5.3 BICYCLOPYRONE (295)

TOXICOLOGY

Bicyclopyrone is the common name approved by the International Organization for Standardization (ISO) for 4-hydroxy-3-[2-(2-methoxy-ethoxymethyl)-6-(trifluoromethyl)-pyridine-3-carbonyl]-bicyclo[3.2.1]oct-3-en-2-one (International Union of Pure and Applied Chemistry [IUPAC] name), with the Chemical Abstracts Service (CAS) number 352010-68-5. Bicyclopyrone is a herbicide that acts by inhibiting 4-hydroxyphenylpyruvate dioxygenase (HPPD), leading to the destruction of chlorophyll in plants. This mode of action is shared with several other herbicide active ingredients, for example, mesotrione, isoxaflutole, topramezone, tembotrione and pyrasulfatole.

Bicyclopyrone has not previously been evaluated by the JMPR and was reviewed by the present Meeting at the request of the CCPR. All critical studies contained statements of compliance with good laboratory practice (GLP), unless otherwise specified.

Biochemical aspects

In metabolism studies conducted in rats, bicyclopyrone was rapidly absorbed (>80%). Times to reach maximum concentrations in blood and plasma (T_{max}) were 1–2 hours at the low and high doses (2 and 200 mg/kg body weight [bw], respectively) and 2–6 hours in tissues. Independent of dose and route (oral or intravenous) of administration, radioactivity declined rapidly in a biphasic pattern. The half-lives of the first phase were 1–3 hours in blood and plasma. The majority of administered radioactivity was excreted in the urine within 24 hours (>80%) and excretion was nearly complete by 7 days after a single dose (98–99%). There was no evidence of bioaccumulation following repeated dosing. Tissue distribution was independent of sex, dose or route of administration. The highest levels of radioactive residues were found in the liver and kidney (up to 4% and 0.4%, respectively). Absorption, pharmacokinetics and total elimination were independent of sex, dose or route of administration. However, males tended to have slightly higher biliary and faecal elimination compared to females.

The levels of radioactivity in the liver following administration of the 200 mg/kg bw dose were only approximately 3 times higher than those following administration of the 2 mg/kg bw dose, despite the 100-fold increase in dose.

Bicyclopyrone is not extensively metabolized with unchanged parent being the principal radioactive component independent of dose or route. The principal routes of biotransformation were via oxidative phase I reactions, namely hydroxylation and *O*-demethylation. Minor routes involved glycine conjugation and cleavage between the pyridinyl and bicyclo rings (each accounting for less than 0.5% of the dose). A quantitative sex difference was apparent in the metabolism of bicyclopyrone; males transformed a higher proportion of parent compound into metabolites than did females. The major component present in the liver was the parent compound.

Toxicological data

The oral and dermal median lethal dose (LD_{50}) for bicyclopyrone in rats was greater than 5000 mg/kg bw. The inhalation median lethal concentration (LC_{50}) was greater than 5.21 mg/L in rats. Bicyclopyrone caused no skin irritation and slight eye irritation in rabbits. It caused no sensitization in the mouse local lymph node assay (LLNA).

Bicyclopyrone inhibits the liver enzyme HPPD, which is involved in the catabolism of tyrosine. The observed ocular effects reported in experimental animals (corneal opacity, keratitis, absent pupillary reflex) are highly correlated with the elevated blood tyrosine levels (tyrosinaemia). Other developmental, thyroid and liver effects may be associated with chemically induced tyrosinaemia, although other mechanisms may also be involved. Severe ocular effects were seen in rats as early as 4 weeks after administration of bicyclopyrone; in dogs the ocular effects were less severe and seen only after 13 weeks at higher dose levels. No ocular effects were observed in mice.

This species-specific sensitivity for ocular opacity and keratitis is related to differences between species in tyrosine clearance. A metabolic pathway to remove tyrosine from the blood involves the liver enzyme tyrosine aminotransferase (TAT). In contrast to rats, mice and humans are unlikely to achieve the levels of plasma tyrosine necessary to produce ocular opacities because murine and human TAT activity is much greater than in rats. Although no data on TAT activity in dogs and rabbits are available, since the ocular effects in dogs are far less severe than in rats and only occur at higher dose levels and after prolonged elevated tyrosine levels, it can be assumed that dogs also have a more efficient metabolic process for handling excess tyrosine than do rats.

In a 90-day oral toxicity study, mice were administered bicyclopyrone in the diet at 0, 100, 3500 or 7000 parts per million (ppm) (equal to 0, 15.4, 543 and 1130 mg/kg bw per day for males and 0, 20.8, 809 and 1340 mg/kg bw per day for females, respectively). The no-observed-adverse-effect level (NOAEL) was 100 ppm (equal to 15.4 mg/kg bw per day) based on increased liver weights at 3500 ppm (equal to 543 mg/kg bw per day).

In a 90-day oral toxicity study, rats were administered bicyclopyrone in the diet at 0, 500, 2000 or 5000 ppm (equal to 0, 51.2, 208, 503 [analytical grade bicyclopyrone] and 518 [technical grade bicyclopyrone] for males and 0, 50.5, 202, 495 [analytical grade bicyclopyrone] and 500 [technical grade bicyclopyrone] for females, respectively). No NOAEL could be identified as ocular toxicity (opacity and keratitis) was observed in males and females at 500 ppm (equal to 50.5 mg/kg bw per day).

In another 90-day oral toxicity study, rats were administered bicyclopyrone in the diet at 0, 2.5, 10, 2500 or 5000 ppm (equal to 0, 0.18, 0.72, 183 and 363 mg/kg bw per day for males and 0, 0.22, 0.88, 229 and 442 mg/kg bw per day for females, respectively). The NOAEL was 10 ppm (equal to 0.72 mg/kg bw per day) based on ocular toxicity (opacities, absent pupillary reflex, keratitis) at 2500 ppm (equal to 183 mg/kg bw per day).

In a 90-day oral toxicity study, dogs were administered bicyclopyrone at 0, 5, 25 or 125 mg/kg bw per day by oral capsule. The NOAEL was 125 mg/kg bw per day, the highest dose tested. Macroscopic and microscopic examinations found no changes in neurological tissues.

In a 1-year oral toxicity study, dogs were administered bicyclopyrone at 0, 2.5, 25 or 125 mg/kg bw per day by oral capsule. Persistent corneal opacity at 25 and 125 mg/kg bw per day was reported from week 13 onwards. Dorsal ganglia chromatolysis and swelling of some neurons was noted at all dose levels without a clear dose–response effect. In addition, degeneration of sciatic nerve and spinal nerve roots was observed in slightly increased incidences in treated animals compared to those of controls. The relevance of these findings in the absence of any clinical neurotoxicity signs is unknown. As these minimal neurological effects could potentially be treatment-related, the lowest-observed-adverse-effect level (LOAEL) was 2.5 mg/kg bw per day, the lowest dose tested.

In an 80-week carcinogenicity study in mice, bicyclopyrone was administered in the diet at 0, 70, 1700 or 7000 ppm (equal to 0, 8.7, 233 and 940 mg/kg bw per day for males and 0, 9.2, 242 and 1027 mg/kg bw per day for females, respectively). The NOAEL for bicyclopyrone was 1700 ppm (equal to 233 mg/kg bw per day) based on decreases in body weight and body weight gain and less efficient feed utilization in males and females treated at 7000 ppm (equal to 940 mg/kg bw per day). There were no tumours considered to be related to treatment with bicyclopyrone.

In a 104-week combined chronic toxicity and carcinogenicity study in rats, bicyclopyrone was administered in the diet at 0, 5, 500, 2500 or 5000 ppm (equal to 0, 0.28, 28.4, 141 and 280 mg/kg bw per day for males and 0, 0.35, 35.8, 178 and 368 mg/kg bw per day for females, respectively, in the carcinogenicity part of the study; the doses in the chronic toxicity study were slightly higher). At 500 ppm (equal to 28.4 mg/kg bw per day) and above, ocular alterations (opacity, keratitis and regenerative hyperplasia of the cornea in males and females, and squamous cell carcinoma and papilloma of the cornea in males only) and focal follicular cell hyperplasia of the thyroid gland in males were observed. No NOAEL could be identified as increased incidences of thyroid hyperplasia were observed after 2 years at the lowest dose, 5 ppm (equal to 0.28 mg/kg bw per day). The NOAEL for carcinogenicity was 5 ppm (equal to 0.28 mg/kg bw per day) based on increased incidences of

squamous cell carcinoma and papilloma of the cornea in males only at 500 ppm (equal to 28.4 mg/kg bw per day) and above.

Several mechanistic studies indicated that bicyclopyrone did not inhibit rat thyroid peroxidase activity in vitro. However, in vivo bicyclopyrone administration in rats resulted in increased levels of tyrosine, decreased triiodothyronine (T_3) and thyroxine (T_4), increased thyroid follicular cell hypertrophy, increased liver weight, increased hepatocellular centrilobular hypertrophy, and increased hepatic uridine diphosphoglucuronosyltransferase (UDPGT) activity. Moreover, dietary treatment of rats for 14 days resulted in a clear dose-dependent increase in both bicyclopyrone and tyrosine plasma concentrations. When the bicyclopyrone dose in rats was increased 20-fold, from 0.5 ppm to 10 ppm, the achieved plasma concentration of bicyclopyrone and tyrosine increased 11.4-fold and 9.7-fold, respectively.

The thyroid effects were observed mainly in male rats and to a lesser extent in female rats; male rats have higher tyrosine levels than female rats and male and female mice, suggesting a relation between thyroid effects and tyrosine plasma concentrations. The relevance of these effects for humans cannot be excluded; however, since the thyroid effects were not observed in mice or dogs, and humans have a more efficient tyrosine clearance mechanism than do rats, humans are unlikely to reach tyrosine levels at which these thyroid effects in rats were observed.

The Meeting concluded that bicyclopyrone is carcinogenic in rats, but not in mice.

Bicyclopyrone did not induce gene mutations in bacteria or mammalian cells in vitro; nor was it clastogenic in human lymphocytes in vitro. It was not clastogenic and did not induce DNA repair in vivo. Bicyclopyrone was tested for genotoxicity in an adequate range of in vitro and in vivo assays. No evidence of genotoxicity was found.

The Meeting concluded that bicyclopyrone is unlikely to be genotoxic.

Ocular tumours have been seen with some but not all HPPD inhibitors, but a progression from corneal damage/repair to ocular tumours has not been demonstrated. Inhibition of HPPD and increase of plasma tyrosine concentrations, as demonstrated for rats and mice in mechanistic studies, could potentially occur in humans. The tumours were only observed in male rats, which have higher plasma tyrosine levels than have female rats and male and female mice, suggesting a relation to tyrosine plasma concentrations. Although the human relevance of these ocular tumours cannot be excluded, the plasma tyrosine levels associated with ocular tumours in rats are unlikely to be achieved in humans because of significantly higher TAT activity.

In view of the lack of genotoxicity, the absence of carcinogenicity in mice and the fact that squamous cell carcinoma and papilloma of the cornea in male rats only were observed at high tyrosine levels unlikely to occur in humans, the Meeting concluded that bicyclopyrone is unlikely to pose a carcinogenic risk to humans from low levels in the diet.

In a two-generation reproduction study, bicyclopyrone was administered to rats in the diet at 0, 25, 500 or 5000 ppm (equal to 0, 1.9, 38.4 and 377 mg/kg bw per day for males and 0, 2.1, 42.2 and 410 mg/kg bw per day for females, respectively). No NOAEL for parental toxicity could be identified as effects on eyes, liver, thyroid and kidney occurred at the lowest dose tested, 25 ppm (equal to 1.9 mg/kg bw per day). The NOAEL for offspring toxicity was 25 ppm (equal to 1.9 mg/kg bw per day) based on effects on eyes, liver, thyroid and kidney at 500 ppm (equal to 38.4 mg/kg bw per day). The NOAEL for reproductive toxicity was 5000 ppm (equal to 377 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study in rats, bicyclopyrone was administered by oral gavage at 0, 100, 500 or 1000 mg/kg bw per day on gestation days 6–20. No NOAEL could be identified for maternal toxicity as transiently reduced lower maternal body weights and body weight gains were observed at 100 mg/kg bw per day, the lowest dose tested. No NOAEL could be identified for embryo/fetal toxicity as skeletal effects consisting of increases in caudal displacement of the pelvic girdle (27 pre-pelvic vertebrae), supernumerary ribs, ossification delays and cartilage changes were

observed at 100 mg/kg bw per day, the lowest dose tested. No increases in malformations were observed.

In a developmental toxicity study in New Zealand White rabbits, bicyclopyrone was administered orally by gavage from gestation days 7 through 28 at 0, 10, 50 or 200 mg/kg bw per day. The NOAEL for maternal toxicity was 50 mg/kg bw per day based on excessive maternal toxicity (mortality, moribundity, abortion, decreased body weight and reduced feed consumption) at the LOAEL of 200 mg/kg bw per day. No NOAEL could be identified for embryo/fetal toxicity as two specific skeletal variations (13th (extra) rib and 27 pre-pelvic vertebrae) were observed at 10 mg/kg bw per day, the lowest dose tested.

In a developmental toxicity study in Himalayan rabbits, bicyclopyrone was administered orally by gavage at 0, 10, 50 or 250 mg/kg bw per day. The NOAEL for maternal toxicity was 50 mg/kg bw per day based on macroscopic findings of irritation in the stomach wall, a possible local effect, at the LOAEL of 250 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 10 mg/kg bw per day based on two specific skeletal variations: costal cartilage asymmetrically aligned at the sternum and 27 pre-pelvic vertebrae, and slight increases in several other skeletal abnormalities at the LOAEL of 50 mg/kg bw per day. At 250 mg/kg bw per day, increases in visceral and skeletal malformations were observed, including heart muscular interventricular septal defects and several cervical vertebral irregularities (e.g. absence, misshapen, fused, supernumerary).

In another developmental toxicity study in Himalayan rabbits, bicyclopyrone was administered orally by gavage at 0, 1, 10 or 250 mg/kg bw per day. The NOAEL for maternal toxicity was 10 mg/kg bw per day based on mortality, decreased body weight gain and signs of stomach irritation at the LOAEL of 250 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 1 mg/kg bw per day based on increases in supernumerary ribs and costal cartilage variations at the LOAEL of 10 mg/kg bw per day. At 250 mg/kg bw per day, increases in external, visceral and skeletal malformations were observed, including jaw and/or palate cleft, heart muscular and/or peri-membraneous interventricular septal defects and several cervical vertebral irregularities (e.g. absence, misshapen, fused).

For rabbits, an overall maternal NOAEL of 50 mg/kg bw per day was identified based on maternal toxicity, including mortality, at 200 mg/kg bw per day.

The Meeting concluded that bicyclopyrone is teratogenic in rabbits but not in rats.

In an acute neurotoxicity study in rats, bicyclopyrone was administered orally by gavage at 0, 20, 200 or 2000 mg/kg bw. The NOAEL was 2000 mg/kg bw, the highest dose tested.

In a 90-day dietary neurotoxicity study, rats were fed bicyclopyrone at 0, 50, 500 or 5000 ppm (equal to 0, 4, 35 and 336 mg/kg bw per day for males and 0, 4, 42 and 415 mg/kg bw per day for females, respectively). No NOAEL for systemic toxicity could be identified; the LOAEL was 50 ppm (equal to 4 mg/kg bw per day) based on the increased incidence of ocular findings at all dose levels. The NOAEL for neurotoxicity was 5000 ppm (equal to 336 mg/kg bw per day), the highest dose tested.

The Meeting concluded that bicyclopyrone is not neurotoxic in rats, but could not exclude the possibility of weak neurotoxicity in the dog.

In a 28-day immunotoxicity study in female mice, bicyclopyrone was administered in the diet at 0, 50, 500 or 5000 ppm (equal to 0, 10.6, 107 and 1190 mg/kg bw per day). No effects were observed in spleen or thymus weights, spleen cell counts or splenic immunoglobulin M (IgM)–antibody-forming cells. The NOAEL for immunotoxicity was 5000 ppm (equal to 1192 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity was 500 ppm (equal to 107 mg/kg bw per day) based on increased liver weights at the LOAEL of 5000 ppm (equal to 1190 mg/kg bw per day).

The Meeting concluded that bicyclopyrone is not immunotoxic.

Toxicological data on metabolites and/or degradates

Some acute toxicity screening data available for SYN503780 indicated that it would not be acutely toxic by the oral and dermal routes, was not sensitizing, and was not mutagenic in a screening Ames test. No toxicological data were available on any other metabolites of bicyclopyrone.

Following application of bicyclopyrone to crops (maize, soya bean and sugar cane), a large number of structurally similar metabolites were detected. The majority of these metabolites were either desmethyl dihydroxylated bicyclopyrone isomers or desmethyl monohydroxylated bicyclopyrone isomers (free and glycoside conjugated forms).

The Meeting noted that the majority of the metabolites are structurally related to bicyclopyrone and fall into one of two groups. The first group comprises compounds that can be hydrolysed to SYN503780; the second group comprises compounds that can be hydrolysed to CSCD686480. All crop fractions also contained numerous other metabolites, all individually less than 10% total radioactive residue (TRR), which would also belong to either of these two groups. Common moiety methods are available to cover these two groups of metabolites.

Based on screening toxicity tests on SYN503780, the detection of several of the quantified metabolites at relatively high levels in the rat and the structural similarities between all the metabolites, the Meeting concluded that these structurally related compounds are unlikely to be more toxic than bicyclopyrone.

The Meeting concluded that the ADI and acute reference dose (ARfD) of bicyclopyrone cover all the structurally related metabolites of SYN503780 and CSCD686480 described above.

Human data

In reports on manufacturing plant personnel, no adverse health effects were noted. No information on accidental or intentional poisoning in humans is available.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.003 mg/kg bw for bicyclopyrone on the basis of a LOAEL of 0.28 mg/kg bw per day for thyroid hyperplasia in the 2-year carcinogenicity study in rats, and using a safety factor of 100 (4×0.83×10×3).

The Meeting considered that the mode of action of bicyclopyrone, inhibition of the liver enzyme HPPD leading to impaired clearance of tyrosine, is relevant to humans. However, humans are less sensitive than rats due to more efficient tyrosine clearance by the liver TAT enzyme. The thyroid effect seen in the 2-year rat study is therefore considered less relevant to human risk assessment, and a safety factor for interspecies variation lower than the usual one of 10 (4×2.5 for kinetic and dynamic factors, respectively) might be used.

Based on the available data and information indicating at least 3-fold higher activity of the human TAT compared to rat TAT, a factor of 2.5/3 = 0.83 could be proposed for toxicodynamic interspecies differences. The resulting interspecies safety factor would be 4×0.83 = 3.3. The intraspecies safety factor would remain at 10, which results in a safety factor of 33.

To convert from a LOAEL to a NOAEL an additional safety factor is needed. There was only a slight dose response in thyroid hyperplasia without an increase in severity and at the low dose without any other thyroid effects such as weight changes or concomitant hypertrophy. Therefore, the Meeting considered that a factor of 3 was adequate and that the total safety factor to be applied to the LOAEL would be 33 × 3 = 100, resulting in the ADI of 0.003 mg/kg bw per day.

This ADI provides a margin of exposure of nearly 10 000 to the ocular tumours in male rats.

This ADI was supported by the LOAEL of 2.5 mg/kg bw per day in the 1-year toxicity study in dogs, based on minimal possible neurological effects.

The Meeting established an ARfD of 0.01 mg/kg bw for bicyclopyrone for women of childbearing age on the basis of the overall NOAEL of 1 mg/kg bw per day for skeletal variations in rabbit fetuses, and using a safety factor of 100.

The Meeting concluded that it was not necessary to establish an ARfD for bicyclopyrone for the remainder of the population in view of its low acute oral toxicity and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

The Meeting concluded that the ADI and ARfD for bicyclopyrone could be applied to all structurally related metabolites of SYN503780 and CSCD686480, expressed as bicyclopyrone.

A toxicological monograph was prepared.

Levels relevant to risk assessment of bicyclopyrone

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighty-week study of carcinogenicity[a]	Toxicity	1 700 ppm, equal to 233 mg/kg bw per day	7 000 ppm, equal to 940 mg/kg bw per day
		Carcinogenicity	7 000 ppm, equal to 940 mg/kg bw per day[b]	–
Rat	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	–	5 ppm, equal to 0.28 mg/kg bw per day[c]
		Carcinogenicity	5 ppm, equal to 0.28 mg/kg bw per day	500 ppm, equal to 28.4 mg/kg bw per day
	Two-generation studies of reproductive toxicity[a,d]	Reproductive toxicity	5 000 ppm, equal to 377 mg/kg bw per day[b]	–
		Parental toxicity	–	25 ppm, equal to 1.9 mg/kg bw per day[c]
		Offspring toxicity	25 ppm, equal to 1.9 mg/kg bw per day	500 ppm, equal to 38.4 mg/kg bw per day
	Developmental toxicity study[d,e]	Maternal toxicity	–	100 mg/kg bw per day[c]
		Embryo/fetal toxicity	–	100 mg/kg bw per day[c]
Rabbit	Developmental toxicity studies [d,e]	Maternal toxicity	50 mg/kg bw per day	200 mg/kg bw per day
		Embryo/fetal toxicity	1 mg/kg bw per day	10 mg/kg bw per day
Dog	One-year study of toxicity[f]	Toxicity	–	2.5 mg/kg bw per day[c]

[a] Dietary administration.

[b] Highest dose tested.

[c] Lowest dose tested.

[d] Two or more studies combined.

[e] Gavage administration.

[f] Capsule administration.

Estimate of acceptable daily intake (ADI) applies to bicyclopyrone and all structurally related metabolites of SYN503780 and CSCD686480, expressed as bicyclopyrone

0–0.003 mg/kg bw

Estimate of acute reference dose (ARfD) applies to bicyclopyrone and all structurally related metabolites of SYN503780 and CSCD686480, expressed as bicyclopyrone

0.01 mg/kg bw (women of childbearing age)

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; information on mode of action and human relevance of thyroid effects; information on TAT activity of dogs and rabbits compared to that of rats and humans

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; extensive (>80%)
Dermal absorption	No data
Distribution	Highest residues in liver and kidneys
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Largely complete within 24 hours; primarily via urine
Metabolism in animals	Mostly excreted unchanged; slight sex difference with males forming more metabolites
Toxicologically significant compounds in animals and plants	Bicyclopyrone and metabolites structurally related to SYN503780 and CSCD686480
Acute toxicity	
Rat, LD_{50}, oral	>5 000 mg/kg bw
Rat, LD_{50}, dermal	>5 000 mg/kg bw
Rat, LC_{50}, inhalation	>5.21 mg/L
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Slightly irritating
Mouse, dermal sensitization	Non-sensitizing (LLNA)
Short-term studies of toxicity	
Target/critical effect	Eye
Lowest relevant oral NOAEL	0.72 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	50 mg/kg bw per day (rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of carcinogenicity	
Target/critical effect	Thyroid
Lowest relevant oral NOAEL	< 0.28 mg/kg bw per day (rat)
Carcinogenicity	Ocular tumours in rats at 28.4 mg/kg bw per day, possibly not relevant to humans[a] Not carcinogenic in mice[a]
Genotoxicity	

	No evidence of genotoxicity in vitro or in vivo[a]
Reproductive toxicity	
Target/critical effect	Eye, liver, thyroid, kidney
Lowest relevant parental NOAEL	<1.9 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	1.9 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	377 mg/kg bw per day (rat)[b]
Developmental toxicity	
Target/critical effect	Maternal mortality, body weight, fetal skeletal abnormalities
Overall maternal NOAEL	50 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	1 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw (rat)[b]
Subchronic neurotoxicity NOAEL	336 mg/kg bw per day (rat)[b]
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity	NOAEL: 1 192 mg/kg bw per day (mouse)[b]
Studies on toxicologically relevant metabolites	SYN503780: screening studies do not indicate acute toxicity (oral, dermal), skin-sensitizing or mutagenic properties
Mechanistic/mode of action studies	
	HPPD inhibition is plausible for humans. The ocular tumours and thyroid hyperplasia could be due to the male rat–specific high tyrosine levels; however, human relevance cannot be excluded. Humans have a significantly higher TAT activity than do male rats and are unlikely to exhibit the toxicities seen in rats
Human data	
	No adverse effects in manufacturing personnel

[a] Unlikely to pose a carcinogenic risk to humans via exposure from the diet.

[b] Highest dose tested.

Summary

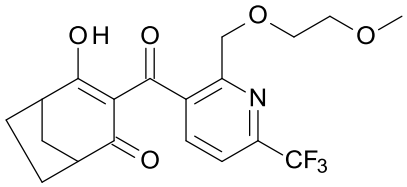
	Value	Study	Safety factor
ADI[a]	0–0.003 mg/kg bw	Two-year study of toxicity (rat)	100
ARfD[a,b]	0.01 mg/kg bw	Developmental studies (rabbit)	100

[a] Applies to bicyclopyrone and all structurally related metabolites of SYN503780 and CSCD686480, expressed as bicyclopyrone.

[b] Applicable to women of childbearing age.

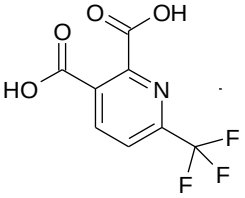
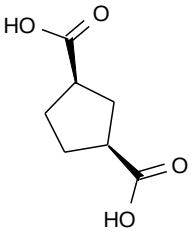
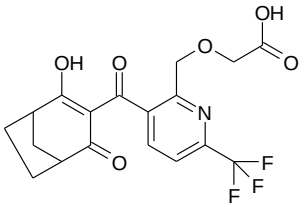
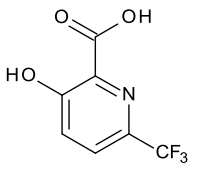
RESIDUE AND ANALYTICAL ASPECTS

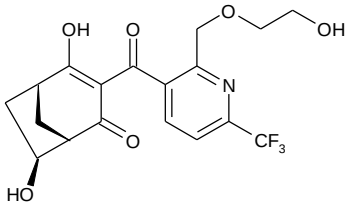
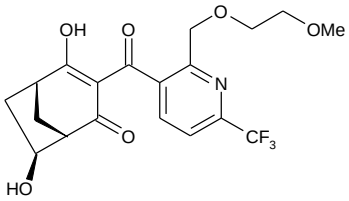
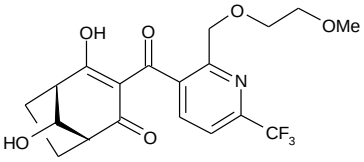
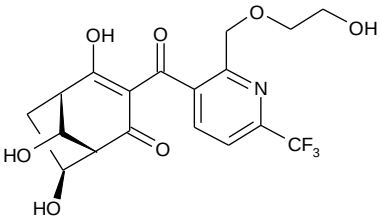
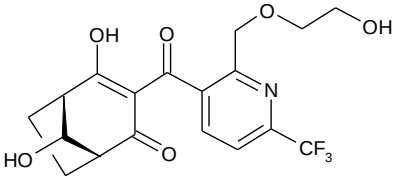
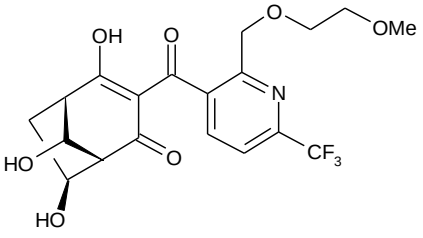
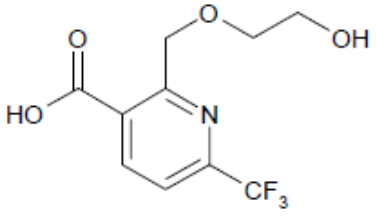
Bicyclopyrone is a selective herbicide developed for the control of broadleaf weeds and perennial grasses in corn, wheat, barley and sugar cane and is registered in a number of countries. At the 48th Session of the CCPR (2016), it was scheduled for toxicological and residue evaluation as a new compound by the 2017 JMPR.

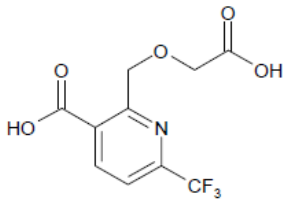
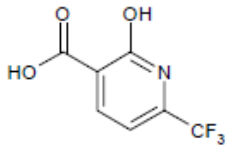
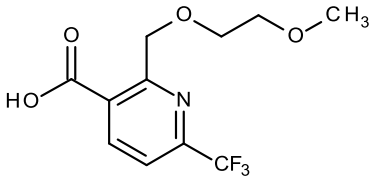
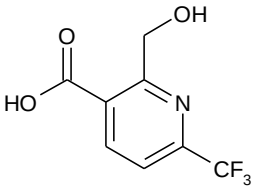
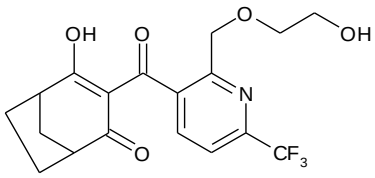


Information on the physical and chemical properties, animal and plant metabolism, environmental fate, analytical methods, storage stability, use patterns, supervised trials, processing and farm animal feeding was received by the present Meeting.

The following abbreviated names were used for the metabolites discussed below.

Compound Name/Code	Chemical name (IUPAC)	Structure	Occurrence in metabolism studies
CSCC163768 SYN504810	6-(trifluoromethyl)pyridine-2,3-dicarboxylic acid		Plants Soil Aqueous photolysis
CSAA589691 (NOA412101)	(1S,3R)-cyclopentane-1,3-dicarboxylic acid		Plants Soil Aqueous photolysis Rat cage wash
CSCD642512 (SYN545859)	2-[[3-(2-hydroxy-4-oxo-bicyclo[3.2.1]oct-2-ene-3-carbonyl)-6-(trifluoromethyl)-2-pyridyl]methoxy]acetic acid		Plants Soil
CSCD656832 (SYN545680)	3-hydroxy-6-(trifluoromethyl)pyridine-2-carboxylic acid		Plants Soil

Compound Name/Code	Chemical name (IUPAC)	Structure	Occurrence in metabolism studies
CSCD675162	*rac*-(1R,5S,6S)-2,6-dihydroxy-3-[2-(2-hydroxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carbonyl]bicyclo[3.2.1]oct-2-en4-one		Plants Rat Goat Hen
CSCD675164	*rac*-(1R,5S,6S)-2,6-dihydroxy-3-[2-(2-methoxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carbonyl]bicyclo[3.2.1]oct-2-en-4-one		Plants Rat Goat Hen
CSCD677306	*rac*-(1S,5R)-2,8-dihydroxy-3-[2-(2-methoxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carbonyl]bicyclo[3.2.1]oct-2-en-4-one		Plants Rat Goat Hen
CSCD677692	*rac*-(1S,5R,6S)-2,6,8-trihydroxy-3-[2-(2-hydroxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carbonyl]bicyclo[3.2.1]oct-2-en-4-one		Plants Rat
CSCD677693	*rac*-(1S,5R)-2,8-dihydroxy-3-[2-(2-hydroxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carbonyl]bicyclo[3.2.1]oct-2-en-4-one		Plants Rats Goat Hen
CSCD677694	*rac*-(1S,5R,6S)-2,6,8-trihydroxy-3-[2-(2-methoxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carbonyl]bicyclo[3.2.1]oct-2-en-4-one		Plants
CSCD686480 (SYN545910)	2-(2-hydroxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid		Plants Goat

Compound Name/Code	Chemical name (IUPAC)	Structure	Occurrence in metabolism studies
CSCD686481 (SYN545911)	2-(carboxymethyloxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid		Plants
CSAA757083 (SYN510579)	2-hydroxy-6-(trifluoromethyl)pyridine-3-carboxylic acid		Plants Soil
CSAA794148 (SYN503780)	2-(2-methoxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid		Rat Soil Aqueous photolysis
CSAA806573 (NOA451778)	2-(hydroxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid		Plants Rat Soil Aqueous photolysis
CSAA915194 NOA454598	2-hydroxy-3-[2-(2-hydroxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carbonyl]bicyclo[3.2.1]oct-2-en-4-one		Plants Rat Goat Hen

Plant metabolism

The Meeting received information on the fate of bicyclopyrone in maize, sugar cane and soya bean.

Maize study

Field grown maize (*Zea mays*) received either a single pre-emergent treatment of 200 g ai/ha or a pre-emergence treatment of 200 g ai/ha followed by a post emergence treatment at 200 g ai/ha at the 8 to 9 leaf stage. Samples were taken from each treatment at three timings: early foliage (28 days after the post emergence application; foliage only), forage (BBCH 75–79; foliage immature cobs and immature grain) and crop maturity (BBCH 89; stover, cobs and grain).

The TRR in early foliage, forage and stover receiving only the pre-emergence application were 0.033, 0.023 and 0.032 mg eq/kg, respectively, for the [bicyclooctenone-6,7-^{14}C2]-labelled experiment and 0.042, 0.083 and 0.077 mg eq/kg, respectively, for the [pyridine-3-^{14}C]-labelled experiment. Values in immature cobs, immature grain, mature cobs and mature grain were ≤ 0.003 mg eq/kg for the [bicyclooctenone-6,7-^{14}C2]-labelled experiment and ≤ 0.005 mg eq/kg for the [pyridine-3-^{14}C]-labelled experiment.

The TRR in early foliage, forage and stover from the combined pre- and post-emergence application regime were 0.35, 0.46 and 0.46 mg eq/kg, respectively, for the [bicyclooctenone-6,7-^{14}C2]-labelled experiment and 0.44, 0.92 and 0.76 mg eq/kg, respectively, for the [pyridine-3-^{14}C]-labelled experiment. Values in immature cobs, immature grain, mature cobs and mature grain were 0.029, 0.037, 0.036 and 0.058 mg eq/kg, respectively, for the [bicyclooctenone-6,7-^{14}C2]-labelled experiment and 0.033, 0.020, 0.018 and 0.025 mg eq/kg, respectively, for the [pyridine-3-^{14}C]-labelled experiment.

Analysis of forage, stover and grain samples from the combined applications showed that bicyclopyrone is extensively metabolised and that no or only very minor residues of bicyclopyrone were present (≤ 4.3% TRR; ≤ 0.009 mg eq/kg). At least four desmethyl dihydroxylated bicyclopyrone isomers were shown to be present which collectively accounted for up to 36% TRR (0.33 mg eq/kg; all in the free metabolite form) and individually up to 21% TRR (CSCD677692: 0.19 mg eq/kg). Two desmethyl monohydroxy isomers of bicyclopyrone were shown to be present which collectively accounted for up to 22% TRR (0.200 mg eq/kg) and individually up to 8% TRR (CSCD677693: 0.07 mg eq/kg; as the free metabolite) or up to 14% TRR (CSCD675162: 0.13 mg eq/kg; total for the free and glycoside conjugated forms). CSAA589691, was shown to be present in immature and mature grain at levels up to 49% TRR (0.024 mg eq/kg).

Sugar cane study

The metabolism of bicyclopyrone in sugar cane was investigated using a single post-emergent treatment of 300 g ai/ha applied to cane plants at the 7–8 leaf stage (BBCH 17–18). Samples of immature foliage were collected 42 days after treatment (BBCH 23–24). Mature foliage (all leaves) and cane were collected 301 days after treatment (BBCH 39).

The TRRs in sugar cane foliage, sampled 42 days after treatment, were 0.78 mg eq/kg and 0.89 mg eq/kg for the [bicyclooctenone-6,7-^{14}C2] and [pyridine-3-^{14}C]-bicyclopyrone labelled experiments, respectively. Residues in foliage at maturity were 0.004 mg eq/kg and 0.003 mg eq/kg respectively. The TRRs in the cane harvested at maturity were 0.002 mg eq/kg and 0.004 mg eq/kg for the [bicyclooctenone-6,7-^{14}C2] and [pyridine-3-^{14}C]-bicyclopyrone labelled experiments respectively.

Extractable residues in immature foliage represented 85% and 88% TRR for the [bicyclooctenone-6,7-^{14}C2]-bicyclopyrone and [pyridine-3-^{14}C]-bicyclopyrone labelled experiments respectively. The mature cane and foliage were not extracted since residues were below 0.01 mg eq/kg.

Bicyclopyrone was not detected in immature foliage. The most significant metabolite detected was the desmethyl monohydroxy metabolite CSCD677693 which was present as both the free form (17 to 18% TRR, 0.14 to 0.16 mg eq/kg) and as a glycoside conjugate (5.6 to 7.1% TRR, 0.05–0.055 mg eq/kg). Two other demethylated metabolites of bicyclopyrone were present, the desmethyl monohydroxy metabolite CSCD675162 (9.9 to 13% TRR, 0.088 to 0.098 mg eq/kg) and the desmethyl dihydroxy metabolite CSCD677692 (5.5 to 6.5% TRR, 0.043 to 0.058 mg eq/kg).

CSCD677306, the monohydroxy metabolite of bicyclopyrone, was present in both the free form and conjugated as the glycoside (4.6 to 5.7% TRR, 0.036 to 0.051 mg eq/kg and 11 to 13% TRR, 0.095 to 0.10 mg eq/kg respectively). Two other glycosides of monohydroxylated bicyclopyrone; including the glycoside of CSCD675164, were detected (2 to 4.2% TRR, 0.018 to 0.033 mg eq/kg and 2.3 to 3.5% TRR, 0.02 to 0.027 mg eq/kg). The dihydroxy metabolite CSCD677694 (8.1 to 9.3% TRR, 0.072 mg eq/kg) was also observed.

Detected metabolites that contained only the pyridine ring of bicyclopyrone were identified as CSCD686480, which was present in both the free form (2.7% TRR, 0.024 mg eq/kg) and as a glycoside (17% TRR, 0.15 mg eq/kg) and CSCD686481 (6.4% TRR, 0.057 mg eq/kg).

The unextracted residues remaining after the initial solvent extraction were further investigated by sequential extraction with 0.1 M and 1M HCl at 40 ºC and 90 ºC respectively after rehydration with water. The water released 0.5–0.7% TRR (0.004 to 0.0005 mg eq/kg), the mild acid

0.6% TRR (0.005 mg eq/kg) and the stronger acid conditions 2.6 to 4.3% TRR (0.023 to 0.03 mg eq/kg). A further 7.8 to 8.9% TRR, corresponding to 0.069 mg eq/kg in each debris fraction, remained unextracted.

Soya bean study

Bicyclopyrone metabolism in greenhouse grown soya bean was investigated following a single pre-emergent application, at a rate of 186.1 g ai/ha.

Harvesting of forage occurred 35 to 36 days after treatment (DAT) at BBCH 16–21, of hay 62–63 DAT at BBCH 65–74 and of mature beans 113–114 DAT at BBCH 89. The TRRs in the beans were 0.19 mg eq/kg and 0.21 mg eq/kg for the [bicyclooctenone-6,7-$^{14}C_2$] and [pyridine-3-^{14}C] labelled experiments, respectively, while the residue in the corresponding hay samples reached 0.15 mg eq/kg and 0.19 mg eq/kg. The TRR in the forage samples were significantly lower, reaching only 0.02 mg eq/kg and 0.031 mg eq/kg for the [bicyclooctenone-6,7-$^{14}C_2$] and [pyridine-3-^{14}C] labelled experiments, respectively.

In mature beans, bicyclopyrone was detected at 15% TRR (0.029 mg eq/kg) and 13% TRR (0.026 mg eq/kg) in the [bicyclooctenone-6,7-$^{14}C_2$] and [pyridine-3-^{14}C] experiments, respectively. The most significant metabolites detected were the monohydroxy metabolite CSCD675164 (14–18% TRR; 0.028–0.034 mg eq/kg) and the demethylated metabolite of bicyclopyrone, CSAA915194 at 7.6–8.5% TRR (0.015–0.018 mg eq/kg).

The desmethyl monohydroxy and dihydroxy metabolites CSCD677306, CSCD677694, CSCD675162, CSCD677693, CSCD642512, CSCD686480, CSCD656832 and CSCC163768 were also present but at low levels.

In bean hay, bicyclopyrone was detected at low levels of 3.3 and 3.9% TRR for the [pyridine-3-^{14}C] and [bicyclooctenone-6,7-$^{14}C_2$] experiments respectively with both corresponding to a residue of 0.006 mg eq/kg. The most significant metabolite detected was the desmethyl monohydroxy metabolite CSCD675162 (14–16% TRR; 0.023–0.027 mg eq/kg). A second desmethyl monohydroxy metabolite CSCD677693 was also present (5.6–6.7% TRR; 0.01–0.011 mg eq/kg) along with the desmethyl metabolite CSAA915194 (4.7–6.2% TRR; 0.009 mg eq/kg). Two monohydroxy metabolites were also observed, CSCD675164 (7.2–9.2% TRR; 0.014 mg eq/kg).

The metabolites CSCD677306, CSAA915194 and CSCD677694 was also present but at low levels. Metabolites that contained only the pyridine ring of bicyclopyrone were identified as CSCD686480 (14.2% TRR; 0.027 mg eq/kg) and CSCD656832 (1.2% TRR; 0.002 mg eq/kg). There were no bicyclooctenone specific metabolites.

In bean forage, residues was detected at low levels from both the radiolabelled experiments (0.02 and 0.031 mg eq/kg for the [bicyclooctenone-6,7-$^{14}C_2$] and [pyridine-3-^{14}C] experiments respectively). In the forage treated with [bicyclooctenone-6,7-$^{14}C_2$]-bicyclopyrone, bicyclopyrone was detected at levels of 10% TRR (0.002 mg eq/kg). The remaining residue consisted of at least four distinct radioactive components none of which exceeded 6.8% TRR (0.001 mg eq/kg). In the [pyridine-3-^{14}C] experiment, bicyclopyrone was also detected at very low levels (9.2% TRR; 0.003 mg eq/kg) and the remainder of the radioactive residue was shown to consist of at least 10 distinct radioactive components none exceeding 11% (0.003 mg eq/kg).

Generally in all investigated plants, the metabolic pathways are similar but with low residues in all soya bean commodities. Unchanged bicyclopyrone was found in corn forage, soya bean seed and hay and was absent in all other samples examined. The majority of the metabolites were formed by hydroxylation on one or more sites on the bicyclic ring or demethylation of the methoxyethoxymethyl side chain followed by hydroxylation. Some glycoside conjugation of the hydroxyl derivatives and some cleavage between the two ring systems was observed. CSAA589691, was found in mature maize grain at levels up to 42% TRR.

Animal metabolism

The Meeting received information on the fate of orally-dosed bicyclopyrone in rat, lactating goats and laying hens. In metabolism studies, total radioactive residues are expressed in mg/kg bicyclopyrone equivalents unless otherwise stated.

Rat

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

Lactating goats

Lactating goats were orally dosed with [Pyridine-3-^{14}C]-bicyclopyrone or [Bicyclooctenone-6,7-^{14}C2]-bicyclopyrone, equivalent to 34 ppm in the feed for 7 consecutive days. The majority of the administered dose was recovered in urine (60% [pyridinyl label] and 62% [bicyclooctenone label] , with moderate amounts recovered in the faeces, 6.5% and 6.2% for the pyridinyl and bicyclooctenone labels, respectively.

Highest TRR levels were found in the liver (2.7 mg eq/kg and 3.0 mg eq/kg for pyridinyl and bicyclooctenone labels, respectively). TRR levels in other samples of kidney, muscle and fat were 0.008–1.33 mg eq/kg and 0.008–1.42 mg eq/kg for pyridinyl and bicyclooctenone labels, respectively.

TRR levels in milk (mean for a 24 hour period) reached a plateau of about 0.008 mg eq/kg for both radiolabels at approximately 2 to 3 days.

Extractability of radioactivity from milk with hexane was high, greater than 95%. In other tissues extractability with solvents (e.g. acetonitrile, acetonitrile:water (4:1, v/v), acetonitrile:water (3:7, v/v) and water) ranged from 86 to 98%, with the exception of renal fat (63 to 71%), where very low levels of residues were found (< 0.016 mg eq/kg). Unextracted residues were either < 10% TRR or < 0.05 mg eq/kg.

Unchanged bicyclopyrone was identified in all samples. The lowest levels of bicyclopyrone were found in the liver (pyridinyl label 16% TRR, 0.44 mg eq/kg), and the highest in kidney (bicyclooctenone label 50% TRR, 0.64 mg eq/kg).

The most abundant metabolite detected in all commodities was CSAA915194. This compound was the principal component of the residue in liver and milk (maximum 70% TRR, 1.92 mg eq/kg (pyridinyl label) and 60% TRR, 0.01 mg eq/kg (bicyclooctenone label) respectively for the two commodities).

Laying hens

Laying hens were orally dosed with [Pyridine-3-^{14}C]-bicyclopyrone and [Bicyclooctenone-6,7-^{14}C2]-bicyclopyrone, at a dose equivalent to 24 or 22 ppm in feed for 10 consecutive days. The majority of the administered dose was recovered in excreta76% of both labels.

More than 84% of radioactivity in tissue samples was extracted by solvents (e.g. acetonitrile, acetonitrile:water (4:1, v/v), acetonitrile:water (3:7, v/v) and water). The majority of tissue-bound radioactivity was found in liver (1.75 mg eq/kg and 1.78 mg eq/kg for pyridinyl and bicyclooctenone labels respectively) and accounted for only ca. 0.3% of the administered dose. Residue levels in the other samples of muscle, egg yolk, egg white, peritoneal fat and skin and subcutaneous fat were 0.084–0.54 mg eq/kg.

Radioactive residues in eggs (mean for a 24 hour period) reached a plateau of 0.1 mg eq/kg for both labels at approximately 6 to 8 days.Eggs contributed a minor route of excretion of radioactivity, with daily recoveries not exceeding 0.017% of dose, equivalent to 0.14 mg eq/kg.

Radioactive residues in edible tissues predominantly consisted of parent bicyclopyrone (> 73% TRR). The metabolite CSAA915194 was detected at up to 3% TRR in egg yolk, egg white, liver, muscle and peritoneal fat. CSCD675164 and CSCD677306 were detected at very low levels in

liver (1.6% TRR, 0.029 mg eq/kg and 2% TRR, 0.035 mg eq/kg respectively) with the bicyclooctenone label.

The metabolite CSCD677692 was detected in liver and excreta but at levels too low to quantify. CSCD675162 was detected at low levels in peritoneal fat (5.4% TRR, 0.01 mg eq/kg) from the bicycloctenone label and in egg yolk (2.2% TRR, 0.002 mg eq/kg) from the pyridinyl label. CSCD677693 was detected in excreta only. All other metabolites detected for both labels were ≤ 0.009 mg eq/kg irrespective of detection method or label.

In summary, the primary metabolic processes observed include O-demethylation, oxidation on one or more sites of the bicyclooctenone ring, a minor amount of bridge cleavage between the rings, and conjugation to some extent. The tissue residues in both animals consisted primarily of parent bicyclopyrone (hen and goat) and CSAA915194 (desmethyl parent) (goat) and several very minor metabolites found in the liver for the goat and several samples for the laying hen. The major metabolites observed in lactating goat and hen were also observed in rats.

Environmental fate

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

Aerobic degradation in soil

Aerobic degradation of [bicyclooctenone] and [pyridine]-^{14}C-bicyclopyrone under laboratory conditions was studied at 20 °C in various soil types treated at 0.27 mg /kg dry soil (200 g ai/ha).

Although the rate of transformation of bicyclopyrone differed between soils, the same transformation products were observed in each soil indicating a similar route of transformation. Bicyclopyrone was extensively mineralised to carbon dioxide. The major metabolites identified in soils were SYN503780, and CSCD642512. The three minor metabolites identified in the tested soil were, CSCD656832, CSCD163768 and CSAA757083

The half-life for bicyclopyrone was estimated at 108 days for clay loam soils, 141–331 days for loamy sand soils, 59–357 days for sandy loam soils, 89 days for silt clay soils, 69 days for silt loam soils, 159 days for silt clay loam soils and 20–59 days for loamy soils.

The Meeting concluded that bicyclopyrone is moderately persistent to persistent in soil.

Aerobic degradation of the major metabolites SYN503780 was investigated in three European soils. There were three extractable metabolites present at ≥ 5% of applied radioactivity (CSCD656832, CSCC163768 and CSAA757083). The half-lives for the metabolite SYN503780 were in range 4–9 days.

The Meeting concluded the metabolite SYN503780 is not persistent in soil.

Soil photolysis

Photolysis of bicyclopyrone was studied in dry and moist soils irradiated with artificial sunlight for the equivalent to 30 summer days.

Dry layer tests

There was no degradation in samples incubated in the dark. In irradiated samples there was only one degradate present at ≥ 5% of applied radioactivity, namely SYN503780 (maximum 17.2% at 12 DAT). The two minor degradates were CSAA589691 (bicyclo label) and CSCC163768 (pyridinyl label). Calculated photodegradation DT_{50} values for bicyclopyrone were 50–64 days (dry soil).

Moist layer tests

Degradation was more significant in irradiated and dark moist soil samples In addition to parent, four known degradates were identified from the pyridinyl label, one of which was present at ≥ 10% of

applied radioactivity, namely SYN503780 (maximum 25%); CSCC163768, CSCD656832 and CSCD642512 were minor degradates. Calculated photodegradation DT_{50} values for bicyclopyrone were 24–25 days (moist soil).

In addition, the photolysis of bicyclopyrone was investigated in moist soil taken from three sites in the US. Under continuous irradiation, photolytic DT_{50} values for bicyclopyrone in the three moist soils were in the range 2–5.7 days. When adjusted to equivalent summer days at latitudes 30–50 °N, the DT_{50} values ranged from 3.9 to 11 days. Degradation involved cleavage of the bridge between the two ring systems and the main photodegradation product was SYN503780.

In summary, the major metabolites identified in soils were SYN503780 (up to 25%), and CSCD642512. In soil photolysis, SYN503780 was present at ≥ 10% of applied radioactivity.

Hydrolysis

Bicyclopyrone was stable to hydrolysis at pH values ranging from 4 to 9. Based on hydrolysis results, the DT_{50} was extrapolated to be > 1 year at 25 °C.

Aqueous Photolysis

Bicyclopyrone was extensively degraded under simulated sunlight. Degradation was pH-dependent in the order pH 5 > pH 7/natural water > pH 9. The two main photodegradation products at pH 5 were CSAA589691 from the bicyclo ring system and CSCC163768 from the pyridine ring system. Based on aqueous photolysis results, the DT_{50} values ranged from 10 to 50 days.

Residues in succeeding crops

A confined rotational crop study was conducted to examine the nature and level of residues of bicyclopyrone in succeeding crops. [^{14}C] - bicyclopyrone was applied to the soil of a planting container by spray application at a nominal rates of 200 g ai/ha or 350 g ai/ha.

Rotational crops (wheat, spinach and turnips) were sown at plant back intervals of 30, 120 and 270 days after application. Due to phytotoxicity of the test item to spinach and turnip, further sowings of both were made at 60 DAA and of spinach only at 180 DAA.

Low levels of bicyclopyrone were detectable in wheat (up to 5.8% TRR and 0.026 mg eq/kg) and turnip foliage (up to 3.8% TRR and 0.001 mg eq/kg). Higher residues were determined in spinach plants exhibiting phytotoxicity (up to 70% TRR and 0.03 mg eq/kg).

Two monohydroxy bicyclopyrone isomers, shown to be present in wheat in both the free and glycoside conjugated metabolite forms, collectively accounted for up to 29% TRR and 0.093 mg eq/kg. Individually these isomers accounted for up to 24% TRR and 0.082 mg eq/kg (CSCD677306) and up to 25% TRR and 0.082 mg eq/kg (CSCD675164). The free metabolites were also found to be present in early rotation turnip foliage but at much lower absolute residue levels, accounted for up to 11% TRR and 0.002 mg eq/kg (CSCD677306) and up to 34% TRR and 0.007 mg eq/kg(CSCD675164).

Two desmethyl monohydroxy-bicyclopyrone isomers, shown to be present in wheat in only the free metabolite form, collectively accounted for up to 27% TRR and 0.11 mg/kg. Individually these metabolites accounted for up to 13% TRR and 0.057 mg eq/kg (CSCD677693) and up to 19% TRR and 0.053 mg eq/kg (CSCD675162).

Two metabolites present in wheat, with structures that retained only the pyridine ring of bicyclopyrone, both of which were found in the free and glycoside conjugated metabolite forms, accounted for up to 21% TRR and 0.10 mg/kg (CSCD686480) and up to 41% TRR and 0.064 mg eq/kg (CSCD656832). CSCD656832 was also present in turnip foliage but at much lower absolute residue levels, accounting for up to 71% TRR and 0.012 mg eq/kg.

A dihydroxy-bicyclopyrone metabolite (CSCD677694), shown to be present in wheat in the free form, accounted for up to 13% TRR and 0.057 mg eq/kg. Significant proportions of the residue in wheat grain (up to 37% TRR) were shown to be attributable to naturally incorporated radioactivity.

CSAA757083, a known soil metabolite was found at very low levels (2% TRR, 0.004 mg eq/kg) in wheat hay from the 120-day plant-back interval. Quantitatively, metabolites resulting from bridge cleavage were more prevalent in the rotational crops than the primary crops and were formed to a larger extent in the later plant-back intervals compared with the crops at the 30 day interval.

In a field rotational crop study with nine trials, bare ground was treated with bicyclopyrone formulated as an emulsifiable concentrate (EC) at a rate of 200 g ai/ha. Radish (root and tuber vegetable), spinach (leafy vegetable) and wheat (cereals) were planted 90, 150, 187, and 270 days after the application of the test substance and harvested at typical intervals reflecting normal farming practice.

No residues of bicyclopyrone or SYN503780 (Method GRM030.03A) were found for any sample at any time interval. The only detectable residues found were either SYN503780 or CSCS686480 (Common Moiety Method – GRM030.05A).

A second study was conducted to determine possible uptake levels in wheat commodities. Bicyclopyrone was applied to bare-ground at a rate of 200 g ai/ha. Winter wheat was planted 90 days after application and spring wheat 270 days after application. The rotational wheat was harvested at normal maturity to provide samples of forage (autumn and/or spring), hay, grain, and straw.

The only residues found above the limit of quantification were of bicyclopyrone, analysed directly using method GRM030.03A, and of common moiety SYN503780, analysed via method GRM030.05A, in autumn forage (45 DAP). In the decline trials, these residues decreased with longer intervals to harvest. All other residues were <LOQ in all matrices, including processed fractions.

In summary, bicyclopyrone related residues in soil could contribute to residues observed in rotational and primary crops.

Methods of analysis

The Meeting received description and validation data for analytical methods of bicyclopyrone related residues in plant and animal commodities.

The metabolism of bicyclopyrone in crops and livestock resulted in numerous different metabolites in the various crop fractions. Most of these metabolites fell into two groups. The first group (compounds structurally related to SYN503780) produce SYN503780 on base hydrolysis and the second group (compounds structurally related to CSC686480) produce CSC686480 on base hydrolysis.

Most of the methods developed to quantify bicyclopyrone residues in plants and animal commodities involve a hydrolysis step to convert bicyclopyrone and its metabolites to either SYN503780 or CSC686480. Any non-metabolised parent bicyclopyrone that might be present would be captured by this method as SYN503780. The analytes SYN503780 and CSC686480 are quantified and expressed in bicyclopyrone equivalents and then added to give a total bicyclopyrone residue.

All of the methods extract residues with acetonitrile/water. The common moiety methods hydrolyse residues with aqueous hydrogen peroxide/sodium hydroxide. The method provided for analysis of bicyclopyrone, SYN503780 and CSCD686480, as single compounds, exclude the hydrolysis step. For all methods, final quantification is achieved using LC-MS/MS, with an LOQ of 0.01 mg/kg for each analyte in high -water and high-starch crops and in animal commodities (thus for an LOQ of 0.02 mg/kg for total bicyclopyrone).

Representative compounds that generate SYN503780 and CSCD686480 on base hydrolysis were used as reference materials for fortification and method validation .

The methods are suitable for the analysis of bicyclopyrone and related metabolites in plants and animal matrices.

Multi-residue methods are currently not available for bicyclopyrone and its metabolites.

Stability of pesticide residues in stored analytical samples

The Meeting received data on storage stability for bicyclopyrone and its metabolites in plant and animal matrices.

Storage stability studies, where bicyclopyrone and SYN503780 were analysed individually, demonstrated that residues were stable for at least 24 months at -18 °C in crop commodities representative of high water, high acid, high oil, high protein, high starch and dry commodity groups. The two compounds were stable for at least 12 months in processed commodities derived from maize, sugarcane and soya beans.

Storage stability studies using common moiety methods, demonstrated that the common moieties SYN503780 and CSCD686480 were stable for at least 26 months at -18 °C in sugar cane commodities, when bicyclopyrone, SYN503780, CSCD686480 or CSAA915914 were added to the samples.

Storage stability studies using common moiety methods demonstrated that total residues captured by the common moieties SYN503780 and CSCD 686480 were stable for at least 13 months at -18 °C in bovine tissues and milk.

The demonstrated periods of stability are sufficient to cover the periods for which samples have been stored during residue analyses.

Definition of the residue

Following application of bicyclopyrone to crops (maize, soya bean and sugar cane) a large number of structurally similar metabolites were detected. The majority of these metabolites were either desmethyl dihydoxylated bicyclopyrone isomers or desmethyl monohydroxylated bicyclopyrone isomers (free and glycoside conjugated forms). In both cases these metabolites are structurally related to bicyclopyrone.

In maize grain the significant residues were CSAA589691 (up to 42% TRR, 0.024 mg eq/kg), CSCD675162 (up to 23% TRR, 0.006 mg eq/kg) and monohydroxy NO449280 (19% TRR, 0.004 mg eq/kg).

In soya bean seeds bicyclopyrone accounted for up to 15% TRR (0.029 mg eq/kg) and the only other major metabolite was CSCD675164 (up to 18% TRR, 0.034 mg eq/kg).

Residues in sugar cane stalks were < 0.01 mg eq/kg.

In animal feed items the major residues were CSCD677692 (up to 21% TRR, 0.19 mg eq/kg, maize forage), CSCD675162 (up to 16% TRR, 0.023 mg eq/kg, soya bean hay), CSCD677693 (up to 18% TRR, 0.163 mg eq/kg, cane forage), CSCD677306 glycoside (14% TRR, 0.105 mg eq/kg, cane forage) and bicyclopyrone (10% TRR, 0.002 mg eq/kg, soya bean forage).

In rotational crops bicyclopyrone was a significant part of the residue in spinach (19 % of TRR, 0.002 mg eq/kg). The metabolite CSCD656832 was found in turnip forage (up to 71% TRR, 0.012 mg eq/kg). The metabolite CSCD675164 was found in turnip tubers (33% TRR, 0.005 mg eq/kg) and turnip foliage (34% TRR, 0.007 mg eq/kg).

The same desmethyl dihydoxylated bicyclopyrone isomers and desmethyl monohydroxylated bicyclopyrone isomers (free and glycoside conjugated forms) were also found in significant levels in rotated crop fractions that are animal feed items. In addition a number of metabolites containing the pyridine ring only were found at significant levels; CSCD656832 (includes conjugates up to 41% TRR, 0.064 mg eq/kg in wheat hay; 16% TRR, 0.009 mg eq/kg in wheat grain) and CSCD686480 (includes conjugates, up to 19% TRR, 0.029 mg eq/kg in wheat hay).

Bicyclopyrone was found to be stable on processing.

Owing to the different metabolites found there is no obvious candidate compound for use as a residue definition for compliance nor is there a small group of compounds that could be usefully monitored to cover the range of metabolites found in the different crop fractions.

The meeting noted that the majority of the metabolites are structurally related to bicyclopyrone and fall into two groups:

The first group are compounds that can be hydrolysed to SYN503780 (bicyclopyrone, monohydroxy N0449280, CSCD675164, CSCD677306 glycoside, CSCD675164).

The second group covers compounds that can be hydrolysed to CSDC686480 (CSCD686480, CSCD675162, CSCD677692 and CSCD677693 and conjugates).

In addition, all crop fractions contained numerous other metabolites, all individually < 10% TRR, which would also belong to these groups.

Common moiety methods are available to cover these two groups of metabolites.

The meeting agreed that the residue definition for enforcement should be the *sum of bicyclopyrone and its structurally-related metabolites determined as sum of compounds hydrolysable with base to SYN503780 (2-(2-methoxyethoxymethyl)-6-(trifluoromethyl) pyridine-3-carboxylic acid) and CSCD686480 (2-(2-hydroxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid), expressed as bicyclopyrone.*

For risk assessment it was concluded that the structurally related compounds to bicyclopyrone (determined as either SYN503780 or CSCD686480) were likely to have a toxicity no greater than bicyclopyrone. Therefore all the metabolites quantified are covered by the toxicological endpoints for bicyclopyrone.

The only other metabolites that need to be considered for inclusion in the residue definition for risk assessment are CSAA589691 and CSCD656832.

Metabolite CSA589691was found in immature and mature maize grain at a level of 0.024 mg eq/kg. The meeting noted that the maize metabolism study was conducted at a rate of 200 g ai/ha applied pre-emergence followed by an application of 200 g ai/ha applied post-emergence. This represents an exaggerated rate compared to the critical GAP for cereals (pre-emergence treatment: maximum application of 200 g ai/ha or pre-emergence/post emergence treatment: maximum application of 50 g ai/ha). The meeting concluded that at the GAP residues of CSAA589691 were likely to be < 0.01 mg/kg and the contribution to the diet would be insignificant.

The metabolite CSCD656832 was a significant residue in rotational crops. The highest levels were wheat forage (0.018 mg/kg), wheat hay (0.064 mg/kg), wheat straw (0.056 mg/kg), wheat grain (0.009 mg/kg) and turnip foliage (0.012 mg/kg). The rotational crop metabolism study was conducted at a rate of 350 g ai/ha to the bare soil. This represents 1.75× the maximum seasonal application rate of 200 g ai/ha. Residues in rotational crop fractions of grain and trunip foliage at the GAP are expected to be < 0.01 mg/kg. Residues in animal feed commodities at the GAP will not contribute significantly to the livestock dietary burden. Consequently, metabolite CSCD656832 does not need to be considered further.

The meeting concluded that the residue definition for risk assessment should be the *sum of bicyclopyrone and its structurally-related metabolites determined as sum of compounds hydrolysable with base to SYN503780 (2-(2-methoxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid) and CSCD686480 (2-(2-hydroxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid), expressed as bicyclopyrone.*

In livestock the major compounds identified were bicyclopyrone and CSAA915194. Bicyclopyrone was identified at levels ranging from 16% TRR to 95% TRR (0.12–0.44 mg eq/kg) in the different animal commodities. CSA915194 was identified at levels from 1–70% TRR (0.001–1.92 mg eq/kg) in the different animal commodities.

Trace levels of CSCD686480 were found in the goat metabolism study whereas in the dairy cow feeding study residues > 0.01 mg/kg were found in liver and kidney; the longer duration of the feeding study is likely to account for cleavage of the ring being more prominent.

A common moiety method is available that will determine bicyclopyrone and CSA915194. A common moiety method is also available for the determination of CSCD686480 and structurally related compounds.

The meeting agreed that based on the livestock metabolism and feeding studies the residue definition for enforcement and monitoring should be;

The *sum of bicyclopyrone and its structurally-related metabolites determined as sum of compounds hydrolysable with base to SYN503780 (2-(2-methoxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid) and CSCD686480 (2-(2-hydroxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid), expressed as bicyclopyrone.*

For risk assessment no additional metabolites were identified at significant levels in any animal tissues. Therefore the meeting concluded that the residue definition for monitoring should apply for risk assessment.

The meeting recommended the following residue definitions for bicyclopyrone:

Definition of the residue for compliance with the MRL and for dietary risk assessment for plant and animal commodities: *sum of bicyclopyrone and its structurally-related metabolites determined as sum of compounds hydrolysable with base to SYN503780 (2-(2-methoxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid) and CSCD686480 (2-(2-hydroxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid), expressed as bicyclopyrone.*

Bicyclopyrone and the structurally related compounds were not found to accumulate in fat. The meeting concluded that the residue is not fat soluble.

Results of supervised residue trials on crops

The Meeting received supervised trial data for the foliar application of bicyclopyrone on barley, maize, wheat, sugar cane and sweet corn. Residue trial data was made available from Belize, Canada, Uruguay and USA. Labels were available from Belize, Uruguay and USA describing the registered uses of bicyclopyrone.

Total bicyclopyrone residues are calculated as the sum of two common moieties (SYN503780 and CSCD686480) expressed as bicyclopyrone equivalents. Where residues have been measured in duplicate samples, a mean total value is presented. Residues and application rates have been reported as provided in the study reports except for finite values below the LOQ, where these have been reported as < 0.01 mg/kg.

The results from trials used for the estimation of maximum residue levels (underlined) have been rounded to two significant digits (or if close to the LOQ, rounded to one significant digit).

Where a residue of both common moieties has been detected, the sum of the two values is used. Where both values are reported as < 0.01 mg/kg, the total bicyclopyrone equivalent residue is reported as < 0.02 mg/kg and where both values are reported as < 0.005 mg/kg, the total bicyclopyrone equivalent residue is reported as < 0.01 mg/kg.

The Meeting noted that some labels included both a latest growth stage for application and a PHI in the use instructions. In interpreting these use instructions, the Meeting decided that trial data reflecting application at the prescribed growth stage and with harvest no earlier than the PHI as suitable for making a recommendation.

Cereal grains

Sweetcorn

Data were available from supervised trials on sweetcorn in Brazil and the USA.

The critical GAP of bicyclopyrone on sweet corn of USA is a soil applied pre-plant pre-emergence application at a maximum rate of 50 g ai/ha with a PHI 45 days when used as pre-plant, pre emergence.

Total bicyclopyrone residue in sweetcorn from the USA trials matching USA GAP were (n=12): < 0.02 (11) and 0.023 mg/kg.

Based on the US trials for sweet corn , the Meeting estimated a maximum residue level of 0.03 mg/kg, STMR of 0.02 mg/kg and HR of 0.023 mg/kg for bicyclopyrone on sweetcorn (corn-on-the cob).

Barley

Data were available from supervised trials on barley in Canada and the USA following GAP of the USA.

The GAP for bicyclopyrone on barley in the USA is as a foliar application at a maximum rate of 50 g ai/ha with the application timing at 2-leaf stage to pre-boot stage, and a PHI of 60 days.

Total bicyclopyrone residues in barley from trials in Canada and USA approximating GAP were (n=10): < 0.01 (4), < 0.011, 0.011, 0.013, 0.014, 0.021, 0.026 mg/kg.

Based on the trials for barley in Canada and USA, the Meeting estimated a maximum residue level of 0.04 mg/kg, a STMR of 0.011 mg/kg for bicyclopyrone on barley.

Maize

Field trials involving maize were performed in Brazil and the USA.

The GAP for maize in the Uruguay is as a single pre-emergence treatment at maximum rate of 200 g ai/ha. The pre-harvest interval is defined by application timings. In field trials from the Brazil matching Uruguay GAP, total residues for maize grains harvested at maturity were < 0.02 (3) mg/kg.

Bicyclopyrone can be applied to maize in the USA as a either a pre-plant, pre-emergence or post-emergence treatment with a yearly maximum rate 50 g ai/ha. The critical US GAP in maize is a single early post-emergence treatment at 50 g ai/ha applied to maize up 30 inches tall or up to the 8-leaf crop growth stage with PHI of 60-days. In 22 US trials conducted on maize, matching the critical GAP (a single application of 50 g ai/ha, PHI 79–113 days) the total bicyclopyroneresidues for maize grains harvested at maturity were < 0.02 (22) mg/kg.

The Meeting noted that residues levels in the three trials conducted in Brazil involving an application of bicyclopyrone at rate of 200 g ai/ha were < 0.02(3) mg/kg. In addition, the meeting noted that in 26 trials conducted in the USA involving application of bicyclopyrone once or twice at a rate of 200g ai/ha, the residues levels were < 0.02 (26) mg/kg.

The Meeting estimated the maximum residue level and STMR of 0.02*and 0 mg/kg, respectively, for bicyclopyrone in maize.

Wheat

Data were available from supervised trials on wheat in Canada and the USA.

The GAP for wheat in the USA is a single treatment at maximum application rate of 50 g ai/ha applied between 2-leaf stage and pre-boot stage with a pre-harvest interval of 60 days.

In field trials from Canada and the USA matching GAP (1× 50 g ai/ha, PHI 57–63 days), total bicyclopyrone residues for wheat grains harvested at maturity were (n=22) < 0.01, 0.01(11), 0.011(4), 0.013, 0.014, 0.015, 0.016 (2), 0.022 mg/kg.

Based on the trials for wheat from Canada and the USA, the Meeting estimated a maximum residue level of 0.04 mg/kg, and STMR of 0.01 mg/kg for bicyclopyrone on wheat grain.

Grasses for sugar or syrup production

Sugar cane

Data were available from supervised trials on sugar cane in Australia and Brazil. The GAP for sugar cane in Belize (South America) is as a pre-emergent treatment, BBCH 00 to BBCH 08 or early post-emergence from BBCH 11 (one true leaf or whorls unfolded) to BBCH 14 (four true leaves or whorls unfolded) treatment at 262.5 g ai/ha. The pre-harvest interval is defined by application timings, i.e., crop growth stage.

In field trials data from Brazil matching the GAP for bicyclopyrone on sugarcane in Belize, total bicyclopyrone residues in sugar cane were < 0.02 (17) mg/kg. Bicyclopyrone residues in sugar cane data from Brazil at exaggerated rates of 900 g ai/ha (3 × GAP rate) were < 0.02 mg/kg and 1500 g ai/ha (5 × GAP rate) were < 0.02 mg/kg.

Total bicyclopyrone residues in sugar cane from trials in Australia with a GAP of up to two applications at a rate of 300 g ai/ha, or one application at 600 g ai/ha applied at a later growth stage (just before row closure, or "out-of-hand" stage) were < 0.02(8) mg/kg.

Based on the available information, the Meeting estimated a maximum residue level, STMR and HR at 0.02*, 0 and 0 mg/kg for bicyclopyrone in sugarcane, respectively.

Animal feedstuffs

Barley, wheat hay and straw

Data were available from supervised trials on barley and wheat (hay and straw) in Canada and the USA.

Trials from Canada on barley hay and straw were reported following the foliar application of an EC formulation (GAP: a post-emergent treatment at plant stage BBCH 12–37 with a maximum rate of 37.5 g ai/ha, and a PHI 30 days for hay and 60 days for straw).

Trials from the USA on barley hay and straw were reported following a foliar application of an EC formulation (GAP: a post-emergent application at plant stage 2-leaf to pre-boot at a maximum rate of 50 g ai/ha, , and a PHI of 30 days for hay and 60 days for straw).

Trials from Canada and the USA on wheat were reported following a foliar application of a EC formulation (GAP: single application at a rate of 50 g ai/ha, from the 2-leaf stage to pre-boot stage, PHI 30 days for forage and hay, 60 days for straw).

Barley and wheat straw and fodder, dry

Total bicyclopyrone residues in barley hay from trials in Canada and USA matching the USA GAP were (n=19): 0.012, 0.013, 0.015, 0.017, 0.018, 0.024(2), 0.026, 0.031, 0.035, 0.036, 0.039, 0.047, 0.048, 0.057, 0.066, 0.081, 0.082, 0.16 mg/kg.

Total bicyclopyrone residues in wheat hay from trials in Canada and USA matching the USA GAP were (n=33): 0.01, 0.011 (3), 0.012 (2), 0.014, 0.015 (2), 0.018, 0.019, 0.02 (2), 0.021, 0.023 (2), 0.025, 0.029, 0.035, 0.039, 0.046, 0.047, 0.063, 0.069, 0.074 (2), 0.092, 0.11, 0.18, 0.20, 0.35, 0.51 and 0.66 mg/kg.

Total bicyclopyrone residues in barley straw from trials in Canada and USA matching the USA GAP were (n=11): 0.010, 0.012, 0.014, 0.016, 0.025, 0.029, 0.056, 0.061, 0.068, 0.085, 0.19 mg/kg as received basis.

Bicyclopyrone residues in wheat straw from trials in Canada and USA matching the USA GAP were (n=20): 0.016, 0.018, 0.031, 0.033, 0.049, 0.060, 0.086, 0.094, 0.097, 0.11, 0.12, 0.13, 0.14, 0.15(2), 0.16, 0.19(2), 0.22 and 0.24 mg/kg (as received).

The Meeting noted that the residues were higher in hay than in straw. Based on the residues in wheat hay from trials in Canada and the USA, the Meeting estimated maximum residue levels of

0.8 mg/kg (dw) for barley straw and fodder, dry and wheat straw and fodder, dry based on a dry matter content of 88%.

Based on the wheat hay data , the Meeting estimated a median residue value and a highest residue value for bicyclopyrone in barley hay and wheat hay of 0.025 and 0.68 (individual value) mg/kg respectively (as received).

Based on the residues in wheat straw from trials in Canada and the USA, the Meeting estimated a median residue value and a highest residue value for bicyclopyrone in barley and wheat straw of 0.115 and 0.25 (individual value) mg/kg, respectively.

Corn (maize and sweet corn) forage

Data for forage were available from supervised trials on corn crops (sweet corn and maize, including popcorn) in Brazil and the USA.

Trials from the USA on sweet corn forage and maize forage were reported for the application of a SL formulation (200 g/L) (GAP: a maximum rate of 50 g ai/ ha pre-emergence (sweet corn) and up to 30 inches tall or up to the 8-leaf growth stage of the crop (maize), PHI 45 days).

Total bicyclopyrone residues in maize forage from data in the USA matching the critical USA GAP were (n=19) <0.02, 0.04, 0.042, 0.079, 0.08, 0.09(2), 0.096, 0.1, 0.11(3), 0.13, 0.14(2), 0.15, 0.16, 0.17, 0.18 mg/kg (as received).

Total bicyclopyrone residues in sweet corn forage from trials in the USA matching GAP (n=4) were 0.05, 0.12, 0.16, 0.23 mg/kg (as received).

The Meeting noted that residues from maize forage and sweetcorn forage from the USA are from similar populations (Mann-Whitney test). As the residues from the USA trials (maize forage and sweetcorn forage) were considered similar, the Meeting decided that the data could be combined, <0.02, 0.04, 0.042, 0.05, 0.079, 0.08, 0.09(2), 0.096, 0.1, 0.11(3), 0.12, 0.13, 0.14(2), 0.15, 0.16(2), 0.17, 0.18, 0.23 mg/kg.

The Meeting estimated median and highest residue for total bicyclopyrone in corn forage of 0.11 and 0.29 (individual value) mg/kg.

Corn (maize and sweet corn) fodder

Data for fodder were available from supervised trials on corn crops (sweet corn and maize, including popcorn) in the USA.

Trials from the USA on sweet corn fodder and maize fodder were reported following the foliar application (GAP: a single application at a rate of 50 g ai/ ha up to V8/8-leaf stage growth stage of the crop, PHI 45 days).

Nineteen trials were avilaible from USA on maize fodder matching US GAP with total bicyclopyrone residues of < 0.02 (3), 0.027, 0.034, 0.047, 0.048, 0.052, 0.053, 0.054(2), 0.06, 0.082, 0.11, 0.13, 0.14, 0.15, 0.22 and 0.28 mg/kg (as received).

Seven trials were available from USA on sweet corn fodder matching US GAP with total bicyclopyrone residues of 0.027, 0.03, 0.079(2), 0.23, 0.30(2) mg/kg (as received).

Three trials were available from USA on popcorn fodder matching US GAP with total bicyclopyrone residues of 0.025, 0.046, 0.12 mg/kg (as received).

As the residues from the USA trials (maize fodder, popcorn fodder and sweetcorn fodder) were considered similar, the Meeting decided to combine the data ; < 0.02 (3), 0.025, 0.027(2), 0.03, 0.034, 0.046, 0.047, 0.048, 0.052, 0.053, 0.054(2), 0.06, 0.079(2), 0.082, 0.11, 0.12, 0.13, 0.14, 0.15, 0.22, 0.23, 0.28 and 0.30(2) mg/kg.

The Meeting estimated a median of 0.054 mg/kg, and highest residue 0.39 mg/kg (individual value). The Meeting estimated a maximum residue level of 0.5 mg/kg (dw) based on a dry matter content of 83% for sweet corn fodder and maize fodder (dry).

Wheat forage

Data were available from supervised trials on wheat in Canada and the USA.

Trials from Canada and the USA on wheat were reported following a foliar application (GAP: single application at a rate of 50g ai/ha, from the 2-leaf stage to pre-boot stage, PHI 30 days for forage).

Total bicyclopyrone residues in wheat forage from trials in Canada and USA matching the USA GAP were (n=32): < 0.01(3), 0.01(6), 0.011(4), 0.012, 0.013(3), 0.019, 0.02, 0.024(2), 0.025(3), 0.045, 0.063, 0.076, 0.082, 0.089, 0.17, 0.27 and 0.34 mg/kg. Based on the residues in wheat forage from trials in Canada and the USA, the Meeting estimated a median residue value and a highest residue value for bicyclopyrone in wheat forage of 0.013 and 0.36 (individual value) mg/kg respectively (as received basis).

Fate of residues during processing

High temperature hydrolysis

The degradation of [^{14}C] bicyclopyrone was studied under hydrolytic conditions at high temperatures in sterile aqueous buffers at pH 4, 5 and 6 for periods of up to 60 minutes so as to simulate common processing practice (pasteurization, baking/brewing/boiling, and sterilization). No degradates were detected at any of the investigated pH and temperature ranges. Bicyclopyrone is stable under hydrolytic conditions at high temperatures.

Residues in processed commodities

The fate of total bicyclopyrone residues has been examined in maize and wheat processing studies.

Based on the results of processing studies conducted in the USA in combination with the residues from supervised trials, the estimated processing factors and the derived STMR-Ps are summarized in the Table below.

Processing factors and STMR-Ps for wheat processed commodities

Crop	Residue value (mg/kg) in raw commodity		Processed Commodity	Calculated PF	PF (Mean or best estimated)*	Residue value (mg/kg) in processed commodity	
	MRL	STMR				MRL**	STMR-P
Wheat	0.04	0.01	AGF	1.8, 17.7	17.7	-	0.177
			Bran	2.7, 1.8	2.3	0.1	0.023
			Flour	0.26, 0.28	0.27	-	
			Middlings	0.91, 0.49	0.7	-	
			Shorts	0.91, 0.47	0.69	-	
			Germ	1.5, 1.3	1.4	0.06	0.014

*The factor is the ratio of the total residue in processed commodity divided by the total residue in the RAC.

** MRLs in processed commodities are only proposed where they are higher than the MRL in the raw grain.

The mean (or best estimated) concentration factors for total bicyclopyrone (bicyclopyrone and its structurally-related metabolites determined as SYN503870 and CSCD686480 by a common moiety method) in wheat aspirated grain fractions, wheat bran and wheat germ were 17.7, 2.3 and 1.4, respectively. Bicyclopyrone residues did not concentrate in any of the other processed fractions.

The Meeting estimated a maximum residue level of 0.1 mg/kg for wheat bran (0.04×2.3= 0.092 mg/kg).

The Meeting decided to extrapolate the processing factor for wheat bran to estimate a maximum residue level and STMR-P for barley bran. The Meeting estimated a maximum residue level of 0.1 mg/kg (0.04×2.3= 0.092 mg/kg) and STMR-P of 0.0253 mg/kg (0.011×2.3= 0.0253 mg/kg) for barley bran.

Residue in animal commodities

Farm animal dietary burden

The Meeting estimated the dietary burden of bicyclopyrone in farm animals on the basis of the diets listed in Appendix IX of the FAO Manual 2016. Calculation from highest residue, STMR (some bulk commodities) and STMR-P values provides 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. The percentage dry matter is taken as 100% when the highest residue levels and STMRs are already expressed on a dry weight basis.

Estimated maximum and mean dietary burdens of farm animals

Dietary burden calculations for beef cattle, dairy cattle, broilers and laying poultry are provided in Annex 6 to the Report. The calculations were made according to the animal diets from US-Canada, EU, Australia and Japan in the Table (Appendix IX of the FAO manual).

Region	Livestock dietary burden, bicyclopyrone, ppm of dry matter diet							
	US - Canada		EU		Australia		Japan	
	Maximum	Mean	Maximum	Mean	Maximum	Mean	Maximum	Mean
Beef cattle	0.13	0.058	0.73	0.223	**1.45**[a]	**0.25**[b]	0.009	0.009
Dairy cattle	0.48	0.13	0.59	0.17	**1.0**[c]	**0.223**[d]	0.37	0.14
Broiler poultry	**0.009**	**0.009**	0.009	0.009	0.002	0.002	0.001	0.001
Laying poultry	0.009	0.009	**0.16**[e]	**0.039**[f]	0.002	0.002	-	-

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

[b] suitable for estimating STMR for meat, fat and edible offal of cattle.

[c] suitable for estimating maximum residue levels for Milk.

[d] suitable for STMR levels for Milk.

[e] suitable for estimating maximum residue levels for poultry meat, offal and eggs.

[f] suitable for STMR levels for poultry meat, offal and eggs.

Livestock feeding studies

The Meeting received a lactating dairy cow feeding studies using bicyclopyrone, which provided information on residues resulting in animal commodities and milk from bicyclopyrone residues in the animal diet.

Lactating dairy cows

Lactating dairy cows were dosed with bicyclopyrone for 28 days at a dose equivalent to 0.15, 0.90 and 3.0 ppm in the diet.

Total bicyclopyrone residues were less than the LOQ (< 0.01 mg/kg) in milk, fat and muscle at The highest feeding level. The residues found in kidney and liver were not linearly-related to the dose administered. The highest residues of total bicyclopyrone equivalents occurring in liver of individual animals (3 in total) were 3.2, 2.6 and 1.4 mg/kg from the 3, 0.9 and 0.15 ppm dose groups, respectively. The highest residues of total bicyclopyrone equivalents occurring in kidney of individual animals were 0.57, 0.65 and 0.50 mg/kg from the 3.0, 0.9 and 0.15 ppm dose groups, respectively, and did not seen to be dose dependent.

Poultry

A poultry feeding study was not available.

Residues in animal commodities

Cattle-STMR, HR and MRLs

For maximum residue level estimation, the high residues in the cattle tissues were calculated by interpolating the maximum dietary burden for beef cattle (1.45 ppm) between the relevant feeding levels (0.92 and 3.0 ppm) in the dairy cow feeding study and using the highest tissue concentrations from individual animals within those feeding groups. For maximum residue level estimation, the high residues in the cattle milk were calculated by interpolating the maximum dietary burden for dairy cattle (1.0 ppm) between the relevant feeding levels (0.92 and 3.0 ppm) in the dairy cow feeding study and using the highest mean milk concentrations from those feeding groups.

The STMR values for the tissues were calculated by interpolating the mean dietary burden for dairy cattle (0.24 ppm) with the 0.15 and 0.92 ppm feeding levels from the dairy cow feeding study and using the mean milk concentrations from those feeding groups. The STMR values for the milk were calculated by interpolating the mean dietary burden for dairy cattle (0.22 ppm) with the 0.15 and 0.92 ppm feeding levels from the dairy cow feeding study and using the mean milk concentrations from those feeding groups.

Bicyclopyrone feeding study	Feed level (ppm) for milk residues	Residues (mg/kg) in milk	Feed level (ppm) for tissue residues	Residues (mg/kg)			
				Muscle	Liver	Kidney	Fat
MRL beef or dairy cattle							
Feeding study	0.90	< 0.02	0.90	< 0.02	2.57	0.57*	< 0.02
	3.00	< 0.02	3.00	< 0.02	3.25		< 0.02
Dietary burden and high residue	1.00	< 0.02	1.45	< 0.02	2.748	0.57	< 0.02
STMR beef or dairy cattle							
Feeding study	0.15	< 0.02	0.15	< 0.02	1.30	0.53*	< 0.02
	0.90	< 0.02	0.90	< 0.02	2.29		< 0.02
Dietary burden and residue estimate	0.22	< 0.02	0.25	< 0.02	1.432	0.53	< 0.02

*Residue levels in kidney were not dose dependent, values are median calculated over all dose levels.

The Meeting estimated the following STMR values: milk 0.02 mg/kg; muscle 0.02 mg/kg; liver 1.415 mg/kg; kidney 0.53 mg/kg and fat 0.02 mg/kg.

The Meeting estimated the following HR values: milk 0.02 mg/kg; muscle 0.02 mg/kg; edible offal (based on liver) 2.75 mg/kg and fat 0.02 mg/kg.

The Meeting estimated the following maximum residue levels: milk 0.02* mg/kg; meat (mammalian except marine mammals) 0.02* mg/kg, edible offal 3 mg/kg and mammalian fats (except milk fats) 0.02* mg/kg.

Poultry-STMR, HR and MRLs

A poultry feeding study was not available. The Meeting used TRR levels from the poultry metabolism study to estimate maximum residue levels, STMRs, and HRs for poultry commodities. To all tissues except liver, TRRs were less than 1 mg eq/kg at a feeding level of approximately 20 ppm. When scaled to the maximum dietary burden of 0.16 ppm, the anticipated residues is 0.008 mg/kg. In liver, the TRR in the metabolism study was approximately 1.8 mg eq/kg, which scales to 0.014 mg/kg. For eggs, the TRR was 0.14 mg eq/kg, which scales to 0.0011 mg/kg.

On the basis of the anticipated residues, the Meeting estimated maximum residue levels of 0.01* mg/kg, STMRs, and HRs each at 0.01 mg/kg for all poultry commodities

RECOMMENDATIONS

On the basis of the data obtained from supervised residue trials the Meeting concluded that the residue levels listed in Annex 1 are suitable for establishing maximum residue limits and for IEDI and IESTI assessment.

Definition of the residue for compliance with the MRL and for dietary risk assessment for plant and animal commodities: sum of bicyclopyrone and its structurally-related metabolites determined as sum of compounds hydrolysable with base to SYN503780 (2-(2-methoxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid) and CSCD686480 (2-(2-hydroxyethoxymethyl)-6-(trifluoromethyl)pyridine-3-carboxylic acid), expressed as bicyclopyrone.

The residue is not fat soluble.

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The International Estimated Daily Intakes (IEDI) for bicyclopyrone was calculated from recommendations for STMRs for raw and processed commodities in combination with consumption data for corresponding food commodities. The results are shown in Annex 3.

The IEDI of the 17 GEMS/Food cluster diets, based on the estimated STMRs represented 3% to 20% of the maximum ADI of 0.003 mg/kg bw/day. The Meeting concluded that the long-term dietary exposure to residues of bicyclopyrone from uses considered by the Meeting is unlikely to present a public health concern.

Short-term dietary exposure

The International Estimated Short term Intake (IESTI) for bicyclopyrone was calculated for all food commodities and their processed fractions for which maximum residue levels were estimated and for which consumption data were available. The results are shown in Annex 4.

The current Meeting established an ARfD of 0.01 mg/kg bw for bicyclopyrone for women of child bearing age. The IESTIs represented 0–100% of the ARfD for women of child bearing age. On the basis of the information provided to the Meeting, it was concluded that the short-term dietary exposure to residues of bicyclopyrone, resulting from the uses considered by the Meeting are unlikely to present a public health concern.