5.4 BIXAFEN (262)

TOXICOLOGY

Bixafen is the ISO-approved common name for *N*-(3′,4′-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) (CAS No. 581809-46-3), a novel fungicide from the pyrazole-carboxamide class. Bixafen exhibits broad fungicidal activity in various crops by inhibition of succinate dehydrogenase, an enzyme of complex II within the mitochondrial respiration chain.

Bixafen has not been evaluated previously by JMPR and was reviewed by the present Meeting at the request of CCPR.

All critical studies were certified as complying with GLP.

Biochemical aspects

In rats given (dichlorophenyl-U-^{14}C)-labelled bixafen orally by gavage, absorption was rapid and accounted for at least 83% of the total administered radioactivity after a single low dose (2 mg/kg bw). The maximum plasma concentrations of radioactivity were reached approximately 2–4 and 8 hours after administration of the low and high doses (2 and 50 mg/kg bw), respectively. Radioactivity was widely distributed throughout the body. Elimination of the radioactivity was mainly via faeces (≥ 91%), whereas elimination via urine accounted for 1–3% of the administered dose. In bile duct–cannulated rats, extensive biliary excretion (up to 83%) was demonstrated. Elimination of the radioactivity from the body was rapid, with a half-life in plasma of 8–9 hours and a mean residence time of 13–19 hours (for the low dose). Residues in tissues at 72 hours after a single oral dose as well as after repeated oral dosing accounted for 0.1–3% of the administered radioactivity, with liver and kidneys containing the highest concentrations of residues.

Metabolism of bixafen in rats was extensive, and more than 30 metabolites were identified. The main metabolic routes included demethylation, hydroxylation of the parent and bixafen-desmethyl, and conjugation with glucuronic acid or glutathione. A minor metabolic reaction was the cleavage of the amide bridge of bixafen.

Toxicological data

The LD_{50} in rats treated orally or dermally with bixafen was greater than 2000 mg/kg bw, and the inhalation LC_{50} in rats was greater than 5.38 mg/L. Bixafen was not a skin irritant in rabbits, was not irritating to the eye of rabbits and was not a skin sensitizer in the local lymph node assay in mice.

Following repeated administration of bixafen, the liver was the primary target organ in mice, rats and dogs. Increased liver weights and hepatocellular hypertrophy were observed in all species tested and were considered to reflect hepatic microsomal enzyme induction. Also, in several studies, there was evidence for liver toxicity based on clinical chemistry changes (increased serum alkaline phosphatase and cholesterol, decreased serum albumin) and histopathological changes (hepatocellular pigmentation, degeneration and necrosis). In mice and rats, the thyroid was an additional target, which was considered to be secondary to the enhanced hepatic clearance of thyroid hormones. This suggestion was supported by a 14-day mechanistic study in rats in which a marked induction of phase I and II hepatic enzymes, a slight reduction of thyroid hormone (T_3, T_4) levels and a significant increase of TSH levels were observed at 150 mg/kg bw per day, the only dose tested.

In a 4-week study in mice using dietary concentrations of 0, 100, 500 and 2500 ppm (equal to 0, 17, 81 and 305 mg/kg bw per day for males and 0, 21, 103 and 424 mg/kg bw per day for females), the NOAEL was 100 ppm (equal to 17 mg/kg bw per day), based on liver toxicity (increased liver weight, clinical chemistry changes, focal coagulative necrosis) at 500 ppm (equal to 81 mg/kg bw per day) and above. In a 13-week study in mice using dietary concentrations of 0, 50, 200 and 500 ppm

(equal to 0, 8.5, 34.3 and 88 mg/kg bw per day for males and 0, 10.4, 42.9 and 110 mg/kg bw per day for females), the NOAEL was 50 ppm (equal to 8.5 mg/kg bw per day), based on liver toxicity in males (increased liver weight, clinical chemistry changes, diffuse hepatocellular vacuolation) and focal/multifocal squamous cell hyperplasia of the stomach in both sexes at 200 ppm (equal to 34.3 mg/kg bw per day) and above.

In a 4-week study in rats using dietary concentrations of 0, 50, 350 and 2000 ppm (equal to 0, 3.5, 25 and 137 mg/kg bw per day for males and 0, 4.1, 28 and 138 mg/kg bw per day for females), the NOAEL was 350 ppm (equal to 25 mg/kg bw per day), based on reduced body weight gain, reduced feed consumption, liver toxicity (increased liver weight, increased cholesterol level) and thyroid effects (hypertrophy of follicular cells) at 2000 ppm (equal to 137 mg/kg bw per day). In a 13-week study in rats using dietary concentrations of 0, 50, 200, 800 and 2000 ppm (equal to 0, 3.2, 12.9, 50.4 and 130 mg/kg bw per day for males and 0, 3.9, 15.0, 59.2 and 153 mg/kg bw per day for females), the NOAEL was 200 ppm (equal to 12.9 mg/kg bw per day), based on liver effects (enlarged liver, increased liver weight) and thyroid effects (hypertrophy of follicular cells) at 800 ppm (equal to 50.4 mg/kg bw per day) and above.

In a 13-week study in dogs testing dose levels of 0, 100, 300 and 1000 mg/kg bw per day by oral gavage, the NOAEL was 100 mg/kg bw per day, based on an increase (> 20%) in absolute and relative liver weights of females at 300 mg/kg bw per day and above. In a 1-year study in dogs testing dose levels of 0, 10, 100 and 1000 mg/kg bw per day by oral gavage, the NOAEL was 10 mg/kg bw per day, based on haematological effects (decrease in red blood cell count, haemoglobin and haematocrit) in males and liver toxicity (increased liver weight, increased alkaline phosphatase and cholesterol levels) in females at 100 mg/kg bw per day and above.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. Due to technical problems in the production of the feed, the vitamin K_3 level of the diet (< 0.3 ppm) used in the first 5–6 months of the studies was significantly lower than the estimated requirement for mice and rats (approximately 1 ppm), with the consequence of a haemorrhagic syndrome and increased mortality, especially in the male animals from the high-dose group of mice or the mid-dose and high-dose groups of rats. After approximately 6 months of treatment, the diet was supplemented with 7–16 ppm of the synthetic vitamin K analogue menadione, and the studies were completed as scheduled, with the exception of the male rats, which were prematurely terminated after approximately 6–8 months of treatment. An additional study of chronic toxicity and carcinogenicity was therefore conducted in male rats (see below).

In the 78-week study of carcinogenicity in mice using dietary concentrations of 0, 50, 150 and 500 ppm (equal to 0, 6.7, 20.4 and 69.0 mg/kg bw per day for males and 0, 8.6, 25.5 and 85.0 mg/kg bw per day for females), there was no evidence for carcinogenicity up to the highest dose tested (500 ppm, equal to 69 mg/kg bw per day). The NOAEL for toxicity was 50 ppm (equal to 6.7 mg/kg bw per day), based on thyroid effects (follicular cell hyperplasia) in females and decreased body weights and liver toxicity (single-cell degeneration/necrosis) in males at 150 ppm (equal to 20.4 mg/kg bw per day) and above.

In the initial 24-month study of toxicity and carcinogenicity in rats, which was completed as planned for females only, dietary concentrations of 0, 50, 300 and 2000 ppm (equal to 0, 2.8, 17.4 and 117 mg/kg bw per day) were tested. There was no evidence for carcinogenicity up to the highest dose tested (2000 ppm, equal to 117 mg/kg bw per day). The NOAEL for toxicity was 50 ppm (equal to 2.8 mg/kg bw per day), based on liver effects (increased cholesterol, higher incidence and/or severity of hepatocellular brown pigments and multinucleated hepatocytes) and thyroid effects (higher incidence and/or severity of follicular cell hypertrophy and colloid alteration) at 300 ppm (equal to 17.4 mg/kg bw per day) and above. In the complementary 24-month study of toxicity and carcinogenicity in male rats using a vitamin K_3–supplemented diet (7–11 ppm) and dietary concentrations of bixafen of 0, 50, 300 and 2000 ppm (equal to 0, 2.0, 12.1 and 80.5 mg/kg bw per day), there was no evidence for carcinogenicity up to the highest dose tested (2000 ppm, equal to 80.5 mg/kg bw per day). The NOAEL for toxicity was 50 ppm (equal to 2.0 mg/kg bw per day), based

on liver effects (increased cholesterol levels, increased liver weights) and thyroid effects (higher incidence and/or severity of colloid alteration) at 300 ppm (equal to 12.1 mg/kg bw per day) and above.

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

Bixafen was tested for genotoxicity in vitro and in vivo in an adequate range of assays. There was no evidence of genotoxicity.

The Meeting concluded that bixafen is unlikely to be genotoxic.

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

In a two-generation study of reproductive toxicity in rats using dietary concentrations of 0, 50, 400 and 2500 ppm (equal to 0, 3.3, 26.4 and 169.2 mg/kg bw per day for males and 0, 3.9, 30.8 and 193.7 mg/kg bw per day for females), the NOAEL for reproductive toxicity was 2500 ppm (equal to 169.2 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was 400 ppm (equal to 26.4 mg/kg bw per day), based on a reduction in body weight and liver effects (liver weight increased > 20%) at 2500 ppm (equal to 169.2 mg/kg bw per day). The NOAEL for offspring toxicity was 400 ppm (equal to 26.4 mg/kg bw per day), based on a slight elevation in stillbirths and reduced pup weight/weight gain during lactation at 2500 ppm (equal to 169.2 mg/kg bw per day).

In a developmental toxicity study in rats testing dose levels of 0, 20, 75 and 250 mg/kg bw per day, the NOAEL for maternal toxicity was 20 mg/kg bw per day, based on decreased body weight gain and feed consumption at 75 mg/kg bw per day and above in the first days of treatment (i.e. gestation days 6–8). The NOAEL for embryo and fetal toxicity was 20 mg/kg bw per day, based on decreased fetal weights at 75 mg/kg bw per day and above.

In a developmental toxicity study in rabbits testing dose levels of 0, 25, 100 and 400 mg/kg bw per day, the NOAEL for maternal toxicity was 25 mg/kg bw per day, based on clinical signs (hair loss, no or reduced excreta) and a reduction in body weight gain and feed consumption at 100 mg/kg bw per day and above. The NOAEL for embryo and fetal toxicity was 25 mg/kg bw per day, based on reduced fetal body weight at 100 mg/kg bw per day and above.

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

A study using high-dose male rats from the initial 24-month study of toxicity and carcinogenicity provided evidence that the low vitamin K_3 level of the diet (< 0.3 ppm) was the cause of the haemorrhagic syndrome, as the prolonged blood coagulation time could be reversed by a vitamin K_3–supplemented diet (16 ppm). This conclusion was supported by the fact that no adverse effects on blood coagulation were observed in the multigeneration studies using diets with an adequate level of vitamin K (vitamin K_1: 0.65 ppm; vitamin K_3: 0.8 ppm).

In a 28-day study in male rats using a vitamin K_3–supplemented diet (16 ppm) and dietary concentrations of bixafen of 0, 2000, 4500 and 10 000 ppm (equal to 0, 162, 375 and 828 mg/kg bw per day), the NOAEL for effects of bixafen on blood coagulation parameters was 10 000 ppm (equal to 828 mg/kg bw per day), the highest dose tested.

Human data

In reports on manufacturing plant personnel, no adverse health effects were noted. Also, there were no reports of poisonings with bixafen.

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

Toxicological evaluation

The Meeting established an ADI for bixafen of 0–0.02 mg/kg bw, based on the NOAEL of 2 mg/kg bw per day for liver and thyroid effects observed at 12.1 mg/kg bw per day in the 24-month study of toxicity and carcinogenicity in male rats. A safety factor of 100 was applied.

The Meeting established an ARfD for bixafen of 0.2 mg/kg bw, based on the NOAEL of 20 mg/kg bw for decreased body weight gain and feed consumption observed in the first days of treatment at 75 mg/kg bw in a developmental toxicity study in rats. A safety factor of 100 was applied.

As the estimated exposures to M18, M20, M44, M45 and M47 are below the respective acute and chronic thresholds of toxicological concern for Cramer class III compounds, there is no concern for these metabolites. Bixafen-desmethyl has been tested in rodents through its formation from the parent compound and is therefore covered by the ADI for bixafen. For M25 and M26, their structural similarity to bixafen-desmethyl is such that the Meeting concluded that they would also be covered by the ADI for bixafen.

A toxicological monograph was prepared.

Levels relevant to risk assessment of bixafen

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 6.7 mg/kg bw per day	150 ppm, equal to 20.4 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 69 mg/kg bw per day[b]	—
Rat	Two-year studies of toxicity and carcinogenicity[a,c]	Toxicity	50 ppm, equal to 2.0 mg/kg bw per day	300 ppm, equal to 12.1 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 80.5 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	2500 ppm, equal to 169.2 mg/kg bw per day[b]	—
		Parental toxicity	400 ppm, equal to 26.4 mg/kg bw per day	2500 ppm, equal to 169.2 mg/kg bw per day
		Offspring toxicity	400 ppm, equal to 26.4 mg/kg bw per day	2500 ppm, equal to 169.2 mg/kg bw per day
	Developmental toxicity study[d]	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[d]	Maternal toxicity	25 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	25 mg/kg bw per day	100 mg/kg bw per day
Dog	One-year study of toxicity[d]	Toxicity	10 mg/kg bw per day	100 mg/kg bw per day

[a] Dietary administration.

[b] Highest dose tested.

[c] Two studies combined.

[d] Gavage administration.

Estimate of acceptable daily intake

0–0.02 mg/kg bw

Estimate of acute reference dose

0.2 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; ≥ 83%
Dermal absorption	No data
Distribution	Widely distributed; highest concentrations in liver and kidneys
Potential for accumulation	None
Rate and extent of excretion	≥ 93% within 72 h (≥ 91% in faeces, including up to 83% in bile; 1–3% in urine)
Metabolism in animals	Extensive (> 30 metabolites identified); demethylation, hydroxylation of parent and bixafen-desmethyl; conjugation with glucuronic acid and glutathione; cleavage of the amide bridge of bixafen as a minor metabolic reaction
Toxicologically significant compounds in animals, plants and the environment	Bixafen
Acute toxicity	
Rat, LD_{50}, oral	> 2000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.38 mg/L (4 h, nose-only exposure)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization	Not sensitizing (local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Liver in mice, rats and dogs; thyroid in rats
Lowest relevant oral NOAEL	8.5 mg/kg bw per day (mouse)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver and thyroid in mice and rats
Lowest relevant NOAEL	2.0 mg/kg bw per day (rat)

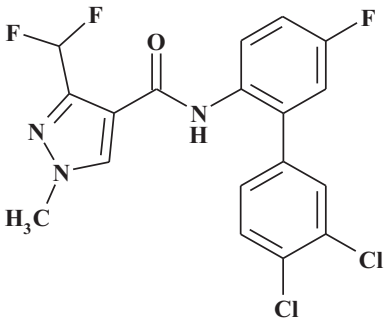
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Target/critical effect	No reproductive toxicity
Lowest relevant parental NOAEL	26.4 mg/kg bw per day
Lowest relevant offspring NOAEL	26.4 mg/kg bw per day
Lowest relevant reproductive NOAEL	169.2 mg/kg bw per day, the highest dose tested
Developmental toxicity	
Target/critical effect	Reduced fetal weights, visceral or skeletal variations at maternally toxic dose (rats and rabbits)
Lowest relevant maternal NOAEL	20 mg/kg bw per day (rat)
Lowest relevant embryo/fetal NOAEL	20 mg/kg bw per day (rat)
Neurotoxicity	
Acute and subchronic neurotoxicity	No specific data, but no indications from repeated-dose studies
Other toxicological studies	
Study on blood coagulation	Vitamin K_3–deficient diet contributed to prolonged blood coagulation times and haemorrhagic effects in male rats
Mechanistic study on thyroid effects	Induction of phase I and II hepatic enzymes was likely the cause of the observed thyroid hormone changes
Medical data	
	No adverse health effects reported in manufacturing plant personnel

Summary

	Value	Study	Safety factor
ADI	0–0.02 mg/kg bw	Two-year study of toxicity and carcinogenicity in rats	100
ARfD	0.2 mg/kg bw	Developmental toxicity study in rats (maternal toxicity)	100

RESIDUE AND ANALYTICAL ASPECTS

Bixafen (ISO common name) is a pyrazole-carboxamide fungicide used to control diseases on rape plants and cereals. Bixafen inhibits fungal respiration by binding to mitochondrial respiratory complex II. It was considered for the first time by the 2013 JMPR for toxicology and residues.

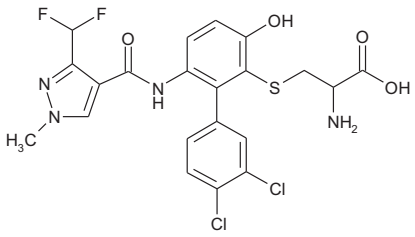


The IUPAC name of bixafen is N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide and the CA name is 1H-pyrazole-4-carboxamide, N-(3',4'-dichloro-5-fluoro[1,1'-biphenyl]-2-yl)-3-(difluoromethyl)-1-methyl-.

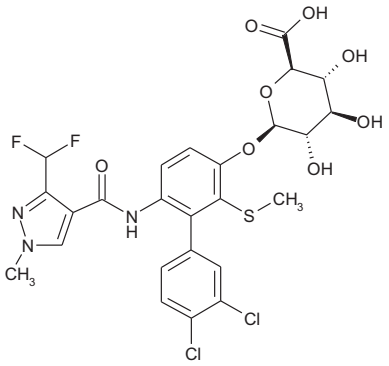
Bixafen labelled either in the pyrazole- or dichlorophenyl-moiety was used in the metabolism and environmental fate studies.

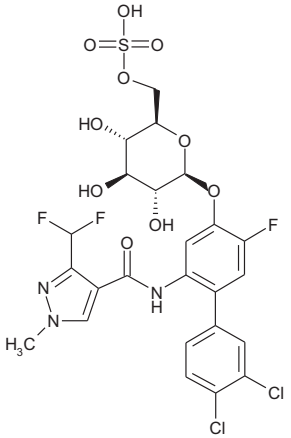
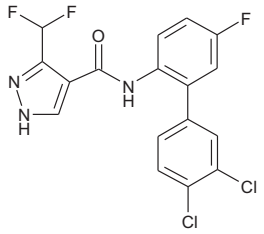
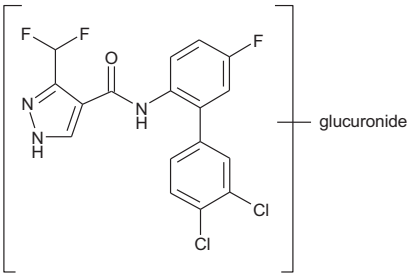
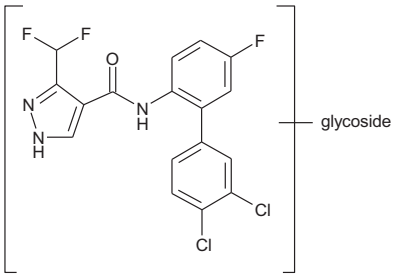
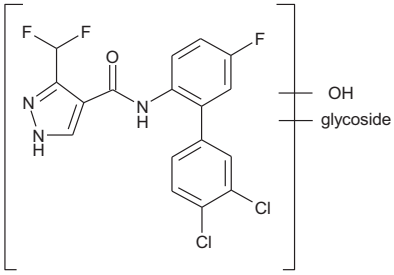
The following abbreviations are used for the metabolites discussed below:

M14 S-[3',4'-dichloro-6-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-3-hydroxybiphenyl-2-yl]cysteine (IUPAC)

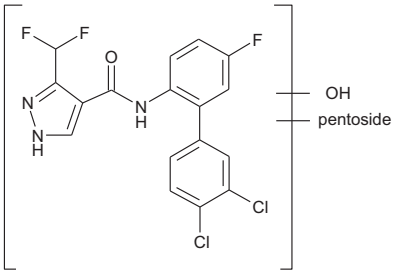


M18 3',4'-dichloro-6-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-2-(methylthio)biphenyl-3-yl beta-L-glucopyranosiduronic acid (IUPAC)

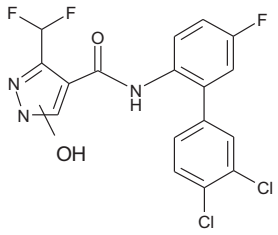


M20	not nomenclature possible - position of hydroxy group not specified	 or isomer
M21 (bixafen-desmethyl)	N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC)	
M23	not nomenclature possible - structure not specified	
M24	not nomenclature possible - structure not specified	
M25	not nomenclature possible - structure not specified	

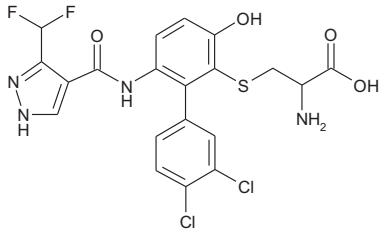
M26 not nomenclature possible - structure not specified



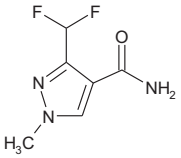
M27 not nomenclature possible - structure not specified



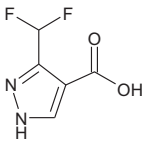
M37 S-[3',4'-dichloro-6-({[3-(difluoromethyl)-1H-pyrazol-4-yl]carbonyl}amino)-3-hydroxybiphenyl-2-yl]cysteine (IUPAC)



M43 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC)

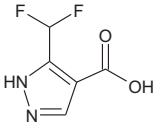


M44 3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid (IUPAC)



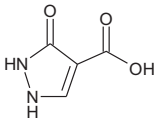
proposal for tautomer 1

M45 5-(difluoromethyl)-1H-pyrazole-4-carboxylic acid (IUPAC)



proposal for tautomer 2

M47 3-hydroxy-1H-pyrazole-4-carboxylic acid (IUPAC)



Animal metabolism

Information was available on the metabolism of bixafen in laboratory animals, lactating goats and laying hens.

In rats given (dichlorophenyl-U-^{14}C)-labelled bixafen orally by gavage, absorption was rapid and accounted for at least 83% of the total administered radioactivity after a single low dose (2 mg/kg bw). The maximum plasma concentrations of radioactivity were reached approximately 2–4 and 8 hours after administration of the low and high doses (2 and 50 mg/kg bw), respectively. Radioactivity was widely distributed throughout the body. Elimination of the radioactivity was mainly via faeces (≥ 91%), whereas elimination via urine accounted for 1–3% of the administered dose. In bile duct–cannulated rats, extensive biliary excretion (up to 83%) was demonstrated. Elimination of the radioactivity from the body was rapid, with a half-life in plasma of 8–9 hours and a mean residence time of 13–19 hours (for the low dose). Residues in tissues at 72 hours after a single oral dose as well as after repeated oral dosing accounted for 0.1–3% of the administered radioactivity, with liver and kidneys containing the highest concentrations of residues.

Metabolism of bixafen in rats was extensive, and more than 30 metabolites were identified. The main metabolic routes included demethylation, hydroxylation of the parent and bixafen-desmethyl, and conjugation with glucuronic acid or glutathione. A minor metabolic reaction was the cleavage of the amide bridge of bixafen.

Two studies on metabolism in lactating goats were available. The goats received five daily doses of [pyrazole-^{14}C]-bixafen or [dichlorophenyl-^{14}C]-bixafen at rates equivalent to 35 ppm and 46 ppm in the diet, respectively. The animals were sacrificed approximately 24 hours after the last dose. In both studies approximately 1.3% of the total dose was recovered from milk or tissues of the animals. Most of the radioactivity was excreted via faeces (74–82% AR) and urine (1.8–5.4% AR).

The metabolic pattern in both studies was comparable. In milk (TRR: 0.064–0.17 mg eq/kg), muscle (TRR: 0.047–0.057 mg eq/kg) and fat (TRR: 0.47–0.61 mg eq/kg) unchanged bixafen was the major residue, representing 74–77%, 56–66% and 89% of the total radioactivity, respectively. M21 (bixafen-desmethyl) was the only major metabolite being present at 16–18% of the TRR in milk, 34–43% in muscle and 10–11% in fat.

For kidney (TRR: 0.14–0.2 mg eq/kg) and liver (TRR: 0.74–1.2 mg eq/kg) parent bixafen was also a major residue, representing 44–46% of the total radioactivity in kidney and 18–23% in liver. Significant metabolites identified were M21 (bixafen-desmethyl), representing 37–38% of the TRR in kidney and 19–21% TRR in liver, followed by the two M23 isomers counting for a total of 9.3–15% of the TRR in kidney and 14–19% in liver.

For laying hens groups of hens received daily doses of [pyrazole-^{14}C]-bixafen or [dichlorophenyl-^{14}C]-bixafen at rates equivalent to 26 ppm and 32 ppm in the diet for 14 consecutive days. The animals were sacrificed ca. 24 hours after the last dose. Approximately 1.5% of the total dose in both studies was recovered from eggs or tissues of the animals. Most of the radioactivity administered was found in the excreta (88–93% AR). Total radioactive residues were 0.53–0.9 mg eq/kg in eggs, 0.032–0.037 mg eq/kg in muscle, 0.23–0.38 mg eq/kg in fat and 0.64–0.81 mg eq/kg in liver.

Parent bixafen was a major residue in eggs and all tissues except liver, representing 51–69% of the TRR in eggs, 23–41% in muscle and 80% in fat. In hens liver, only minor amounts of bixafen were detected (4.5–6.7% TRR).

M21 (bixafen-desmethyl) was the only major metabolite found in poultry tissues and eggs. It was found at levels of 26–39% of the TRR in eggs, 35–51% in muscle, 19–20% in fat and 24–26% in liver. In liver, M14, M18, M24, M25, M26, M27 and M37 were identified as minor metabolites, representing 1.0–8.8% of the TRR (0.007–0.067 mg eq/kg) each.

In summary bixafen is the major residue in most tissues, milk and eggs. It is moderately metabolized in goats and hens mainly resulting in M21 (bixafen-desmethyl). All major metabolites

were also identified in the rat. The metabolites M18, M25 and M26, mainly found in poultry liver, were not directly identified in the rat.

Plant metabolism

The Meeting received plant metabolism studies for bixafen following foliar application of either [pyrazole-^{14}C]-bixafen or [dichlorophenyl-^{14}C]-bixafen to soya beans or wheat.

Soya beans were independently treated with both bixafen-labels with three foliar applications of 0.06 kg ai/ha each when the first flowers opened (BBCH 60), at the end of flowering (BBCH 69) and finally when approximately 80% of the pods were ripe (BBCH 88). Samples were collected containing forage (5 days after 2nd application), hay (29 days after 2nd application), straw and seed (26 days after the 3rd application). Total radioactive residues were 4.0–5.3 mg eq/kg for forage, 2.8–4.0 mg eq/kg for hay, 9.5–13 mg eq/kg for straw and 0.005–0.024 mg eq/kg for seeds.

In all plant parts directly affected by the spray solution, unchanged bixafen was the major residue representing 96–98% of the TRR in forage, 92% in hay and 90–92% in straw. The only other metabolite identified was M21 (bixafen-desmethyl), present at 0.5–2.6% of the TRR.

For soya bean seeds, only samples following application of the pyrazole-label contained sufficient total radioactive residues for further investigation. Bixafen was the major residue with 30% of the TRR. Metabolites identified were M44 and M45 (19% TRR, 0.004 mg eq/kg) and M47 (12% TRR, 0.003 mg eq/kg). M21 (bixafen-desmethyl) was not identified in soya bean seeds.

Wheat was independently treated with both bixafen-labels using one foliar application of 0.125 kg ai/ha at the end of tillering / beginning of stem elongation (BBCH 29–31) followed by a second spraying with 0.15 kg ai/ha at the end of flowering (BBCH 69). Forage was harvested 9 days after the 1st application, hay 9 days after the 2nd application and straw and grain at maturity (50 days after the 2nd application). Total radioactive residues were 1.6–1.7 mg eq/kg for forage, 6.6–7.6 mg eq/kg for hay, 23–24 mg eq/kg for straw and 0.16–0.23 mg eq/kg for seeds.

In all samples unchanged bixafen was the major residue, representing > 90% of the TRR. The only other metabolite identified was M21 (bixafen-desmethyl) at 0.8–2.4% of the TRR.

In summary the plant metabolism of bixafen in the plants investigated is very limited. In plant parts directly affected by the spray solution, unchanged bixafen was the only residue significant. M21 (bixafen-desmethyl) was present at low levels up to 2.6% of the TRR.

In soya bean seeds, which were protected by the pod during treatment, bixafen was present at lower concentrations of 0.007 mg eq/kg (30% TRR). Major metabolites in soya bean seeds were M44, M45 and M47, probably taken up from the soil and distributed systemically, however at low levels not exceeding 0.004 mg eq/kg. All three of these metabolites were not identified in the rat.

Environmental fate in soil

The Meeting received information on the fate of bixafen after aerobic degradation in soil and after photolysis on the soil surface. In addition, the Meeting received information on the uptake and metabolism of bixafen soil residues by rotational crops, its dissipation under field conditions and long-term accumulation in soil.

In soil photolysis studies degradation of bixafen was not observed.

In aerobic soil metabolism studies under laboratory conditions bixafen was highly persistent with 80–90% remaining after 120 days. The only metabolite found was M44 (maximum 2.9% of AR), while the rest of the radioactivity remained unextracted or was recovered as $^{14}CO_2$. DT_{50} values could not be calculated due to the minimal degradation observed within 120 days.

In soil samples from the confined rotational crop metabolism studies mentioned below, bixafen was also slowly degraded. The only metabolite found was identified as M21 (bixafen-

desmethyl), slowly increasing from 0.5% (day 30) to 2.3% TRR at the end of the study (day 418). M44 was not detected.

In summary it can concluded that bixafen is persistent in soil, being degraded to a very minor extent.

Confined rotational crop studies on Swiss chard, turnips and wheat were conducted at rates equivalent to 0.79 kg ai/ha (pyrazole-label) and 0.85 kg ai/ha (dichlorophenyl-label). In plant commodities bixafen (11–78% TRR) and M21 (bixafen-desmethyl, 3–73% TRR) were the major residue components found for both labels. Quantified concentrations for the sum of both analytes were 0.016–0.024 mg eq/kg for Swiss chard, 0.005–0.035 mg eq/kg in turnip roots and tops and 0.011–0.041 mg eq/kg, 0.106–0.18 mg eq/kg and 0.152–0.462 mg eq/kg for wheat forage, hay and straw, respectively. In grain TRR levels were too low for identification (0.001–< 0.01 mg eq/kg).

Following application of the pyrazole-labelled active substance, the cleavage products M43 (3–15% TRR), M44 (0.3–37% TRR) and M45 (2–23% TRR) were identified as major metabolites. Concentrations were between 0.001–0.015 mg eq/kg each.

Following treatment with the dichlorophenyl-label, M20 was found in Swiss chard only at levels of 25–38% of the TRR (0.007–0.016 mg eq/kg).

The residue concentrations of bixafen and M21 in plants declined moderately in animal feed commodities while in food commodities only a slow decline of the residue was observed over the three crop rotations investigated. In all commodities investigated, except for wheat grain, detectable residues above the LOQ of 0.01 mg/kg were found for bixafen.

Field rotational crop studies were conducted at four locations in Europe. Bixafen was either applied to bare soil to simulate crop failure (0.28 kg ai/ha) or to barley as a primary crop (0.16 kg ai/ha at BBCH 56 plus 0.125 kg ai/ha at BBCH 69). Turnip/carrots, lettuce and wheat were planted as rotational crops at three rotations. Samples analysed for residues of bixafen and M21 were below the LOQ of 0.01 mg/kg for each analyte, except for one sample of wheat straw (M21: 0.02 mg/kg) and lettuce at a pre-mature growth stage (BBCH 46; bixafen: 0.05 mg/kg).

Field dissipation studies at six locations in Europe (four in the north, two in the south) confirmed the slow degradation of bixafen in soil observed in the aerobic metabolism studies. Within the first 100 days, a significant degradation of the residue concentration in soil was observed, leaving 42–63% of the initial concentrations. However, the decline after this period up to 730 days was minimal, leaving 17–47% of the initial concentration. M21 was not found above the LOQ of the analytical method.

The Meeting observed that the degradation of bixafen in soil follows a bi-phasic kinetics, starting with a fast decline within the first 100 days. After that initial interval, bixafen is highly persistent in soil, accumulating with subsequent treatments over multiple years.

In a long-term soil accumulation study under field conditions residues of bixafen and M21 (bixafen-desmethyl) in soil were investigated involving five and seven years of annual treatment with 0.14 kg ai/ha to the ground. In the first location in France a plateau for the bixafen concentration in soil was reached after five years, resulting in concentrations of up to 0.18 mg per kg soil. In Germany, the study was terminated after 7 years, due to technical reasons, without reaching a plateau, showing a bixafen peak concentration of 0.35 mg per kg soil. Most of the residue (> 95%) was present as unchanged parent substance located in the initial 10 cm soil layer. Based on an average density of 1.5 g/cm³ for soil these concentrations are equivalent to single application rates to the bare soil of 0.27 kg ai/ha in the French trial and 0.53 kg ai/ha in the German trial.

The Meeting concluded that bixafen residues accumulate in soil after annual treatments. Under consideration of the highest annual application rate reported in the authorised GAPs of 0.25 kg ai/ha, soil residue concentrations equivalent to single application rates to bare soil of 0.9 kg ai/ha could be reached.

In summary the Meeting concluded that bixafen is persistent in soil, accumulating after subsequent years of annual treatment. Confined rotational crop studies indicate a potential uptake of residues for bixafen and M21 (bixafen-desmethyl) into plant commodities. The Meeting also recognized that field rotational crop studies involved soil treatment rates not addressing the soil concentrations expected after subsequent annual treatment.

Methods of residue analysis

The Meeting received analytical methods for the analysis of bixafen and M21 (bixafen-desmethyl) in plant and animal matrices. The basic principle employs extraction by homogenisation with acetonitrile/water (4/1, v/v) or n-hexane with acetonitrile partitioning for fatty samples. The extracts were cleaned with filtration and C18 solid-phase extraction. Residues are determined by liquid chromatography (LC) in combination with tandem mass spectroscopy (MS/MS). Mass-transitions are m/z 414→394 (m/z 414→266 for confirmation) for bixafen and m/z 398→378 (m/z 398→358 for confirmation) for M21 (bixafen-desmethyl). The methods submitted are suitable for measuring residues of bixafen and M21 in plant and animal commodities with a LOQ of 0.01 mg/kg for each analyte.

The extraction efficiency with acetonitrile/water (4/1, v/v) was tested for wheat (forage, grain straw) obtained from plant metabolism and confined rotational crop studies. Extraction rates were of > 90% for primary treated commodities and 68–73% (corresponding to 72–99% of the TTR) for commodities from rotational crops.

For the application of multi-residue methods the DFG S-19 was tested, but found to be unsuitable for analysing bixafen or M21 in plant matrices.

Stability of residues in stored analytical samples

The Meeting received information on the storage stability of bixafen and M21 (bixafen-desmethyl) in plant matrices. In wheat grain, wheat straw, wheat green material, lettuce head, potato tuber, rape seed and in soil, no significant degradation of both analytes was observed within 24 months.

For animal matrices no storage stability data were provided. Samples in livestock metabolism or feeding studies were analysed within one month of sampling.

Definition of the residue

Livestock animal metabolism studies were conducted on laying hens (36–32 ppm) and lactating goats (35–46 ppm).

In goats parent bixafen and M21 (bixafen-desmethyl) were the major residue. Bixafen represented 74–77% TRR in milk, 56–66% TRR in muscle, 44–46% TRR in kidney, 18–23% TRR in liver and 89% TRR in fat. M21 was the major metabolite present at 16–18% TRR in milk, 34–43% TRR in muscle, 37–38% TRR in kidney, 19–21% TRR in liver and 10–11% TRR in fat. In kidney and liver the two isomers of M23 were found at 9–15% of the TRR in kidney (isomer 1: 2.8–4.3%; isomer 2: 6.5–10% TRR) and of 14–19% in liver (isomer 1: 8.6–13% TRR; isomer 2: 5.2–5.8% TRR).

In laying hens again bixafen was the major residue in eggs and all tissues except liver, representing 51–69% TRR in eggs, 23–41% in muscle and 80% in fat. In liver only minor amounts of bixafen were detected (4.5–6.7% of the TRR). M21 (bixafen-desmethyl) was the major metabolite found in poultry tissues and eggs. It accounted for 26–39% of the TRR in eggs, 35–51% in muscle, 19–20% in fat and 24–26% in liver. In liver, M14, M18, M24, M25, M26, M27 and M37 were identified as minor metabolites, representing 1.0–8.8% of the TRR (0.007–0.067 mg eq/kg) each. Of these, M18, M25 and M26 were not identified in the rat, however the exposure of M18 was below the respective acute and chronic TTCs for Cramer class III while M25 and M26 have structural similarity

to bixafen-desmethyl and are covered by the ADI for bixafen. As a consequence no consideration of dietary intake is required.

M21 (bixafen-desmethyl) was identified as the major residue in rat studies, suggesting that it is covered by toxicological reference values for parent bixafen. The Meeting concluded that parent bixafen and M21 (bixafen-desmethyl) are suitable marker compounds in animal commodities and should be included into the residue definition for compliance with MRLs and for the estimation of the dietary intake. Analytical methods are capable of measuring both analytes.

In livestock feeding studies the distribution of bixafen and M21 between skim milk/cream and egg white/egg yolk was investigated. The average ratio for cream/skim milk was > 93 for bixafen and 39 for M21. Egg yolk concentrations of bixafen were three times higher than in egg white while M21 showed ratios of 12–20. For the parent substance a log P_{ow} of 3.3 was measured.

The data for milk and eggs suggests that bixafen and M21 partition in the fat portion. In addition, residues in fat tissues were about ten times higher when compared to muscle. The Meeting decided that residues of bixafen are fat-soluble.

The fate of bixafen in plants was investigated following foliar application to soya beans and wheat. In all samples unchanged bixafen was the major residue, normally representing at least 90% of the TRR. M21 was present at very low levels, not exceeding 3% of the TRR. In soya bean seeds, which were not directly exposed to the spray solution due to the pods, only 30% of the TRR (0.007 mg eq/kg) was present as bixafen. Further major metabolites in soya bean seeds were identified as the tautomers M44 and M45 (19% TRR, 0.004 mg eq/kg) and M47 (12% TRR, 0.003 mg eq/kg).

In confined rotational crop studies, plant commodities Swiss chard, wheat and turnips contained concentrations of radioactive residues as high as 0.49 mg eq/kg, in wheat straw. Bixafen (11–78% TRR) and M21 (bixafen-desmethyl, 3–73% TRR) were the major compounds identified. In addition, M43 (3–15% TRR), M44 (0.3–37% TRR) and M45 (2–23% TRR) were identified as major metabolites in all rotational crops. M20 was only found in Swiss chard (25–38% TRR, 0.007–0.016 mg eq/kg). Wheat grain did not contain TRR levels allowing further identification (0.001–< 0.01 mg eq/kg).

The Meeting concluded that parent bixafen is a suitable maker for compliance with MRLs in all plant commodities (primary treated or rotational). For the estimation of the dietary intake M21 was insignificant in wheat and soya beans directly treated, but was identified in high relative amounts in rotational crops. Therefore, the Meeting decided to include M21 (bixafen-desmethyl) into the residue definition for dietary intake with the parent substance. The metabolites M20, M44, M45 and M47, mainly found in rotational crops, were not identified in the rat. However, the estimated exposure levels based on the confined rotational crop study are below the respective acute and chronic TTCs for Cramer class III. As a result, no consideration is required for dietary intake.

Analytical methods are capable of measuring bixafen and M21 (bixafen-desmethyl) in plant matrices.

Definition of the residue for compliance with MRL for plant commodities: *bixafen*

Definition of the residue for compliance with MRL for animal commodities and (for the estimation of dietary intake) for plant and animal commodities: *sum of bixafen and N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (bixafen-desmethyl), expressed as bixafen*

The residue is fat-soluble.

Results of supervised residue trials on crops

The Meeting received supervised European trial data for applications of bixafen to rape seed, barley and wheat.

Residue values referred to as "total" describe the sum of bixafen and M21 (bixafen-desmethyl), expressed as bixafen.

The Meeting concluded that field rotational crop studies do not address residues in soil expected after subsequent annual application of bixafen. Confined rotational crop studies available are not considered representative of field conditions. In the absence of suitable data, residue concentrations in plant commodities taken up from the soil by annual crops could not be estimated. Therefore, the Meeting decided, that no recommendations on maximum residue levels and median/highest residues could be made for bixafen in non-permanent crops.

Nevertheless, for the benefit of potential future assessments of bixafen uses, the Meeting decided to evaluate GAPs and residue data following direct application.

Rape seed

Bixafen is registered in the UK for use on rape seed at rates of 2 × 0.075 kg ai/ha with a PHI of 56 days. Supervised field trials conducted in northern Europe, according to this GAP, were submitted.

For MRL compliance purposes residues of parent bixafen in rape seeds were (n=10): < 0.01(6), 0.01(3), 0.017 mg/kg.

For dietary intake purposes the total residues in rape seeds were (n=10): < 0.02(5), 0.02(4), 0.028 mg/kg.

Barley and oats

For barley and oats the maximum GAP in northern Europe was reported from the UK involving two foliar applications of up to 0.125 kg ai/ha each. The last application is conducted at BBCH 61 and the PHI is covered by the growth between treatment and harvest. Supervised field trials conducted in northern Europe according to this GAP were submitted.

For MRL compliance purposes residues of parent bixafen in barley grain in northern Europe were (n=10): 0.02, 0.04(3), 0.05, 0.07, 0.08, 0.09, 0.09, 0.1 mg/kg.

For dietary intake purposes the total residues in barley grain in northern Europe were (n=10): 0.03, 0.05(3), 0.06, 0.08, 0.1, 0.1, 0.11, 0.11 mg/kg.

In Southern Europe a GAP for barley and oats was reported from France with one application of 0.075 kg ai/ha up to BBCH 61 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for the residue data originating from southern Europe against the GAP of the UK.

For MRL compliance purposes residues of parent bixafen in barley grain in southern Europe according to the UK GAP were (n=9): 0.03, 0.04, 0.04, 0.06, 0.06, 0.08, 0.1, 0.25, 0.34 mg/kg.

For dietary intake purposes the total residues in barley grain in southern Europe according to the UK GAP were (n=9): 0.04, 0.05, 0.05, 0.08, 0.08, 0.1, 0.11, 0.29, 0.38 mg/kg.

The Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for barley:

For MRL compliance purposes residues of parent bixafen in barley grain in whole Europe (n=19): 0.02, 0.03, 0.04(5), 0.05, 0.06, 0.06, 0.07, 0.08, 0.08, 0.09, 0.09, 0.1, 0.1, 0.25 and 0.34 mg/kg.

For dietary intake purposes the total residues in barley grain in whole Europe were (n=19): 0.03, 0.04, 0.05(5), 0.06, 0.08(3), 0.1(3), 0.11(3), 0.29 and 0.38 mg/kg.

Wheat, rye, triticale and spelt

For wheat, rye and triticale the maximum GAP in Northern Europe was reported from the UK and involved two foliar applications of up to 0.125 kg ai/ha each. The last application is conducted at BBCH 69 and the PHI is covered by the growth between treatment and harvest. Supervised field trials conducted in Northern Europe according to this GAP were submitted.

For MRL compliance purposes residues of parent bixafen in wheat grain in northern Europe were (n=10): < 0.01(6), 0.01, 0.01, 0.03, 0.03 mg/kg.

For dietary intake purposes the total residues in wheat in northern Europe were (n=10): < 0.02(6), 0.02, 0.02, 0.04, 0.04 mg/kg.

In southern Europe a GAP for wheat, rye and triticale was reported from France with one application of 0.094 kg ai/ha up to BBCH 69 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for residue data from southern Europe against UK GAP:

For MRL compliance purposes residues of parent bixafen in wheat grain in Southern Europe according to the UK GAP were (n=10): < 0.01(6), 0.01, 0.02, 0.02, 0.03 mg/kg.

For dietary intake purposes the total residues in wheat grain in southern Europe according to UK GAP were (n=10): < 0.02(6), 0.02, 0.03, 0.03, 0.04 mg/kg.

The Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for wheat:

For monitoring purposes residues of parent bixafen in wheat grain in whole Europe (n=20): < 0.01(12), 0.01(3), 0.02, 0.02, 0.03, 0.03 and 0.03 mg/kg.

For dietary intake purposes the total residues in wheat grain in whole Europe were (n=20): < 0.02(12), 0.02(3), 0.03, 0.03, 0.04, 0.04 and 0.04 mg/kg.

Animal feeds

Oilseed rape, forage

The Meeting noted that the only authorisation submitted for bixafen in rape was from UK explicitly relating to oilseed rape. This GAP involves late treatment of the crop 56 days before harvest, which is normally beyond the common timeframe for utilization of oilseed rape as a forage crop, i.e., before winter and up to BBCH 39. This is supported by supervised field trials in northern Europe, where last applications were conducted at growth stages at the end of flowering or at early maturity.

The Meeting concluded that the reported GAP for bixafen is not relevant for the utilization of oilseed rape as an animal forage crop.

Barley, oats, rye, triticale and wheat – forage of cereals

GAPs for barley and oats in the UK are for a maximum of two foliar applications up to flowering (BBCH 61) with 0.125 kg ai/ha each. The PHI is covered by the interval between treatment and harvest (covered by growth stage).

For the calculation of the livestock animal dietary burden the total residues in barley forage (fresh) in northern Europe were (n=10): 2.1, 2.5, 2.6, 2.9, 3.5, 3.9, 4.0, 4.4, 4.5, 7.3 mg/kg.

In southern Europe a GAP for barley and oats was reported from France with one application of 0.075 kg ai/ha up to BBCH 61 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for residue data from southern Europe against the UK GAP:

For the calculation of the livestock animal dietary burden the total residues in barley forage (fresh) in southern Europe were (n=9): 2.7, 3.0, 3.2, 3.4, 3.4, 3.7, 3.8, 4.3, 6.0 mg/kg.

The Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for barley and oat forage:

For the calculation of the livestock animal dietary burden the total residues in barley forage (fresh) in whole Europe were (n=19): 2.1, 2.5, 2.6, 2.7, 2.9, 3.0, 3.2, 3.4, 3.4, 3.5, 3.7, 3.8, 3.9, 4.0, 4.3, 4.4, 4.5, 6.0, 7.3 mg/kg.

For wheat, rye and triticale the maximum GAP in northern Europe was reported for the UK involving two foliar applications of up to 0.125 kg ai/ha. The last application is at BBCH 69 with the PHI covered by growth between treatment and harvest. Supervised field trials conducted in northern Europe according to this GAP were submitted.

For the calculation of the livestock animal dietary burden the total residues in wheat forage (fresh) in northern Europe were (n=10): 1.5, 2.4, 2.8, 2.9, 3.1, 3.4, 3.8, 4.7, 4.8, 7.3 mg/kg.

In southern Europe a GAP for wheat, rye and triticale was reported from France with one application of 0.094 kg ai/ha up to BBCH 69 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for residue data from southern Europe against the UK GAP.

For the calculation of the livestock animal dietary burden the total residues in wheat forage (fresh) in southern Europe were (n=10): 2.6, 2.7, 2.9, 3.0, 3.6, 3.9, 4.2, 4.5, 5.2, 5.5 mg/kg.

The Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for wheat forage.

For the calculation of the livestock animal dietary burden the total residues in barley and wheat forage (fresh) in Europe were (n=20): 1.5, 2.4, 2.6, 2.7, 2.8, 2.9, 2.9, 3.0, 3.1, 3.4, 3.6, 3.8, 3.9, 4.2, 4.5, 4.7, 4.8, 5.2, 5.5, 7.3 mg/kg.

Barley, oats, rye, triticale and wheat – straw and fodder

GAPs for barley and oats in the UK are for a maximum of two foliar applications up to flowering (BBCH 61) with 0.125 kg ai/ha each. The PHI is covered by the interval between treatment and harvest (covered by growth stage).

For MRL compliance purposes residues of parent bixafen in barley straw (fresh) in northern Europe (n=10): 0.64, 0.7, 0.77, 0.86, 1.1, 1.1, 3.7, 4.8, 5.4, 10 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in barley straw (fresh) in northern Europe were (n=10): 0.72, 0.74, 0.85, 1.0, 1.2, 1.2, 3.9, 5.2, 5.6, 11 mg/kg.

In southern Europe a GAP for barley and oats was reported from France with one application of 0.075 kg ai/ha up to BBCH 61 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for residue data from Southern Europe against the UK GAP.

For MRL compliance purposes residues of parent bixafen in barley straw (fresh) in southern Europe (n=9): 0.46, 0.76, 1.2, 1.5, 1.9, 3.1, 5.2, 5.7, 6.2 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in barley straw (fresh) in southern Europe were (n=9): 0.5, 1.0, 1.3, 1.6, 2.1, 3.3, 5.6, 6.1, 6.7 mg/kg.

Since both datasets are not significantly different (Mann-Whitney-U-testing), the Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for barley and oat straw.

For MRL compliance purposes residues of parent bixafen in barley straw (fresh) in whole Europe (n=19): 0.46, 0.64, 0.7, 0.76, 0.77, 0.86, 1.1, 1.1, 1.2, 1.5, 1.9, 3.1, 3.7, 4.8, 5.2, 5.4, 5.7, 6.2 and 10 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in barley straw (fresh) in whole Europe were (n=19): 0.5, 0.72, 0.74, 0.85, 1.0, 1.0, 1.2, 1.2, 1.3, 1.6, 2.1, 3.3, 3.9, 5.2, 5.6, 5.6, 6.1, 6.7, 11 mg/kg.

For wheat, rye and triticale the maximum GAP in Northern Europe was reported from the UK and involved two foliar applications of up to 0.125 kg ai/ha. The last application is at BBCH 69 and the PHI is covered by growth between treatment and harvest. Supervised field trials conducted in northern Europe according to this GAP were submitted.

For MRL compliance purposes residues of parent bixafen in wheat straw (fresh) in northern Europe (n=10): 0.52, 0.93, 0.95, 1.3, 1.8, 1.9, 3.6, 4.1, 8.4 and 10 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in wheat straw (fresh) in northern Europe were (n=10): 0.78, 1.2, 1.3, 1.5, 2.1, 2.5, 3.9, 4.4, 9.6 and 11 mg/kg.

In southern Europe a GAP for wheat, rye and triticale was reported from France with one application of 0.094 kg ai/ha up to BBCH 69 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for residue data from southern Europe against the UK GAP:

For MRL compliance purposes residues of parent bixafen in wheat straw (fresh) in southern Europe (n=10): 0.79, 1.4, 1.7, 1.8, 2.6, 3.2, 3.3, 3.6, 5.4 and 5.7 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in wheat straw (fresh) in southern Europe were (n=10): 1.2, 1.9, 1.9, 2.2, 3.2, 3.7, 3.9, 4.1, 6.0 and 6.2 mg/kg.

The Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for wheat, rye and triticale straw:

For MRL compliance purposes residues of parent bixafen in wheat straw (fresh) in whole Europe (n=20): 0.52, 0.79, 0.93, 0.95, 1.3, 1.4, 1.7, 1.8, 1.8, 1.9, 2.6, 3.2, 3.3, 3.6, 3.6, 4.1, 5.4, 5.7, 8.4 and 10 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in wheat straw (fresh) in whole Europe were (n=20): 0.78, 1.2, 1.2, 1.3, 1.5, 1.9, 1.9, 2.1, 2.2, 2.5, 3.2, 3.7, 3.9, 3.9, 41, 4.4, 6.0, 6.2, 9.6 and 11 mg/kg.

Residues in rotational crops

Bixafen is highly persistent in soil, showing accumulation over subsequent years of treatment. In field rotational crop studies conducted at rates corresponding to the highest annual application rates registered, no significant residues were found in plant commodities. However, the long-term field accumulation study submitted suggests plateau residues in soil after up to seven years of annual treatment are equivalent to 2–3 times the soil residues expected after a single treatment at the registered maximum annual application rate. In confined rotational crop studies approximating this plateau level observed in soil, residues above the LOQ of 0.01 mg/kg were found for bixafen and M21 (bixafen-desmethyl) in all plant commodities investigated except for wheat grain.

The Meeting concluded that the accumulation of bixafen in soil results in residue concentrations in follow crops which are relevant for MRL compliance, dietary intake assessment

and the estimation of livestock dietary burden. However, the Meeting recognized that the available field rotational crop studies were underdosed compared to the soil concentrations following long-term use of bixafen, while confined rotational crop studies are not considered representative for field conditions.

The Meeting decided that further information on bixafen in rotational crops under field conditions are required involving application rates approximating the plateau levels in soil after subsequent years of treatment. The estimation of maximum residue levels and median or highest residues for annual crops is not possible without considering the contribution of residues taken up from soil and will be postponed to a future meeting when new data becomes available to assess the rotational crop situation.

Fate of residues during processing

The Meeting received information on the hydrolysis of radio-labelled bixafen as well as processing studies using unlabelled material on grown residues in oilseed rape, barley and wheat.

In a hydrolysis study using radio-labelled bixafen typical processing conditions were simulated (pH 4,5 and 6 with 90 °C, 100 °C and 120 °C for 20, 60 and 20 minutes). In duplicate samples of sterile buffer solution no degradation was observed.

The Meeting concluded that no recommendations on bixafen residues in plant commodities can be made (see residues in rotational crops section) and therefore no processing factors are required. For an overview of the available information on the fate of bixafen during processing please refer to the 2013 Evaluation.

Residues in animal commodities

Farm animal feeding studies

The Meeting received feeding studies involving bixafen on lactating cows and laying hens.

Three groups of lactating cows were dosed daily at levels of 4, 12 and 40 ppm in the diet (0.15, 0.45 and 1.5 mg/kg bw) for 28 consecutive days. Milk was collected throughout the whole study and tissues were collected on day 29 within 24 hours of the last dose.

In milk highest mean total residues were 0.039 mg/kg for the 4 ppm group, 0.077 mg/kg for the 12 ppm group and 0.218 mg/kg for the 40 ppm group. Investigation of the distribution of the residue in cream gave a 9.9 fold higher concentration than in whole milk (15 between whole milk and milk fat).

Total residues in muscle for the 4, 12 and 40 ppm groups were 0.039–0.065 mg/kg (mean: 0.052 mg/kg), 0.081–0.26 mg/kg (mean: 0.162 mg/kg) and 0.63–1.0 mg/kg (mean: 0.82 mg/kg), respectively. In liver residues were 0.42–0.69 mg/kg (mean: 0.57 mg/kg) for the 4 ppm group, 1.2–1.7 mg/kg (mean: 1.4 mg/kg) for the 12 ppm group and 4.8–5.4 mg/kg (mean: 5.0 mg/kg) for the 40 ppm group. Kidney contained total residues of 0.1–0.15 mg/kg (mean: 0.14 mg/kg), 0.28–0.37 mg/kg (mean: 0.34 mg/kg) and 1.0–1.3 mg/kg (mean: 1.2 mg/kg) for the for the 4, 12 and 40 ppm group.

For fat three different tissues were analysed (perirenal, mesenteric and subcutaneous fat). Highest residues were found in perirenal fat with 0.14–0.21 mg/kg (mean: 0.18 mg/kg) for the 4 ppm group, 0.33–0.48 mg/kg (mean: 0.43 mg/kg) for the 12 ppm group and 0.8–1.9 mg/kg (mean: 1.4 mg/kg) for the 40 ppm group.

For laying hens three groups of animals were dosed with rates of 1.5, 4.5 and 15 ppm in the dry weight feed (0.1, 0.3 and 1.0 mg/kg bw) for 28 consecutive days. Eggs were collected throughout the whole study and tissues were collected on day 29 after the last dose.

In eggs total residues at the plateau phase were < 0.02-0.02 mg/kg (highest daily mean: 0.02 mg/kg) for the 1.5 ppm group and ranged between 0.05 to 0.07 mg/kg (highest daily mean: 0.063 mg/kg) for the 4.5 ppm and between 0.13 to 0.22 mg/kg (highest daily mean: 0.178 mg/kg) for the 15 ppm group.

In tissues no residues above the LOQ were found in muscle. Total residues in fat for the 1.5, 4.5 and 15 ppm groups were < 0.02–0.02 mg/kg (mean: 0.02 mg/kg), 0.05–0.06 mg/kg (mean: 0.057 mg/kg) and 0.06–0.09 mg/kg (mean: 0.07 mg/kg), respectively. In liver residues were < 0.02–0.02 mg/kg (mean: 0.02 mg/kg) for the 1.5 ppm group, 0.02–0.04 mg/kg (mean: 0.03 mg/kg) for the 4.5 ppm group and 0.03–0.05 mg/kg (mean: 0.04 mg/kg) for the 15 ppm group.

Estimated maximum and mean dietary burdens of livestock and animal commodities maximum residue levels

The Meeting noted that the uptake of bixafen and M21 (bixafen-desmethyl) from soil contributes significantly to the overall residues in annual crops. Based on the information available (see residues in rotational crops), no estimation on livestock animal dietary burdens and the corresponding residue levels in animal commodities can be made.

RECOMMENDATIONS

Definition of the residue for compliance with MRL for plant commodities: *bixafen*

Definition of the residue for compliance with MRL for animal commodities and (for the estimation of dietary intake) for plant and animal commodities: *sum of bixafen and N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (bixafen-desmethyl), expressed as bixafen*

The residue is fat-soluble.

FURTHER WORK OR INFORMATION

The Meeting considered that the currently available information on residues in rotational crops was not sufficient to make recommendations on maximum residue levels in plant and animal commodities. For future recommendations field rotational crop studies approximating plateau concentrations of bixafen in soil are required.

DIETARY RISK ASSESSMENT

The Meeting concluded that the contribution of residues in plant commodities from soil uptake cannot be estimated based on the available data. Thus no estimations on median or highest residues in food commodities of plant and animal origin could be made, precluding both long and short-term dietary risk assessments for bixafen.

Consequently, the dietary risk assessment will be undertaken at a future meeting when the residues derived from both direct application and those taken up from the soil in a rotational crop situation can be evaluated together.