5.16 ISOPYRAZAM (249)

TOXICOLOGY

Isopyrazam is the provisional International Organization for Standardization (ISO)–approved name for a mixture of two *syn*-isomers of 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*RS*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and two *anti*-isomers of 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*SR*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide (Chemical Abstracts Service No. 881685-58-1). It is a new broad-spectrum fungicide of the ortho-substituted phenyl amides, acting by inhibition of succinate dehydrogenase. It has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present Meeting at the request of the Forty-second Session of the Codex Committee on Pesticide Residues.

All the pivotal studies met the requirements of the relevant Organisation for Economic Co-operation and Development or national test guidelines and were certified as complying with good laboratory practice.

Biochemical aspects

Isopyrazam administered to rats at 1 or 75 mg/kg body weight (bw) was rapidly absorbed, with peak levels of radiolabel occurring in plasma within 3–6 hours post-dosing. Approximately 70% of the dose was absorbed at 1 and 75 mg/kg bw. The terminal half-life of the labelled material was 5–9 hours. No saturation of absorption was observed, but the area under the curve increased disproportionately with dose, indicating saturation of elimination at higher doses. In the low-dose group, 91–97% of the absorbed dose was excreted within 48 hours after administration. Highest residues were identified in the liver, kidney, thyroid and adrenals. The major route of excretion was by bile, accounting for approximately 65–90% of the absorbed dose, with the rest being excreted in urine within 48 hours. After repeated dosing, no accumulation of radioactivity in rats was observed. There were no significant differences between the toxicokinetic parameters of the two diastereoisomers *syn* and *anti*. The predominant metabolic pathway for isopyrazam and its *N*-demethylated metabolite is hydroxylation in the bicyclic-isopropyl moiety, followed by further oxidation to form the carboxylic acid or to give rise to multiple hydroxyl moieties with subsequent formation of glucuronic acid or sulfate conjugates. The structure of isopyrazam provides the potential for stereoisomerization of most metabolites.

Toxicological data

Isopyrazam technical with a *syn*:*anti* ratio of up to 69.7:30.3 is of low acute oral toxicity. The oral median lethal dose (LD_{50}) was greater than 2000 mg/kg bw in female rats. However, as the oral LD_{50} of pure *anti* isomer or of a 50:50 *syn*:*anti* batch was 310.2 mg/kg bw in female rats, there seems to be an isomeric difference in toxicity at very high dose levels. By dermal application, the LD_{50} of a 92.8:7.2 *syn*:*anti* batch was greater than 5000 mg/kg bw, and the median lethal concentration (LC_{50}) in an inhalation study was greater than 5.28 mg/L. Isopyrazam was not irritating to the skin and, initially, only slightly irritating to the eye. Isopyrazam showed skin sensitizing potential in a mouse local lymph node assay.

In repeated-dose studies in mice, rats and dogs, the main effects were changes in clinical chemistry (plasma protein, cholesterol, triglycerides, liver enzymes) and haematological parameters (red blood cell counts, haemoglobin, haematocrit), effects on the liver (hepatocellular hypertrophy) and body weight changes.

In a 13-week mouse feeding study with dietary concentrations up to 7000 ppm, reduced body weight gain in spite of higher feed consumption was observed. Red blood cell parameters were

reduced and platelet counts were increased, particularly at the high dose. In females, a few clinical chemistry parameters were elevated. Relative liver weights were increased, and hepatocellular hypertrophy was observed. For one male and one female high-dose animal, necrotic liver nodules were noted. The no-observed-adverse-effect level (NOAEL) was 2500 ppm (equal to 390.8 mg/kg bw per day), based on haematological changes at 7000 ppm (equal to 1328.8 mg/kg bw per day).

In a study to compare the toxicity of the *syn* and *anti* conformation isomers of isopyrazam, pure *syn* epimer, pure *anti* epimer and 1:1 *syn*:*anti* epimer were administered to rats at up to 5000 ppm for 4 weeks in the diet. With pure *anti* and 1:1 *syn*:*anti*, feed intake and body weight gain were reduced. Haematological and clinical chemistry parameters were affected with all three compounds, but more severely with pure *anti* and 1:1 *syn*:*anti*. Liver weights were increased in females in all dosed groups and in males at 2000 ppm and above. The Meeting considered the liver findings as adaptive effects and not toxicologically relevant. All compounds in both sexes mildly elevated the total hepatic cytochrome P450 (CYP) content and mildly increased 7-ethoxyresorufin-*O*-deethylase (EROD; CYP1A) activity, but markedly increased pentoxyresorufin-*O*-deethylase (PROD; CYP2B) activity. The NOAEL for pure *syn* was 2000 ppm (equal to 176.7 mg/kg bw per day), based on reduced body weight gain and increased cholesterol levels at 5000 ppm (equal to 437.3 mg/kg bw per day). The NOAEL for pure *anti* and 1:1 *syn*:*anti* was 500 ppm (equal to 45.2 mg/kg bw per day), based on reduced body weight gain and increased cholesterol levels at 2000 ppm (equal to 176.7 mg/kg bw per day). In two further 4-week rat feeding studies with slightly different isopyrazam batches (*syn*:*anti* ratios of 89:11 and 92.8:7.2), the NOAELs were 500 ppm (equal to 46.1 mg/kg bw per day) and 300 ppm (equal to 28.1 mg/kg bw per day), based on body weight changes, clinical chemistry and haematological changes at 2000 ppm (equal to 174.9 mg/kg bw per day) and above.

In a 13-week rat feeding study with dietary concentrations of isopyrazam (92.8:7.2 *syn*:*anti*) of up to 6000 ppm, reduced feed consumption and body weight gain were noted. Relative liver weight increases were accompanied by hepatocellular hypertrophy. Relative brain weights were decreased in both sexes at the highest dose level. Triglyceride and bilirubin levels were decreased at 1500 ppm and above. The NOAEL was 300 ppm (equal to 21.3 mg/kg bw per day), based on clinical chemistry changes at 1500 ppm (equal to 106.3 mg/kg bw per day). In a comparative 13-week rat feeding study with dietary concentrations of two batches of isopyrazam (*syn*:*anti* ratios 92.8:7.2 and 69.7:30.3) up to 2000 ppm, the NOAEL for both compounds was 250 ppm (equal to 20.5 mg/kg bw per day), based on body weight effects and hepatocellular hypertrophy and vacuolation at 2000 ppm (equal to 161.0 mg/kg bw per day).

In dogs, two 13-week gelatine capsule gavage studies were performed with two batches of isopyrazam (*syn*:*anti* ratios 92.8:7.2 and 69.7:30.3). In the first study with isopyrazam *syn*:*anti* 92.8:7.2, several behavioural changes, reduced feed consumption and body weight gain, changes in clinical chemistry parameters and increases in liver weights were noted. The NOAEL in this study was 30 mg/kg bw per day, based on behavioural changes and liver weight increases at 100 mg/kg bw per day. In the second study with isopyrazam *syn*:*anti* 69.7:30.3, the NOAEL was 30 mg/kg bw per day, based on clinical observations and initial body weight loss at 250 mg/kg bw per day. In a 52-week gelatine capsule gavage study with isopyrazam (*syn*:*anti* 92.8:7.2), no clinical signs were observed. Initially reduced feed consumption and lower body weights were noted throughout the study. Some clinical chemistry parameters showed occasional modest changes but were without any histopathological correlates or other signs of toxicity. The NOAEL in this study was 25 mg/kg bw per day, based on changes in clinical chemistry parameters and in liver weight at higher dose levels. The overall NOAEL for the effects of isopyrazam with a *syn*:*anti* ratio down to 69.7:30.3 in the 3-month and 1-year studies in dogs was 30 mg/kg bw per day.

In an 18-month feeding study in mice with dietary concentrations of isopyrazam (*syn*:*anti* 92.8:7.2) up to 3500 ppm, the incidence of males with eye discharge was elevated at 3500 ppm. Also at 3500 ppm, body weight gain was reduced in both sexes, body weight–adjusted spleen weights were decreased and liver weights were increased. At 500 ppm, the incidence of periportal hepatocellular hypertrophy in females was increased. At 3500 ppm, the incidences of epithelial eosinophilic droplets

in the nasal cavity of males and in the gall bladder of females were elevated. The incidences of benign or malignant tumours were not increased at any dose. The NOAEL was 70 ppm (equal to 9.9 mg/kg bw per day), based on periportal hepatocellular hypertrophy in females at 500 ppm (equal to 56.2 mg/kg bw per day).

Isopyrazam was not carcinogenic in mice.

In a 104-week feeding study in rats with dietary concentrations up to 3000 ppm isopyrazam (*syn*:*anti* 92.8:7.2), body weight gain was decreased in all dosed females and in high-dose males. In both sexes, haematological parameters were changed and the prothrombin time was reduced, and in females, the activated partial thromboplastin time was prolonged at 500 ppm and above. There were changes in some clinical chemistry parameters (e.g., urea, triglyceride and bilirubin levels in females) in all dose groups. At terminal kill, female brain weights at 3000 ppm were increased, and liver weights in both sexes were increased at 500 and 3000 ppm. Adrenal weights in females were decreased at 3000 ppm. The incidences of foci of eosinophilic hepatocytes were increased statistically significantly at 500 ppm and above in both sexes. The hepatocellular pigmentation in all dosed females and in high-dose males was not considered to be of toxicological relevance at the lowest dose because it was of minimal severity. The increased centrilobular hepatocellular hypertrophy observed at all dose levels in both sexes was considered to represent adaptive changes and not to be of toxicological significance. In females at 3000 ppm, there was an increase in hepatocellular adenoma (17%), and at 3000 ppm, one hepatocellular carcinoma was found in each sex. In the high-dose females, the incidence of uterine endometrial adenocarcinoma was increased (23%). The NOAEL was 100 ppm (equal to 5.5 mg/kg bw per day), based on reduced body weight gain in females and foci of eosinophilic hepatocytes and clinical chemistry changes of equivocal toxicological significance in both sexes at 500 ppm (equal to 27.6 mg/kg bw per day).

Isopyrazam was carcinogenic in female rats at the highest dose tested.

The potential genotoxicity of isopyrazam was tested in an adequate range of in vitro and in vivo studies, providing no evidence of genotoxic potential.

The Meeting concluded that isopyrazam is unlikely to be genotoxic.

In mechanistic studies to evaluate possible modes of action for liver tumours in female rats, isopyrazam was shown to induce CYP2B and CYP3A activities and replicative deoxyribonucleic acid (DNA) synthesis in female rat hepatocytes in vitro with a significantly higher potency than phenobarbital. CYP3A was also induced with a high potency in female human hepatocytes in vitro, whereas phenobarbital showed a weak induction potential. In a 14-day feeding study in rats, CYP1A- and CYP2B-dependent activities were induced significantly at all three time points (3, 8 and 14 days after treatment with 500 or 3000 ppm), whereas CYP3A- and CYP4A-dependent activities were not induced. The significant in vivo CYP1A induction and the very high potency CYP2B (in vitro and in vivo) and CYP3A induction (in vitro) for isopyrazam suggest more than phenobarbital-like enzyme induction. Although microsomal enzyme induction was observed, no clear mode of action was identified that could be causally linked to the liver adenoma in female rats.

In an in vitro test with human estrogen receptor α, isopyrazam did not show significant binding capacity. Isopyrazam was negative in a rat uterotrophic assay. Therefore, an estrogen-like mode of action as a possible explanation for the uterine endometrial adenocarcinoma is not supported.

On the basis of the absence of genotoxicity and the absence of carcinogenicity in mice and the fact that an increase in the incidences of hepatocellular adenoma and uterine endometrial adenocarcinoma in female rats occurred only at the highest dose tested, the Meeting concluded that isopyrazam is unlikely to pose a carcinogenic risk to humans at dietary exposure levels.

In a two-generation study of reproductive toxicity in rats at dietary concentrations up to 3000 ppm isopyrazam (*syn*:*anti* 92.8:7.2), F_0 and F_1 rats had decreased body weight gains at 500 and 3000 ppm. Hepatocellular hypertrophy and increases in liver weights were noted in F_0 and F_1 animals at 500 ppm and above. In F_0 males at 500 ppm and above, thyroid weights were increased statistically

significantly. In F_0 and F_1 females at 3000 ppm, weights of the uterus with cervix were decreased statistically significantly. Kidney weights in F_1 animals were dose-relatedly increased at all dose levels in both sexes, statistically significantly in females at all dose levels and in males at 3000 ppm. Ovary weights in high-dose F_0 and F_1 females were statistically significantly decreased. F_{1A} and F_{2A} pup body weights were reduced. At 500 ppm and above, mean total litter weights were reduced. F_1 males at 3000 ppm showed statistically significantly delayed preputial (2.3 days) separation, and F_1 females at 3000 ppm showed statistically significantly delayed vaginal opening (2 days). Whereas the males showed statistically significantly reduced body weights (-7%), the body weights of females were unchanged. The NOAEL for parental toxicity was 100 ppm (equal to 8.1 mg/kg bw per day), based on decreased body weight gain and organ weight changes at 500 ppm (equal to 40.6 mg/kg bw per day) in parental F_0 and F_1 animals. The NOAEL for postnatal developmental toxicity was 100 ppm (equal to 8.1 mg/kg bw per day), based on decreased mean total litter weights at 500 ppm (equal to 40.6 mg/kg bw per day). The NOAEL for reproductive performance was 3000 ppm (equal to 239.1 mg/kg bw per day), the highest dose tested.

In a study on the developmental toxicity of isopyrazam (*syn*:*anti* 92.8:7.2) in rats at dose levels up to 250 mg/kg bw per day administered by gavage, two high-dose dams were killed in extremis on gestation days (GD) 20 and 21 because they were showing severe signs of toxicity. The high dose group animals showed reduced feed consumption and reduced body weight gain, and fetal body weights were decreased in this group. In the 250 mg/kg bw per day group, one fetus with hydroencephalus and microphthalmia was observed, and in another litter, a fetus with hydrocephalus only was noted. Non-ossified cervical centra and incomplete xiphoid cartilage were noted at 75 mg/kg bw per day and above. The NOAEL for maternal toxicity was 75 mg/kg bw per day, based on reduced body weight gain and clinical signs of toxicity at 250 mg/kg bw per day. The NOAEL for developmental toxicity was 20 mg/kg bw, based on delayed or absent ossification in cervical centra at 75 mg/kg bw per day.

In a study on the developmental toxicity of isopyrazam (*syn*:*anti* 69.7:30.3) in rats at dose levels up to 200 mg/kg bw per day administered by gavage, ventral recumbency and sedation were noted in all dams at 200 mg/kg bw per day, from the first day of treatment (GD 4) throughout the first week. Feed consumption and body weight gain were reduced from GD 4 in animals at 75 mg/kg bw per day and above. At 75 mg/kg bw per day and above, fetal body weights were lower than those of controls. One fetus at 200 mg/kg bw per day was found with diaphragmatic hernia. Increased incidences of delayed or absent ossification of cervical vertebral bodies were observed at 200 mg/kg bw per day and of incompletely ossified sternebrae at 75 mg/kg bw per day and above. Additionally, non-ossified structures in forelimbs and hindlimbs were identified. The NOAEL for maternal and developmental toxicity was 20 mg/kg bw per day, based on clinical signs and reduced body weight gain in dams and lower fetal body weights at 75 mg/kg bw per day.

In two range-finding studies on the developmental toxicity of isopyrazam (*syn*:*anti* 92.8:7.2) in Himalayan rabbits at dose levels up to 1000 mg/kg bw per day, no maternal toxicity was observed. In fetuses, the incidences of small eyes, malrotated and flexed limbs and changes in the skull were increased at 400 mg/kg bw per day and above.

In a third range-finding study on the developmental toxicity of isopyrazam (*syn*:*anti* 92.8:7.2) in New Zealand White rabbits at dose levels up to 1000 mg/kg bw per day, maternal toxicity was noted at all dose levels from 400 to 1000 mg/kg bw per day, as dams had decreased body weight gain, increased liver weights, hepatocellular hypertrophy and changes in clinical chemistry parameters. At the high dose, fetal body weight was reduced and early resorptions were increased. Small eyes were noted in fetuses at 1000 mg/kg bw per day. Furthermore, absent gall bladders, extra papillary muscle in the heart and variations of major blood vessels were observed.

In a definitive study on the developmental toxicity of isopyrazam (*syn*:*anti* 92.8:7.2) in New Zealand White rabbits at dose levels up to 500 mg/kg bw per day, hepatocellular vacuolation was observed at 500 mg/kg bw per day. One fetus in the 500 mg/kg bw per day group had bilateral microphthalmia. The NOAEL for maternal toxicity was 150 mg/kg bw per day, based on

hepatocellular vacuolation at 500 mg/kg bw per day. The developmental NOAEL was 150 mg/kg bw per day, based on a single observation of bilateral microphthalmia at 500 mg/kg bw per day.

A low incidence of microphthalmia was consistently observed in dose range–finding and main studies in two different rabbit strains. Microphthalmia is a very rare finding in the rabbit strains used. Thus, the Meeting concluded that the low incidences of microphthalmia in treated rabbits could not be discounted.

The Meeting concluded that isopyrazam was teratogenic in rabbits.

In an acute neurotoxicity study in rats administered isopyrazam (*syn*:*anti* 69.7:30.3) at doses ranging from 30 to 2000 mg/kg bw, nonspecific and transient effects were apparent within 3 hours after dosing in all dose groups, with a dose-dependent increase in the incidence and severity of rigidity. The NOAEL was 30 mg/kg bw, based on clinical signs of toxicity at 250 mg/kg bw. The NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose tested.

In a 13-week rat feeding study of the neurotoxicity of isopyrazam (*syn*:*anti* 69.7:30.3) with dietary concentrations up to 6000 ppm, no behavioural or histological evidence for neurotoxicity was observed. The NOAEL was 1500 ppm (equal to 98.01 mg/kg bw per day), based on decreased body weight gain in females at 6000 ppm (equal to 382.26 mg/kg bw per day). The NOAEL for subchronic neurotoxicity was 6000 ppm (equal to 382.26 mg/kg bw per day), the highest dose tested.

Toxicological data on metabolites

CSCD465008, a soil and plant metabolite, and CSCD459488, a rat, soil, plant and aquatic metabolite, were investigated in acute and subacute toxicity studies and an adequate range of in vitro genotoxicity studies. CSCD459488 was also investigated in a developmental toxicity study in rabbits.

CSCD465008 and CSCD459488 were both of low acute oral toxicity, with LD_{50} values greater than 2000 mg/kg bw, and did not give any evidence of genotoxic potential.

In a 4-week rat feeding study with dietary concentrations of CSCD465008 up to 12 000 ppm, no evidence for toxicity or for induction of EROD or PROD activity was observed. The NOAEL was 12 000 ppm (equal to 1018 mg/kg bw per day), the highest dose tested.

In a 4-week rat feeding study with dietary concentrations of CSCD459488 up to 10 000 ppm, the liver weights were increased at 300 ppm and above, and increased incidences of centrilobular hepatocyte hypertrophy and follicular cell hypertrophy of the thyroid were noted at 4000 ppm and above. Total hepatic microsomal P450 content was approximately doubled in males in the 4000 and 10 000 ppm groups and only slightly elevated in females in the same dose groups. EROD and PROD activities were statistically significantly increased at all dose levels. The NOAEL was 300 ppm (equal to 27 mg/kg bw per day), based on liver weight changes greater than 10% at higher doses.

In a study on the developmental toxicity of CSCD459488 in New Zealand White rabbits at dose levels up to 1000 mg/kg bw per day, maternal liver weights were increased at all dose levels. In the high-dose group, late resorptions per litter were increased primarily due to three resorptions in one female. The maternal NOAEL was 150 mg/kg bw per day, based on significant liver weight increases (> 20%) at higher dose levels. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested.

No reports on exposure of personnel working with isopyrazam were submitted.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.06 mg/kg bw derived from the NOAEL of 5.5 mg/kg bw per day in the 104-week rat feeding study on the basis of decreased body weight gain in females and foci of eosinophilic hepatocytes and clinical chemistry changes (triglycerides, bilirubin) of equivocal toxicological significance in both sexes at 27.6 mg/kg bw per day. A safety factor of 100 was applied. The ADI is supported by the NOAEL of 9.9 mg/kg bw per day in the mouse 80-week feeding study, based on periportal hepatocellular hypertrophy in females at 500 ppm (equal to 56.2 mg/kg bw per day). The margin between the maximum ADI and the LOAEL at 232.8 mg/kg bw per day for uterine and liver tumours in female rats is approximately 3900.

The Meeting established an ARfD of 0.3 mg/kg bw derived from the NOAEL of 30 mg/kg bw in the rat acute neurotoxicity study, on the basis of nonspecific clinical signs of toxicity (weak appearance and decreased activity) at 250 mg/kg bw. A safety factor of 100 was applied. In a rat developmental toxicity study, the NOAEL of 20 mg/kg bw per day for maternal and developmental toxicity was based on reduced body weight gain in dams only on day 4 of treatment. The margin between the ARfD and the LOAEL at 500 mg/kg bw per day for teratogenic effects (microphthalmia) in rabbits is approximately 1700.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	70 ppm, equal to 9.9 mg/kg bw per day	500 ppm, equal to 56.2 mg/kg bw per day
		Carcinogenicity	3500 ppm, equal to 432.6 mg/kg bw per day[b]	—
Rat	Acute neurotoxicity[c]	Toxicity	30 mg/kg bw	250 mg/kg bw
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 5.5 mg/kg bw per day	500 ppm, equal to 27.6 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 34.9 mg/kg bw per day	3000 ppm, equal to 232.8 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	3000 ppm, equal to 239.1 mg/kg bw per day[b]	—
		Parental toxicity	100 ppm, equal to 8.1 mg/kg bw per day	500 ppm, equal to 40.6 mg/kg bw per day
		Offspring toxicity	100 ppm, equal to 8.1 mg/kg bw per day	500 ppm, equal to 40.6 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	20 mg/kg bw per day	75 mg/kg bw per day
		Embryo and fetal toxicity	20 mg/kg bw per day	75 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	150 mg/kg bw per day	500 mg/kg bw per day
		Embryo and fetal toxicity	150 mg/kg bw per day	500 mg/kg bw per day
Dog	Thirteen-week and 1-year studies of toxicity[c,d]	Toxicity	30 mg/kg bw per day	100 mg/kg bw per day

[a] Dietary administration.

[b] Highest dose tested.

[c] Gavage administration.

[d] Three studies combined.

Estimate of acceptable daily intake for humans

0–0.06 mg/kg bw

Estimate of acute reference dose

0.3 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 70%
Dermal absorption	No data
Distribution	Extensive, highest levels in liver
Potential for accumulation	Low, no evidence of accumulation
Rate and extent of excretion	Rapid, close to 100% within 48 h, mainly via bile
Metabolism in animals	Extensive, primarily via hydroxylation at bicyclic-isopropyl moiety
Toxicologically significant compounds (animals, plants and the environment)	Isopyrazam, CSCD459488
Acute toxicity	
Rat, LD_{50}, oral	> 2000 mg/kg bw (69.7:30.3 *syn*:*anti*)
Rat, LD_{50}, oral	310.2 mg/kg bw (50:50 *syn*:*anti* and pure *anti*)
Rat, LD_{50}, dermal	> 5000 mg/kg bw (92.8:7.2 *syn*:*anti*)
Rat, LC_{50}, inhalation	> 5.28 mg/L (69.7:30.3 *syn*:*anti*)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slightly irritating
Mouse, skin sensitization (local lymph node assay)	Sensitizing potential
Short-term studies of toxicity	
Target/critical effect	Body weight changes and liver toxicity (rat)
Lowest relevant oral NOAEL	250 ppm, equal to 20.3 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEL	No data

Genotoxicity	
	Not genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Clinical chemistry, body weight (rat)
Lowest relevant NOAEL	5.5 mg/kg bw per day (rat)
Carcinogenicity	Unlikely to pose a carcinogenic risk at dietary exposure levels
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects
Lowest relevant reproductive NOAEL	239.1 mg/kg bw per day (rat), highest dose tested
Developmental target/critical effect	Decreased fetal body weights (rat), microphthalmia (rabbit)
Lowest relevant developmental NOAEL	20 mg/kg bw per day (rat), 150 mg/kg bw per day (rabbit)
Neurotoxicity/delayed neurotoxicity	
	No evidence in acute or subchronic neurotoxicity studies
Other toxicological studies	
Studies on metabolites	In rat 4-week feeding studies, CSCD465008 was less toxic than the parent and CSCD459488 was of similar toxicity to the parent
Medical data	
	No reports submitted

Summary

	Value	Study	Safety factor
ADI	0–0.06 mg/kg bw	Two-year study in rats	100
ARfD	0.3 mg/kg bw	Acute neurotoxicity study in rats	100

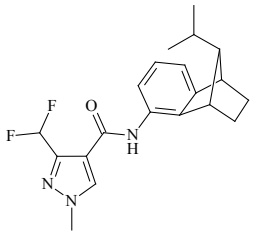
RESIDUE AND ANALYTICAL ASPECTS

Isopyrazam is a broad-spectrum foliar fungicide belonging to the chemical class of ortho-substituted phenyl amides. It controls a wide range of fungal pathogens.

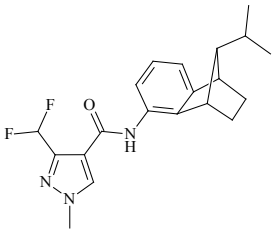
The Meeting received information on physical and chemical properties, animal and plant metabolism, environmental fate, analytical methods, storage stability, use patterns, processing and farm animal feeding.

Isopyrazam contains two diastereoisomers designated syn- and anti-isomers. Both of these isomers are biologically active and the specification for technical isopyrazam covers the range of syn:anti isomer ratios from 70:30 to 100:0.

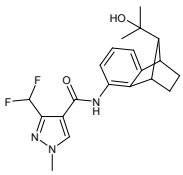
syn-isomer



anti-isomer

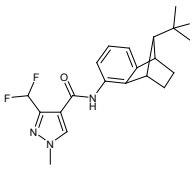


In this Appraisal, the following abbreviated names were used.



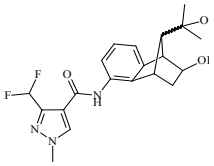
CSCD459488

Hydroxylated syn-isomer (tertiary alcohol)



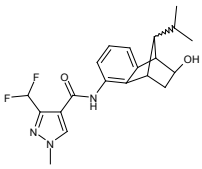
CSCD459489

Hydroxylated anti-isomer (tertiary alcohol)



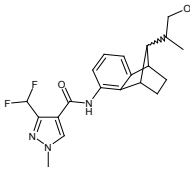
CSCD656800

Dihydroxylated isopyrazam



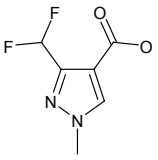
CSCD563692

Hydroxylated isopyrazam (secondary alcohol)



CSCD563691

Hydroxylated isopyrazam (primary alcohol)



CSAA798670

3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid

Animal metabolism

The Meeting received information on the fate of orally-dosed isopyrazam in rats, lactating goats and laying hens.

For the animal metabolism studies, three types of radioactive isopyrazam were used: isopyrazam uniformly labelled with ^{14}C on phenyl ring and with syn/anti ratio of 70:30 or 95:5; and one with ^{14}C at position 5 of pyrazole ring with syn/anti ratio of approximately 95:5. In addition, in a goat metabolism study, CSCD 459488 labelled at position 5 of pyrazole ring with ^{14}C was used.

In metabolism studies, total radioactive residues are expressed in mg/kg isopyrazam equivalents unless otherwise stated.

Metabolism of isopyrazam in rats

Metabolism studies on laboratory animals including rats were reviewed in the framework of toxicological evaluation by the current Meeting.

When radio-labelled isopyrazam was administered once at 1 or 75 mg/kg bw to rats, approximately 70% of the dose was absorbed. Most of the absorbed dose was excreted within 24 hours after administration, 65–90% via bile and the rest via urine. Highest residues were identified in the liver, kidney, thyroid and adrenals. After repeated dosing, no accumulation of radioactivity was observed in rats. There were no significant differences between the toxicokinetic parameters of syn- and anti isomers. The predominant metabolic pathway for isopyrazam or its N-demethylated metabolite is hydroxylation in the bicyclic-isopropyl moiety, followed by further oxidation to form

carboxylic acid and/or to give rise to multiple hydroxylated metabolites with subsequent formation of glucuronic acid or sulphate conjugates.

Metabolism of isopyrazam in lactating goats

The three types of [^{14}C]isopyrazam were administered orally to three groups of lactating goats (each type of radio-labelled isopyrazam to each different group) at a dose equivalent to a dietary concentration of 30 ppm (in dry matter) daily for seven consecutive days. Of the total administered radioactivity (TAR), 60–63% and 12–13% was eliminated via faeces and urine, respectively. Administration of radio-labelled isopyrazam with different ^{14}C position or syn/anti ratio did not reveal any significant difference in the excretion. Total recovered radioactivity was 80–84% of the TAR.

Radioactivity in the Day 5 am milk was 0.055–0.076 mg/kg from the use of three different types of radioactive isopyrazam.

Total radioactive residues (TRR) in tissues except liver after sacrifice (16 hours after the last dose) were similar regardless of the label position or the syn/anti ratio. In the liver, TRR (0.331 mg/kg) after administration of phenyl-labelled isopyrazam of syn/anti ratio of 70:30 was about one half of the TRR (0.604 and 0.612 mg/kg) after administration of pheyl-labelled or pyrazole-labelled isopyrazam of syn/anti ratio of 95:5. In other tissues, TRR were 0.143–0.189 mg/kg in the kidney, 0.022–0.032 mg/kg in the muscle, and 0.012–0.020 mg/kg in the fat.

In the milk, liver, kidney and muscle extracts, parent compound was a minor component with the maximum of 0.0063 mg/kg (1.9% TRR) in the liver or 8.6% of TRR (0.0019 mg/kg) in the muscle from the administration of three different types of radio-labelled isopyrazam.

In the fat, the parent compound was predominant at 40–51% of TRR but the concentration was very low at 0.005–0.010 mg/kg.

The major residue was CSCD656800, dihydroxylated isopyrazam, in the extracts of milk (15–32% of TRR; 0.008–0.019 mg/kg), liver (6–17% of TRR; 0.020–0.104 mg/kg), kidney (13–25% of TRR; 0.023–0.038 mg/kg) and muscle (29–44% of TRR; 0.007–0.013 mg/kg). CSCD656800 was not detected in the fat extracts. In the liver, CSCD656800 existed as glucuronide or sulphate conjugates.

No other identified metabolites existed at quantifiable concentrations. All identified metabolites contained both the pyrazole and phenyl moieties indicating that there was no or little cleavage of amide in metabolism.

Treatment of the liver extraction debris with protease released a significant portion of radioactivity which was composed of multiple minor metabolites.

Metabolism of CSCD459488 in lactating goats

Metabolism of CSCD459488, hydroxylated syn-isomer of isopyrazam and major metabolite in wheat, grapes and lettuce, was studied by orally administering pyrazole-labelled CSCD459488 at a dose equivalent to dietary concentration of 12 ppm to lactating goats daily for seven consecutive days. The major portion of administered radioactivity was excreted via faeces (57% of TAR) and urine (30% of TAR).

Radioactivity in the Day 6 pm milk was 0.123 mg/kg in CSCD459488 equivalents. After sacrifice, the highest radioactivity was found in the liver at 0.457 mg/kg followed by kidney at 0.246 mg/kg, muscle at 0.038 mg/kg, and fat at 0.007 mg/kg.

In this study, the predominant metabolite was CSCD656800 in the milk and all the tissues tested (33–56% of TRR). The highest concentration was found in the liver at 0.159 mg/kg (36% of TRR) where the majority of CSCD656800 existed as conjugates.

CSCD459488 was detected in the milk and all the tissues tested but at only very low levels (< 0.001–0.007 mg/kg; 0.1–6.2% of TRR) and in conjugated forms.

Metabolism of isopyrazam in laying hens

The three types of [^{14}C]isopyrazam were administered orally to three groups of laying hens (each type of radio-labelled isopyrazam to each different group) at a dose equivalent to a dietary concentration of 11 ppm (in dry matter) daily for 14 consecutive days. Of the total administered radioactivity (TAR), 88–93% was eliminated in excreta. Administration of radio-labelled isopyrazam with different ^{14}C position or syn/anti ratio did not reveal any significant difference in the excretion. Total recovered radioactivity was 91–95% of the TAR.

TRR in the composite egg white and egg yolk samples obtained in Days 7–14 from hens dosed with radio-labelled isopyrazam with syn/anti ratio of 95:5 were 0.017 and 0.039–0.042 mg/kg, respectively. On the other hand, when phenyl-labelled isopyrazam with syn/anti ratio of 70:30 was administered, TRR in the composite egg white and egg yolk samples were higher at 0.024 and 0.080 mg/kg respectively.

The TRR in tissues after sacrifice (16 hours after the last dose) were 0.119–0.143 mg/kg, 0.004–0.006 mg/kg, 0.008–0.020 mg/kg and 0.011–0.020 mg/kg in liver, muscle, skin and attached fat, and peritoneal fat, respectively.

Parent isopyrazam was detected in all fat samples and in egg yolk samples (from administration of pyrazole-labeled isopyrazam with the syn/anti ratio of 95:5 and phenyl-labelled isopyrazam with the syn/anti ratio of 70:30) and in liver (from administration of pyrazole-labelled isopyrazam with the syn/anti ratio of 95:5) but the concentrations were < 0.01 mg/kg (< 5% of TRR in egg yolk and liver; up to 21% TRR in fat).

From the egg and liver samples, three metabolites were identified: CSCD656800 (dihydroxylated isopyrazam), hydroxy CSCD459489 and unsaturated carboxylic acid. None of them existed in a concentration higher than 0.012 mg/kg but CSCD656800 contributed up to 29% of TRR in egg white. Detection of hydroxyl CSCD459489 (anti configuration) in egg and liver samples from treatment with the syn/anti ratio of 70:30 was consistent with the larger proportion of anti-isomer administered in the experiment.

Treatment of the liver extraction debris with protease followed by 0.1 NHCl released a significant portion of radioactivity which was composed of multiple minor metabolites.

No other identified metabolites existed at quantifiable concentrations. All identified metabolites contained both the pyrazole and phenyl moieties indicating that there was no or little cleavage of amide in metabolism.

The metabolic pathway in lactating goats and laying hens was similar to the one in rats. The primary metabolism of isopyrazam in these animals, in relation to edible tissues, milk and eggs, proceeded through hydroxylation of the isopropyl group and bicyclic portion of the molecule; and then oxidation of primary alcohols to form carboxylic acids and/or multiple hydroxyl moieties which subsequently converted into glucuronic acid or sulphate conjugates.

No significant inter-conversion of the two isomers of isopyrazam occurred in metabolism.

Plant metabolism

The Meeting received information on the fate of isopyrazam after foliar applications on wheat, grape and lettuce.

For the plant metabolism studies, three types of radioactive isopyrazam were used: isopyrazam uniformly labelled with ^{14}C on phenyl ring and with syn/anti ratio of 70:30 or 96:4; and one with ^{14}C at position 5 of pyrazole ring with syn/anti ratio of 96:4.

Wheat

Wheat grown in pots in glasshouse was treated three times (approximating BBCH 31, BBCH 39 and BBCH 69) with foliar spray application of the three types of isopyrazam separately at a rate of 125 g ai/ha.

Total radioactive residues (TRR) in forage collected 13 days after the second application were 4.75–7.09 mg/kg, and in grain and straw collected 4–48 days after the last application were 0.031–0.059 mg/kg and 14.1–20.8 mg/kg respectively. This indicates that translocation to grains is very small.

Application of radio-labelled isopyrazam with different ^{14}C position did not result in significant difference in the TRR while the application of isopyrazam with the syn/anti ratio of 70:30 resulted in significantly lower TRR.

Parent isopyrazam was the major residue: 4.50–5.81 mg/kg (79–91% of TRR) in forage, 8.56–15.5 mg/kg (61–69% of TRR) in straw, and 0.021–0.037 mg/kg (53–66% of TRR) in grain.

In grain, no identified metabolites existed in excess of 0.0013 mg/kg (< 5% of TRR).

In straw, the most significant metabolite was CSCD459488 (tertiary alcohol) at 1.01–1.94 mg/kg (7.3–9.7% of TRR). This compound was also found in forage and grain but at lower concentrations: 0.020–0.15 mg/kg (0.4–2.4% of TRR) in forage and 0.0004–0.0008 mg/kg (1.2–1.4% of TRR) in grain.

In straw, CSCD563692 (secondary alcohol) and CSCD563691 (primary alcohol) were also identified but both of them were less than 5% of TRR (up to 0.760 and 0.540 mg/kg respectively). These compounds were also found in forage at lower levels.

A dihydroxylated compound was also identified but accounted for less than 5% of TRR in forage, straw and grain. A number of additional components were identified as the N-demethylated isopyrazam and unsaturated products (< 5% of TRR).

Major portions of the above mentioned metabolites were released after pectinase treatment indicating their presence in conjugated form.

Grapes

Grape vines in the field were given a single foliar application of either of phenyl-labelled or pyrazole-labelled isopyrazam (syn/anti ratio of 70:30) at a nominal rate of 400 g ai/ha. Grape berries were harvested 21 days after the application.

Total radioactive residues (TRR) in unwashed grape berries were 0.156 and 0.147 mg/kg for the pheny-labelled and pyrazole-labelled isopyrazam application respectively. The TRR in the vine leaves were 11.0 and 3.77 mg/kg for the pheny-labelled and pyrazole-labelled isopyrazam application respectively. Residue concentrations were much lower in berries than in leaves.

Parent isopyrazam is the major component of the residues from the both treatments at 0.116–0.131 mg/kg (89–90% of TRR) in grape berries. In leaf samples, parent compound was present at 10.1 mg/kg (91% of TRR) from treatment with phenyl-labelled isopyrazam and 3.25 mg/kg (86% of TRR) from pyrazole-labelled isopyrazam. The syn/anti ratio of isopyrazam in fruit and leaf fractions was approximately 70:30 in the HPLC analysis indicating no significant change in ratio from the applied isopyrazam.

The metabolites CSCD563692 (secondary alcohol), CSCD610195 (primary alcohol) and CSCD459488 (syn-form tertiary alcohol) were found in berries. Corresponding anti-form CSCD459489, dihydroxylated metabolite CSCD656800 and N-demethylated metabolites were found in leaves only. None of them accounted for more than 5% of TRR (individually up to 0.110 mg/kg in leaf).

Lettuce

Lettuce grown outdoors was treated three times (BBCH < 40, 42 and 46) with phenyl-labelled or pyrazole-labelled isopyrazam at a nominal application rate of 125 g ai/ha. Lettuce was harvested 3 (early harvest) or 14 days (normal harvest) after the last application.

The total radioactive residues in lettuce were 1.54–1.56 mg/kg 3 days after the last application but decreased to 0.22–0.31 mg/kg at full maturity, 14 days after the last application.

Also in lettuce, parent isopyrazam was the major component of residues: 1.03–1.09 mg/kg (66–71% of TRR) in early harvest and 0.100–0.108 mg/kg (35–45% of TRR) in normal harvest.

CSCD459488 (tertiary alcohol), mostly in a conjugated form, was the most significant metabolite: 0.009–0.022 mg/kg (0.6–1.4% of TRR) in early harvest and 0.031–0.053 mg/kg (14–17% of TRR) in normal harvest. This indicates biotransformation of isopyrazam into CSCD459488.

There were a number of minor metabolites characterized in lettuce from both harvest timings from the both treatments. They were hydrolyzed, N-demethylated or cleaved compounds and existed at very low levels.

The studies on wheat, grape and lettuce indicate that the metabolism of isopyrazam in these plants was qualitatively the same. The position of radiolabel or the syn/anti ratio of isopyrazam did not reveal significant difference in metabolic profiles.

In all plants tested, parent compound was the major residue component with a number of hydrolyzed or N-demethylated metabolites identified or characterized.

In plants, isopyrazam undergoes hydroxylation of the isopropyl group to produce a variety of alcohol products with CSCD459488 as the major metabolite; conjugation of these metabolites with natural carbohydrates; or N-demethylation of the pyrazole ring. None of the metabolites, except CSCD459488 in lettuce leaf (14–17% of TRR) and wheat straw (7.3–9.7% of TRR), accounted for more than 5% of TRR.

Environmental fate in soil

Since isopyrazam is a fungicide with foliar applications and its uses are currently limited to cereals and bananas, the Meeting reviewed hydrolysis and succeeding crop studies.

Hydrolysis

Isopyrazam was stable for 30 days at 25 °C at pH 5, 7 and 9 and for 5 days at 50 °C at pH 4, 5, 7 and 9.

Residues in succeeding crops

A confined study was conducted to examine the nature and levels of residues of isopyrazam in succeeding crops. A single application of either pyrazole-labelled or pheyl-labelled isopyrazam (syn/anti ratio of 96:4) was made to sandy loam soil in containers at a nominal rate of 375 g ai/ha, higher than the proposed maximum annual application rate of 250 g ai/ha.

At each rotational interval of 30, 90 and 300 days after treatment (DAT), spring wheat, lettuce and turnips were sown into the treated soil, grown in glasshouses, and harvested at maturity.

Following application of isopyrazam to soil and aging of the treated soil for up to 300 days, uptake of radioactivity into rotational crops was most significant in straw, hay and forage of wheat (0.80–1.02 mg/kg for 30-day plant back interval, 0.35–0.40 mg/kg for 90-day plant back interval (no harvest for 30-day plant back interval), and 0.073–0.154 mg/kg for 30-day plant back interval, respectively). The uptake gradually decreased in samples as plant back interval increased. In wheat grain, turnip roots and leaves, and lettuce, uptake of radioactivity was much smaller (0.012–

0.023 mg/kg, 0.016–0.018 mg/kg, 0.026–0.051 mg/kg, and 0.010–0.023 mg/kg respectively for 30-day plant back interval).

In general, the metabolism of isopyrazam in succeeding crops was similar to that in primary crops but parent compound accounted for a much lower percentage of the residue in the confined study (< 10% of TRR) and was not predominant residue. The only exception was turnip roots, where unchanged parent accounted for up to 34% TRR, but this represented a residue of only 0.0055 mg/kg. CSCD459488 was detected in all rotational crop commodities and reached 22–25% TRR in wheat straw and hay (0.17 mg/kg in straw and 0.090 mg/kg in hay). Pyrazole-specific half-molecule metabolites (CSAA798670 and its N-demethylated compound) were relatively more abundant in the confined succeeding crop study (CSAA798679 up to 48% TRR and 0.025 mg/kg in turnip foliage; and CSCD465008 up to 14% TRR and 0.003 mg/kg in turnip root) indicating that cleavage of the amide bond of isopyrazam seem to play some role in succeeding crops while they were negligible in the primary crops receiving foliar applications.

Four field trials were conducted to investigate the magnitude of the residues of isopyrazam and its metabolites in succeeding or rotational crops. Isopyrazam was applied three times to primary crops of wheat at a nominal rate of 125 g ai/ha, giving a total application rate of 375 g ai/ha, i.e., higher than the proposed maximum annual application rate of 250 g ai/ha.

Neither syn-isomer nor anti-isomer of isopyrazam was detected at or above the LOQ of 0.005 mg/kg in any of the succeeding crops (barley, carrots and spinach) from all plant-back intervals up to about one year, except in one trial where residues of syn-isomer were found at very low levels (0.005–0.006 mg/kg) in the carrot roots, barley forage and whole barley plant samples from the first (28-day) plant-back interval. CSCD459488 (hydroxylated syn-isomer) was found at low levels (up to 0.054 mg/kg in barley straw) while CSCD459489 (hydroxylated anti-isomer) or CSAA798670 was not found in any of the rotational crops.

CSCD465008 was found in all crops and at slightly higher levels (up to 0.15 mg/kg in carrot leaves).

The metabolic pathway in rotational crops is similar to that in primary crops but parent compound represented a much lower percentage of the residue and pyrazole-specific metabolites account for a higher proportion of the residue in rotational crops. Of these, CSAA798670 was not found in any of the field rotational crop studies.

The Meeting concluded that isopyrazam residue was not expected to be found above the LOQ in barley grains, carrot roots and spinach leaves. CSCD459488 or CSCD465008 were not detected more than 0.01 mg/kg in barley grains and carrot roots while these were found in spinach at 0.015 and 0.06 mg/kg respectively. These residues were found at higher concentrations in barley forage, hay and straw and carrot leaves.

Analytical methods

Analytical methods for determination of residues of isopyrazam and its metabolites were developed for a wide range of matrices of plant and animal origin.

In general, the methods for data generation employ extraction by homogenization with a mixture of acetonitrile and water (mostly 80:20 v/v), clean-up with solid phase extraction or a process of centrifugation and dilution, and determination of analytes using LC-MS/MS or, in one method, GC-MS/MS.

A number of methods for plant matrices were successfully validated for each isomer of isopyrazam at LOQ (0.005 mg/kg for each analyte) and higher concentrations in barley grain, forage and straw, ryegrass, apples, carrots, spinach, tomatoes, oranges, potatoes, lentils, sunflower seeds, rapeseed, bran bread and beer.

One method was successfully validated for determination of monohydroxylated metabolites (CSCD459488 and CSCD459489) of isopyrazam at LOQ of 0.005 mg/kg and above for barley grain, forage, straw, apples, carrots, spinach, rapeseed, lentils, bran, bread and beer. This method involves hydrolysis with 0.1 M HCl at 60 °C for 3 hours.

Another method was successfully validated for CSCD465008 and CSAA798670 at LOQ of 0.01 mg/kg and above in barley grain, forage and straw, carrot leaves and roots, and spinach. This method involves hydrolysis with pectinase at pH 5 at 37 °C for 16–20 hours and partition with hexane.

For commodities of animal origin, one method was successfully validated for the determination of each isomer of isopyrazam at 0.005 mg/kg and above in eggs, milk, muscle, liver, kidney and fat. Another method determines isopyrazam and its metabolites in commodities of animal origin as the common moiety CSAA798670; i.e., this method determines not only isopyrazam but any metabolites hydrolysable to CSAA798670. The analytical procedure involves hydrolysis of acetonitrile + water extract with 12 M potassium hydroxide solution at 100 °C for 3 hours. The method was successfully validated for the determination of isopyrazam and its metabolites hydrolysable to CSAA798670 at 0.005 mg/kg in CSAA798670 equivalents and above in eggs, milk, muscle, liver, kidney and fat.

For enforcement, a multi-residue method DFG-S19 was investigated for monitoring of isopyrazam in plant and animal commodities using GC-MSD (selected ion monitoring) or LC-MS/MS (positive or negative multiple reaction monitoring). DFG-S19 using negative multiple reaction monitoring LC-MS/MS was successfully validated in-house and independently for the determination of isopyrazam (determined as syn- and anti-isomer separately) at 0.005 mg/kg and above (plant commodities) or 0.0025 mg/kg and above (animal commodities) for each isomer, In the case of wheat grain, the method could only be validated at 0.05 mg/kg when ion m/z 316 was monitored with GC-MSD in an independent laboratory validation.

Both GC-MSD and LC-MS/MS are specific and either of them can be used for quantification of residues. However, only the negative multiple reaction monitoring LC-MS/MS received full validation and independent laboratory validation.

Stability of pesticide residues in stored analytical samples

The stability of isopyrazam residues during storage of samples frozen at approximately -15 to -20 ºC was investigated in a range of plant and animal matrices: tomato fruit, rape seeds, lentil seeds, potato tubers, barley grain, barley straw, ryegrass forage and spinach leaves; and milk, eggs, liver, kidney, muscle and fat.

Compounds tested were: both isomers of isopyrazam, CSCD459488, CSCD459489, CSCD465008 and CSAA798670. Each compound was spiked to matrices at 0.5 mg/kg.

All of the compounds tested were found stable (> 70% remaining) at least for the following storage periods tested: in plant commodities, both isomers of isopyrazam, 24 months; CSCD459488, 11 months; CSCD459489, 28 months; and CSCD45008 and CSAA798670, 12 months; and in animal commodities, both isomers of isopyrazam, 14 months; and isopyrazam and metabolites hydrolysable to CSAA798670, 12 months.

The storage durations of samples from the supervised field trials were within the above storage periods.

Definition of the residue

In animal metabolism studies, parent isopyrazam was detected in all the tissues tested, milk and eggs at concentrations < 0.01 mg/kg. It was metabolized extensively and accounted for < 9% of TRR in milk, eggs and tissues other than fat, and up to 51% of TRR in fat.

Sufficiently validated multi-residue LC-MS/MS or GC-MSD method was available for determining the parent compound as the two separate isomers in animal commodities for enforcement. A number of LC-MS/MS methods were validated for analysing isopyrazam in animal commodities.

CSCD656800 (dihydroxylated metabolite) was found in all tissues (except fat), milk and eggs as a major metabolite at significant concentrations (up to 0.104 mg/kg in goat liver) and accounted for 6–44% of TRR in goats and 1.0–29% of TRR in hens. While CSCD656800 was the major metabolite in animals, it is difficult to obtain analytical standard material for this compound and CDCD656800 was not separately analysed in the animal feeding study due to the lack of validated specific analytical methods.

An LC-MS/MS method was validated for analysing parent compound and any metabolites (including CSCD656800) hydrolysable to the common moiety (CSAA798670) in animal commodities. However, the Meeting noted that CSAA798670 moiety is not specific to isopyrazam and may arise from the use of other pesticides containing this moiety, such as sedaxane.

The Meeting therefore concluded that the parent isopyrazam was suitable residue definition for enforcement.

For estimation of dietary intakes, the Meeting considered inclusion of CSCD656800 in the residue definition for animal commodities but it was not possible to include it as no specific analytical method was available. Its contribution to dietary exposure would be no more than 1% of the maximum ADI or ARfD even when uses were expanded.

In plant metabolism, parent isopyrazam was the predominant residue component (53–66% in wheat grain, 86–91% in grape berries and 35–45% in lettuce).

While CSCD459488 was the most significant metabolite in wheat foliage (< 10% of TRR), grapes (< 5% of TRR) and lettuce (14–17% of TRR), it was found at levels below 5% of TRR in wheat grain and grape berries. However, CSCD459488 was found in the supervised residue trials on bananas, barley and wheat, where, in some trials, CSCD459488 was found at levels approaching that of isopyrazam (e.g., 0.026 mg/kg of isopyrazam and 0.022 mg/kg of CSCD459488 in barley grain). CSCD459488 was considered to be of similar toxicity as the parent.

Sufficiently validated multi-residue LC-MS/MS or GC-MSD methods were available for determining the parent compound as the two separate isomers in plant commodities for enforcement.

A number of LC-MS/MS methods were successfully validated for analysing parent or CSCD459488 in plant commodities.

The Meeting therefore concluded that the parent isopyrazam was a suitable residue for enforcement.

For estimation of dietary intakes, the Meeting decided to include CSCD459488, the major metabolite, in the residue definition for plant commodities.

The syn-isomer of isopyrazam has log P_{ow} of 4.1 and the anti-isomer 4.4. In the goat metabolism studies, the isopyrazam concentrations in fat were ~ 5–9 times higher than those in muscle. In the cattle feeding study, the isopyrazam concentrations in cream were about eight times those in whole milk. In the hen metabolism study, isopyrazam was found only in egg yolk samples but not in egg white samples. The Meeting considered isopyrazam residues to be fat-soluble.

The Meeting recommended the following residue definition for plant and animal commodities:

Definition of the residue for plant commodities (for compliance with the MRL): *Isopyrazam (sum of syn-isomer and anti-isomer)*

Definition of the residue (for estimation of dietary intake) for plant commodities: *Sum of isopyrazam and 3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid [9-(1-hydroxyl-1-*

methylethyl)-(1RS, 4RS, 9RS)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]amide expressed as isopyrazam

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for animal commodities: *Isopyrazam (sum of syn-isomer and anti-isomer)*

The residue is considered fat-soluble.

Results of supervised trials on crops

The Meeting received supervised trial data for isopyrazam on bananas, barley, and wheat.

For all matrices, the LOQ was 0.005 mg/kg for each isomer of isopyrazam and CSCD459488. In the summing of the total residues, if syn- and anti-isomers and CSCD459488 were below the LOQ, the LOQ value of each was used for the calculation.

The OECD MRL calculator was used as a tool to assist in the estimation of maximum residue levels from the selected residue data set obtained from the supervised residue trials. As a first step, the Meeting reviewed trial conditions and other relevant factors related to each data set to arrive at a best estimate of the maximum residue level using expert judgement. Then, the OECD calculator was employed. If the statistical calculation spreadsheet suggested a different value, a brief explanation of the derivation was supplied.

Bananas

A total of 12 supervised trials were conducted on bananas in 2008 in Columbia, Costa Rica, Guatemala and Honduras. Isopyrazam was applied five times (or in one case, six) at a rate of 75 g ai/ha, with an interval between applications of 10 days. Applications were made to both bagged and unbagged bananas. Results from unbagged bananas in each trial were used to estimate a maximum residue level and STMR/HR as in all trials conducted, residue concentrations in bagged banana were never higher than those in unbagged bananas.

The GAP in Columbia allows five foliar applications at a rate of 75 g ai/ha with PHI of 0 days.

Residues of isopyrazam from trials matching he Columbian GAP in banana fruit were: < 0.01, < 0.01, < 0.01, 0.011, 0.012, 0.013, 0.015, 0.016, 0.017, 0.022, 0.034 and 0.04 mg/kg.

The Meeting estimated a maximum residue level of 0.06 mg/kg.

Corresponding total residues of isopyrazam and CSCD459488 in the pulp of unbagged banana were all < 0.015 mg/kg. After six applications instead of five applications, total residues in pulp were < 0.015 mg/kg regardless of whether banana fruit was bagged or unbagged.

The Meeting estimated an STMR and HR for banana pulp at 0.015 and 0.015 mg/kg respectively.

Barley

A total of 21 trials were conducted on barley: three in 2008 in New Zealand, two in 2006 in Switzerland, eight in 2006 and 2007 in France, three in 2006 and 2007 in Germany, one in 2007 in the United Kingdom, two in 2006 and 2007 in Italy, and two in 2006 and 2007 in Spain.

The registered use on barley in New Zealand allows two foliar applications per season prior to BBCH growth stage 59 (ear emergence) at a rate of 75 g ai/ha with a PHI of not shorter than 42 days.

In the three trials conducted in New Zealand, isopyrazam was applied twice at rates approximating 75, 125 and 250 g ai/ha with PHI of 41 or 42 days.

Residues of isopyrazam from trials matching GAP in New Zealand were: < 0.01, < 0.01 and 0.018 mg/kg.

The registered use of isopyrazam on barley in the United Kingdom (UK) allows two foliar applications per season between growth stages 30 and 61 (before beginning of flowering), each at a rate of 125 g ai/ha isopyrazam.

Residues of isopyrazam from the trials conducted in Northern France, Germany, Switzerland and the United Kingdom matching GAP of the UK were (n = 8): 0.014, 0.016, 0.017, 0.020, 0.024, 0.026, 0.026 and 0.035 mg/kg.

The Meeting estimated a maximum residue level of 0.07 mg/kg for barley. The Meeting also estimated a median residue of 0.022 mg/kg for the purpose of calculating animal dietary burdens.

Corresponding total residues of isopyrazam and CSCD459488 were: 0.020, 0.022, 0.029, 0.032, 0.043, 0.046, 0.048 and 0.058 mg/kg.

The Meeting estimated an STMR at 0.0375 mg/kg.

Wheat

A total of 25 trials were conducted on wheat: three in 2008 in New Zealand, ten in 2006 and 2007 in France, five in 2006 and 2007 in Germany, one in 2007 in the United Kingdom, two in 2006 and 2007 in Italy, and four in 2006 and 2007 in Spain.

The registered use of isopyrazam on wheat in New Zealand allows two foliar applications per season prior to BBCH growth stage 69 (end of flowering) at rates of 75–125 g ai/ha and a PHI of not shorter than 42 days.

In the three trials conducted in New Zealand, isopyrazam was applied twice at rates approximating 75, 125 and 250 g ai/ha with PHI of 42 days.

Residues of isopyrazam from trials matching GAP in New Zealand were: < 0.01, < 0.01 and 0.020 mg/kg.

The registered use of isopyrazam on wheat, rye and triticale in the United Kingdom allows two foliar applications per season between growth stages 30 and 71 (before grain watery ripe stage), each at a rate of 125 g ai/ha isopyrazam.

In most of the trials, isopyrazam was applied three times instead of twice. Therefore, the trials were not in compliance with the GAP of the UK. The isopyrazam concentrations in whole plants immediately before the third application were on average about 15% of those on the day of the third application. The Meeting decided to use data from these trials for estimating a maximum residue level in wheat if the contribution of isopyrazam from the second application was below 25% of residues after the third application.

Residues of isopyrazam from these trials conducted in Northern France, Germany and the United Kingdom were (n = 11): < 0.01 (7), 0.012, 0.012, 0.014 and 0.017 mg/kg.

The Meeting estimated a maximum residue level of 0.03 mg/kg for wheat.

The Meeting estimated a median residue level of 0.01 mg/kg for the purpose of calculating animal dietary burdens.

Corresponding total residues of isopyrazam and CSCD459488 were: < 0.015 (7), 0.018, 0.019, 0.019 and 0.026 mg/kg.

The Meeting estimated an STMR at 0.015 mg/kg.

As GAP in the UK covers not only wheat but also rye and triticale, the Meeting decided to extrapolate the maximum residue level, median residue and highest residue for wheat to rye and triticale.

Barley straw and fodder, dry, and forage

The registered use on barley in New Zealand allows two foliar applications per season prior to BBCH growth stage 59 (ear emergence) at a rate of 75 g ai/ha. The PHI is 28 days for forage and 42 days for straw.

Residues of isopyrazam in straw from trials matching GAP in New Zealand were: < 0.01, 0.925 and 1.37 mg/kg.

Residues of isopyrazam in forage from trials matching GAP in New Zealand were: 0.13, 0.304 and 0.655 mg/kg.

Residues of isopyrazam in straw from appropriate trials conducted in Northern Europe matching UK GAP were (n = 8): 0.076, 0.129, 0.349, 0.362, 0.495, 0.679, 0.838 and 1.06 mg/kg.

The Meeting estimated a highest and median residue at 1.06 and 0.356 mg/kg, respectively, for the purpose of calculating animal dietary burdens.

Although a maximum residue level for barley straw and fodder would be 2 mg/kg, as barley and wheat straw are not distinguishable in trade, the Meeting recommended to use a maximum residue level for wheat straw and fodder at 3 mg/kg to cover barley straw and fodder, dry (see next section).

As for forage, since there is no description about PHI for forage, the Meeting selected the highest residue concentration from each trial conducted in Northern Europe in compliance with UK GAP. These residue concentrations were (n = 7): 2.14, 2.26, 2.30, 2.45, 2.93, 3.26 and 3.63 mg/kg.

The Meeting estimated a highest residue and median residue at 3.63 mg/kg and 2.45 mg/kg (as received) respectively for the purpose of calculating animal dietary burdens.

Wheat straw and fodder, dry, and forage

The registered use on barley in New Zealand allows two foliar applications per season prior to BBCH growth stage 69 (end of flowering) at rates of 75–125 g ai/ha. PHI is 28 days for forage and 42 days for straw.

Residues of isopyrazam in straw from trials matching GAP in New Zealand were: 0.284, 0.993 and 1.79 mg/kg.

Residues of isopyrazam in forage from trials matching GAP in New Zealand were: 0.9, 0.397 and 0.835 mg/kg.

Residues of isopyrazam in straw from trials conducted in Northern Europe approximating UK GAP were (n = 11): 0.113, 0.260, 0.288, 0.921, 0.947, 0.952, 0.977, 1.06, 1.11, 1.41 and 1.51 mg/kg.

The Meeting estimated a maximum residue level of 3 mg/kg. The Meeting estimated a highest and median residue at 1.51 and 0.952 mg/kg for the purpose of calculating animal dietary burdens.

As for forage, since there is no description about PHI for forage, the Meeting selected the highest residue concentration from each trial conducted in compliance with GAP. These residue concentrations were (n = 9): 1.17, 1.33, 1.53, 1.88, 2.10, 2.22, 2.25, 2.46 and 2.95 mg/kg.

The Meeting estimated a highest residue and median residue at 2.95 mg/kg and 2.10 mg/kg (as received) respectively for the purpose of calculating animal dietary burdens.

As GAP in the UK covers not only wheat but also rye and triticale, the Meeting decided to extrapolate the maximum residue level for wheat straw and fodder, dry to rye straw and fodder, dry. The median and highest residues for wheat straw and fodder, dry, and for forage were extrapolated to straw and fodder, and forage of rye and triticale.

Fate of residues during processing

High temperature hydrolysis

A high-temperature aqueous hydrolysis study was conducted to determine the nature of degradates of isopyrazam in processed commodities or by-products under conditions typical of common processing practices.

After heating at 90, 100 or 120 °C in acetate buffers of pH 4 and 6 for 20 minutes or in a buffer of pH 5 for 60 minutes, about 95% of recovered radioactivity (> 95% of the initial radioactivity) was isopyrazam. This indicated that isopyrazam was stable against hydrolysis under the above mentioned conditions.

Processing

The Meeting received information on processing of barley to beer and pot barley, and wheat to flour, bread, germ and related by-products.

Processing factors were calculated for the processed commodities of barley and wheat and are shown in the table below. STMR-Ps were calculated for processed commodities of barley and wheat for which maximum residue levels were estimated.

Processed Orange Product	Median Processing factor		STMR-P
	Isopyrazam	Isopyrazam and CSCD459488	
Barley			(0.0375)
Malt	0.55	0.59	0.022
Beer	< 0.13	< 0.12	0.0045
Pot barley	0.37	0.33	0.012
Wheat			(0.015)
Bran (unprocessed)	4.07	4.39	0.066
White flour	0.20	0.23	0.0035
Wholemeal flour	0.73	0.81	0.012
Wholemeal bread	0.50	0.55	0.0083
Wheat germ	0.19	0.25	0.0038

As the residue concentration is higher in bran than in wheat grain, the Meeting estimated a maximum residue level of 0.15 mg/kg by multiplying the maximum residue level for wheat (0.03 mg/kg) by 4.07. A median residue was calculated to be 0.041 mg/kg for the purpose of estimating animal dietary burdens.

Residues in animal commodities

Farm animal dietary burden

Grain, straw and forage of barley, wheat, rye and triticale, and wheat bran may be fed to dairy cattle, beef cattle, broilers and layers. The maximum and mean dietary burdens were calculated using the highest residues or median residues of isopyrazam estimated at the current Meeting on a basis of the OECD Animal Feeding Table.

Summary of livestock dietary burdens (ppm of dry matter diet)

	US-Canada		EU		Australia		Japan	
	max	mean	max	Mean	max	mean	Max	mean
Beef cattle	0.20	0.14	3.65	2.52	12.0[a]	8.40[b]	0.04	0.04
Dairy cattle	2.39	1.71	3.65	2.52	12.0[c]	7.84[d]	0.12	0.09

	US-Canada		EU		Australia		Japan	
	max	mean	max	Mean	max	mean	Max	mean
Broilers	0.04	0.04	0.03	0.03	0.02	0.02	0.00	0.00
Layers	0.04	0.04	1.21[e]	0.87[f]	0.02	0.02	0.01	0.01

[a] Suitable for estimating maximum residue levels for meat, fat and edible offal of cattle.

[b] Suitable for estimating STMRs for meat, fat and edible offal of cattle.

[c] Suitable for estimating maximum residue levels for milk.

[d] Suitable for estimating STMRs for milk.

[e] Suitable for estimating maximum residue levels for meat, fat and edible offal of poultry and eggs.

[f] Suitable for estimating STMRs for meat, fat and edible offal of poultry and eggs.

Residues in milk and cattle tissues

Lactating dairy cows were dosed daily for 28 consecutive days via gelatin capsules containing isopyrazam (15–137 ppm in diet corresponding to 1×, 3× and 10×). The syn/anti ratio was approximately 70:30.

In whole milk samples from the 1× and 3× groups, isopyrazam residues were not found above LOQ. However, isopyrazam was found at a slightly higher level than LOQ in milk samples from the 10× group. Isopyrazam was found in cream samples at < 0.01–0.010, 0.018–0.040 and 0.048–0.141 mg/kg from 1×, 3× and 10× group respectively.

The isopyrazam residues in liver were < 0.01–0.010, 0.019–0.036 and 0.092–0.174 mg/kg from the 1×, 3× and 10× groups, respectively, and the isopyrazam residues in kidney were < 0.01,0.01–0.012 and 0.018–0.042 mg/kg from the 1×, 3× and 10× groups, respectively.

The highest mean residues of isopyrazam in muscle occurred in diaphragm muscle, where residues were < 0.01, 0.010 and 0.024 mg/kg from the 1×, 3× and 10× groups, respectively.

The highest mean residues of isopyrazam in fat were detected in renal fat, where residues were < 0.01, 0.034 and 0.120 mg/kg from the 1×, 3× and 10× groups, respectively.

Residues of CSAA798670, resulting from the hydrolysis of isopyrazam and structurally-related metabolites were also analysed.

CSAA798670 was present in all milk and tissue samples from treated cows and were generally dose dependent. No residues of this common moiety above the limit of quantification of the method (0.005 mg/kg) were seen in any samples of milk or tissues from the control animal.

Residues of CSAA798670 in whole milk, expressed as isopyrazam equivalents, reached a maximum after 3 days of dosing in all three dose groups with mean CSAA798670 residues of 0.039, 0.120 and 0.340 mg/kg from the 1×, 3× and 10× groups, respectively. The mean residues decreased by day 5 to 0.026, 0.067 and 0.184 mg/kg from the 1×, 3× and 10× groups, respectively, and remained approximately at that level during the remainder of the dosing period.

Residue levels in cream were higher than in skimmed milk. The mean residues of CSAA798670 in skimmed milk, expressed as isopyrazam equivalents, were 0.022, 0.069 and 0.189 mg/kg, from the 1×, 3× and 10× groups, respectively. The mean residues in cream were 0.024, 0.081 and 0.262 mg/kg from the 1×, 3× and 10× groups, respectively.

The mean CSAA798670 residues in liver, expressed as isopyrazam equivalents, were 0.219, 0.597 and 1.907 mg/kg from the 1×, 3× and 10× groups, respectively, and the mean residues in kidney were 0.060, 0.162 and 0.658 mg/kg from the 1×, 3× and 10× groups, respectively.

The highest mean residues of CSAA798670 in muscle expressed as isopyrazam equivalents were detected in diaphragm muscle, where residues were 0.022, 0.052 and 0.174 mg/kg from the 1×, 3× and 10× groups, respectively.

The highest mean residues of CSAA798670 in fat expressed as isopyrazam equivalents were detected in renal fat, where residues were 0.028, 0.089 and 0.346 mg/kg from the 1×, 3× and 10× groups, respectively.

Using the dietary burdens for beef and dairy cattle and the results in the lactating cattle feeding study, the maximum residue levels and STMRs were estimated. The calculated residues in cattle tissues and milk are summarized below.

	Feed level (ppm) for milk residues	Residues in milk (mg/kg)	Residues in cream (mg/kg)	Feed level (ppm) for tissue residues	Residues (mg/kg) in			
					Muscle	Liver	Kidney	Fat
Maximum residue level, beef or dairy cattle								
Feeding study [a]	15	< 0.01	0.01	15	< 0.01	0.01	< 0.01	< 0.01
Dietary burden and residue estimate	12	< 0.008	< 0.008	12	< 0.008	0.008	< 0.008	< 0.008
STMR beef or dairy cattle								
Feeding study [b]	15	< 0.01	< 0.01	15	< 0.01	< 0.01	< 0.01	< 0.01
Dietary burden and residue estimate	7.8	0.0042	0.0042	8.4	0.0056	0.0056	0.0056	0.0056

[a] Highest residues for tissues and mean residue for milk

[b] Mean residues for tissues and milk

The Meeting estimated a maximum residue level for isopyrazam in milks, mammalian meat and mammalian fats (except milk fats) at 0.01* mg/kg, and for milk fats at 0.02 mg/kg. The Meeting also estimated a maximum residue level of 0.02 mg/kg for edible offal (mammalian) on a basis of residues in liver.

STMRs were estimated to be 0.0056 mg/kg for mammalian meat, liver, kidney and mammalian fats (except milk fats) and 0.0042 mg/kg for milks and milk fats. HRs were estimated to be 0.008 mg/kg for mammalian meat, liver, kidney and mammalian fat (except milk fats).

Residues in eggs and poultry tissues

No feeding study on laying hens was conducted as the expected dietary burden for hens was low.

In the hen metabolism study conducted at an actual dose of 11 ppm dry matter in the feed, the highest residue (as total radioactive residue, TRR) found was 0.164 mg/kg in parent equivalent in liver.

In the extracts of egg white, egg yolk, liver, skin and attached fat, and peritoneal fat, the highest concentration of isopyrazam observed was 0.004 mg/kg. Muscle was not subject to characterization or identification of radioactive residues as the TRR in muscle was 0.004–0.006 mg/kg in isopyrazam equivalents.

As the calculated maximum and mean dietary burden for estimating a maximum residue level and STMR/HRs for poultry were 1.21 and 0.87 ppm, significantly lower than 11 ppm, the Meeting estimated a maximum residue level of 0.01* mg/kg for isopyrazam in eggs, poultry meat, edible offal of poultry and fat.

STMRs were estimated to be at LOQ of 0.01 mg/kg for eggs, poultry meat, liver and fat. HRs were also estimated to be 0.01 mg/kg (same level as the maximum residue levels) for these commodities.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Dietary Intakes (IEDIs) of isopyrazam were calculated for the 13 GEMS/Food cluster diets using STMRs and STMR-Ps estimated by the current Meeting (Annex 3). The ADI is 0–0.06 mg/kg bw and the calculated IEDIs were 0% of the maximum ADI. The Meeting concluded that the long-term intake of residues of isopyrazam resulting from the uses considered by the current JMPR is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-Term Intakes (IESTI) of isopyrazam were calculated for food commodities and their processed commodities using HRs/HR-Ps or STMRs/STMR-Ps estimated by the current Meeting (Annex 4). The ARfD is 0.3 mg/kg bw and the calculated IESTIs were 0% of the ARfD. The Meeting concluded that the short-term intake of residues of isopyrazam, when used in ways that have been considered by the JMPR, is unlikely to present a public health concern.