR estimates for poultry meat and eggs.

Animal commodity maximum residue levels

Based on the linear relationship observed for the maximum residues in various tissues of cattle and the results of the metabolism studies, the expected maximum prothioconazole-residue derived from feeding 10.4 mg/kg feed were: 0.095 mg/kg in liver, 0.065 mg/kg in kidney, 0.009 mg/kg in meat, and 0.01 mg/kg in fat.

In milk the highest dose resulted in a maximum of 0.02 mg/kg residue, and no residue (< 0.004 mg/kg) could be detected at lower dose levels. Consequently, no residue is expected in milk where the feed contains residues up to 7.8 mg/kg.

The STMR residues estimated from the 3.84 mg/kg median residue intake are: < 0.01 in liver and kidney, meat and fat.

The Meeting estimated maximum residue levels of 0.2 mg/kg for edible offal, 0.01 mg/kg in meat and fat and 0.004* mg/kg in milk. The STMR and HR values are 0.01 mg/kg for meat, 0.004 mg/kg for milk and edible offal the values are 0.05 and 0.1 respectively.

A metabolism study was carried out on laying hens with the parent prothioconazole at an exaggerated rate of 171 mg/kg feed indicated a total residue of 4 mg/kg in liver. Assuming a proportional residue level the 1.05 mg/kg residue in feed would result in 0.025 mg/kg residue in the liver.

A feeding study performed with parent prothioconazole at maximum rate of 2.59 mg/kg feed showed that the total residue would be below the LOQ in eggs (0.005 mg/kg), liver, meat and fat

(0.01 mg/kg). The Meeting also noted that the study designs do not reflect the residue composition in feed and the results cannot be used for estimation of maximum residue levels or STMR values.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Daily Intake (IEDI) for prothioconazole-desthio was calculated from the recommendations for STMR-s for raw agricultural commodities in combination with consumption data for corresponding food commodities. The results are shown in Annex 3.

The IEDI of the 13 GEMS/Food cluster diets were in the range of 0-1% of the maximum ADI of 0.01 mg/kg bw. The Meeting concluded that the long-term intake of residues from uses of prothioconazole considered by the Meeting is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-term Intake (IESTI) for prothioconazole-desthio was calculated from the recommendations for STMRS and HRs for raw agricultural commodities in combination with consumption data for corresponding food commodities. The results are shown in Annex 4.

The IESTI for women of child bearing age is 0-6% of the ARfD of 0.01 mg/kg bw. The IESTI for children and general populations is 0% of the ARfD of 1 mg/kg bw.

The Meeting concluded that the short-term intake of residues from uses of prothioconazole considered by the Meeting is unlikely to present a public health concern.