5. EVALUATION OF DATA FOR ACCEPTABLE DAILY INTAKE AND ACUTE DIETARY INTAKE FOR HUMANS, MAXIMUM RESIDUE LEVELS AND SUPERVISED TRIALS MEDIAN RESIDUE VALUES

5.1 AMETOCTRADIN (260)

TOXICOLOGY

Ametoctradin is the common name provisionally approved by the International Organization for Standardization (ISO) for 5-ethyl-6-octyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine, for which the Chemical Abstracts Service (CAS) number is 865318-97-4. Ametoctradin is a fungicide that inhibits zoospore differentiation within the zoosporangium, the release of zoospores from the zoosporangium, the motility of any released zoospores and the germination of encysted zoospores. It acts by reducing the adenosine triphosphate (ATP) content in these stages of development by binding to and inhibiting complex III of the respiratory chain in mitochondria of oomycetes.

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

All studies evaluated were performed by good laboratory practice (GLP)–certified laboratories and complied with the relevant OECD and/or United States Environmental Protection Agency (USEPA) test guidelines.

Biochemical aspects

Following oral administration, ^{14}C-labelled ametoctradin underwent limited and saturable absorption from the gastrointestinal tract, but was quite widely distributed. Maximum plasma concentrations (C_{max}) were observed within 1–2 hours after administration, and initial half-lives ranged from 2 to 3 hours. The area under the plasma concentration–time curve (AUC) values indicate that internal exposure was not different in males and females. Excretion of ametoctradin occurred rapidly and independently of sex. Most of the administered dose (91–110%) was recovered within 168 hours after a single low or high dose and repeated high doses, with faeces as the main elimination route. Based on the amount of radioactivity excreted via bile and urine, the bioavailability of ametoctradin in rats was calculated to be about 40% of the administered dose at 50 mg/kg bw and about 20% of the applied dose at 500 mg/kg bw. The parent compound is metabolized by terminal oxidation of the octyl side-chain to the respective carboxylic acid (M650F09), with subsequent degradation of the carboxylic side-chain to give M650F06 and M650F01. In addition, conjugation of the respective oxidized side-chain with taurine and/or glucuronic acid occurs, leading to metabolites M650F10 (taurine conjugate of M650F09), M650F11 (glucuronic acid conjugate of M650F06) and M650F12 (taurine conjugate of M650F06). Also, a minor metabolic step leads to the formation of M650F05 (ω-hetarylpentanoic acid). Several metabolites of ametoctradin were found in liver, kidneys, plasma and bile, with metabolite M650F06 being the most abundant.

Toxicological data

Ametoctradin has low acute oral and dermal toxicity (median lethal doses [LD_{50}s] > 2000 mg/kg bw) and low toxicity by inhalation (median lethal concentration [LC_{50}] > 5.5 mg/L). No skin or eye irritation was observed after ametoctradin exposure. Ametoctradin was not a sensitizer in a Magnusson & Kligman maximization test or in the murine local lymph node assay.

In repeated-dose toxicity studies in mice, rats and dogs, no consistent toxicological findings were evident in any of the species at any dose tested up to the limit dose (around 1000 mg/kg bw per day) or after any study duration. Ametoctradin was extensively tested in a comprehensive set of current guideline studies, including short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, studies of reproductive and developmental toxicity, neurotoxicity studies and an immunotoxicity study.

In the long-term studies of toxicity and carcinogenicity, no treatment-related changes in tumour incidence were observed.

The Meeting concluded that ametoctradin was not carcinogenic in mice or rats.

Ametoctradin was tested for genotoxicity in an adequate range of in vitro and in vivo studies. No evidence for genotoxicity was observed in any of these tests.

The Meeting concluded that ametoctradin was not genotoxic.

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

The toxicity of several soil metabolites of ametoctradin was examined. Ninety-day dietary toxicity studies in rats were performed with metabolites M650F03 and M650F04, metabolites that were not found in the rat. In the 90-day dietary toxicity studies, no adverse effects were observed after exposure to either M650F03 or M650F04 up to the limit dose (i.e. 15 000 parts per million [ppm], equivalent to about 1000 mg/kg bw per day). The genotoxic potential of three soil metabolites, M650F02, M650F03 and M650F04, was tested in several in vitro and in vivo studies. All were negative for genotoxicity.

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

Toxicological evaluation

From the animal studies with ametoctradin, no adverse effects were observed at or near the limit dose of approximately 1000 mg/kg bw per day. The Meeting concluded that it was not necessary to establish an ADI for ametoctradin. This was based on a reasonable estimate of a likely maximal intake of the residues of a pesticide arising from the daily diet. In the 2004 JMPR report and in more detail in the 2005 publication by Solecki et al. on guidance on setting ARfDs[1], a maximum cut-off of 5 mg/kg bw for the ARfD was suggested, based on food consumption estimates and maximum residue levels in foods. This cut-off would equate to a NOAEL of 500 mg/kg bw per day in an animal study, with the application of the default uncertainty factor of 100. A similar principle was considered by the Meeting to be applicable in setting an extreme upper bound for the ADI, noting that the long-term daily dietary exposure for the residues of a particular pesticide will be less than the IESTI for the residues of that pesticide. A cut-off for the ADI could be refined, taking into account long-term high-level consumption.

The Meeting concluded that it was not necessary to establish an ARfD for ametoctradin in view of the absence of acute toxicity or any other effect that could be elicited by a single dose.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	6000 ppm, equal to 1099 mg/kg bw per day[b]	—
		Carcinogenicity	6000 ppm, equal to 1099 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	15 000–22 500 ppm, equal to 871 mg/kg bw per day[b]	—
		Carcinogenicity	15 000–22 500 ppm,	—

[1] Solecki R *et al*. (2005). Guidance on setting of acute reference dose (ARfD) for pesticides. Food and Chemical Toxicology, 43:1569–1593.

Species	Study	Effect	NOAEL	LOAEL
			equal to 871 mg/kg bw per day[b]	
	Two-generation study of reproductive toxicity[a]	Parental toxicity	939 mg/kg bw per day[b]	—
		Offspring toxicity	939 mg/kg bw per day[b]	—
		Reproductive toxicity	939 mg/kg bw per day[b]	—
	Developmental toxicity study[c]	Maternal toxicity	1000 mg/kg bw per day[b]	—
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	—
	Acute neurotoxicity study[c]	Neurotoxicity	2000 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity study[c]	Maternal toxicity	1000 mg/kg bw per day[b]	—
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	—
Dog	One-year study of toxicity[a]	Toxicity	30 000 ppm, equal to 848 mg/kg bw per day[b]	—

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.

Estimate of acceptable daily intake for humans

Unnecessary

Estimate of acute reference dose

Unnecessary

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 ametoctradin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Approximately 20% at high dose (500 mg/kg bw) and 40% at low dose (50 mg/kg bw)
Dermal absorption	No information on the pure active substance
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid and complete
Metabolism in animals	Limited; several metabolites, with M650F06 being most abundant
Toxicologically significant compounds in animals, plants and the environment	None
Acute toxicity	
Rat, LD_{50}, oral	> 2000 mg/kg bw per day
Rat, LD_{50}, dermal	> 2000 mg/kg bw per day
Rat, LC_{50}, inhalation	> 5.5 mg/L air (4 h, nose only)
Rabbit, dermal irritation	Non-irritant
Rabbit, ocular irritation	Non-irritant
Dermal sensitization	Not sensitizing (Magnusson & Kligman and local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	No adverse effects at the limit dose
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	No adverse effects at the limit dose
Carcinogenicity	No carcinogenic potential

Genotoxicity	
	No genotoxic potential
Reproductive toxicity	
Target/critical effect	No adverse effects at the limit dose
Developmental toxicity	
Target/critical effect	No adverse effects at the limit dose
Neurotoxicity	
Acute neurotoxicity	No neurotoxicity at the limit dose
Other toxicological studies	
Immunotoxicity studies	No immunotoxicity at the limit dose
Studies performed on metabolites or impurities	M650F02: Not genotoxic M650F03: Not genotoxic; no effects at the limit dose in 90-day rat study M650F04: Not genotoxic; no effects at the limit dose in 90-day rat study
Medical data	
	Limited information; new compound

Summary

	Value	Study	Safety factor
ADI	Unnecessary	—	—
ARfD	Unnecessary	—	—

RESIDUE AND ANALYTICAL ASPECTS

Residue and analytical aspects of ametoctradin were considered for the first time by the present Meeting. The residue evaluation was scheduled for the 2012 JMPR by the Forty-third Session of the CCPR.

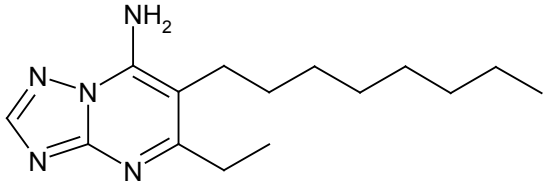
Ametoctradin is a fungicide of the chemical class triazolo-pyrimidylamines. Ametoctradin strongly inhibits zoospore differentiation within the zoosporangium, the release of zoospores from the zoosporangium, the motility of any released zoospores and the germination of encysted zoospores. The inhibition caused by ametoctradin reduces the ATP content in these stages of development by binding to and inhibiting complex III of the respiratory chain in mitochondria of Oomycetes.

The Meeting received information from the manufacturer on identity, metabolism, storage stability, residue analysis, use pattern, residues resulting from supervised trials on grapes, bulb vegetables, Brassica vegetables, fruiting vegetables, leafy vegetables, celery, potatoes and hops, fate of residue during processing, and livestock feeding studies.

Chemical name:

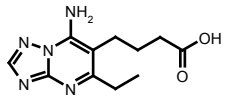
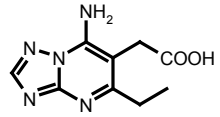
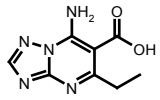
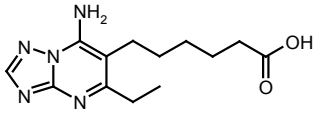
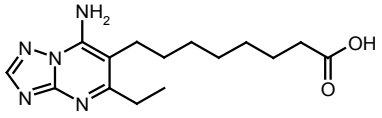
Ametoctradin or IUPAC: 5-ethyl-6-octyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine

Structural formula:



Metabolites referred to in the appraisal by codes:

M650F01	4-(7-amino-5-ethyl[1,2,4]triazolo[1,5-a]pyrimidin-6-yl)butanoic acid or ω-hetarylbutanoic acid

	
M650F03	(7-amino-5-ethyl [1,2,4]triazolo [1,5-a]pyrimidin-6-yl) acetic acid or hetarylacetic acid 
M650F04	7-amino-5-ethyl [1,2,4]triazolo [1,5-a]pyrimidine-6-carboxylic acid or hetarylcarboxylic acid 
M650F06	6-(7-amino-5-ethyl[1,2,4]triazolo[1,5-a]pyrimidin-6-yl)hexanoic acid or ω-hetarylhexanoic acid 
M650F09	8-(7-amino-5-ethyl[1,2,4]triazolo[1,5-a]pyrimidin-6-yl)octanoic acid or ω-hetaryloctanoic acid 

Animal metabolism

The Meeting received results of animal metabolism studies in lactating goats and laying hens. Experiments were carried out with [2,7-^{14}C]-ametoctradin.

Metabolism in laboratory animals was summarized and evaluated by the WHO panel of the JMPR in 2012. Oral administration of radiolabelled ametoctradin in rats results in a rapid absorption and high degree of biotransformation, as indicated by low amounts of parent compound found in urine and bile. A considerable part of the applied ametoctradin was excreted unchanged via faeces. In liver, kidneys, plasma and bile, several metabolites of ametoctradin were found, with metabolite M650F06 being the most abundant. The parent compound is metabolized by terminal oxidation of the octyl side chain to the respective carboxylic acid (M650F09) with subsequent degradation of the carboxylic side chain (M650F06 and M650F01). In addition, conjugation of the respective oxidised side chain with taurine and/or glucuronic acid occurs, leading to metabolites M650F10 (taurine conjugate of M650F09), M650F11 (glucuronic acid conjugate of M650F06), and M650F12 (taurine conjugate of M650F06), respectively. Also a minor metabolic step leads to the formation of M650F05 (ω-hetarylpentanoic acid).

Two lactating goats, orally treated once daily for 10 consecutive days with [2,7-^{14}C]-ametoctradin, were sacrificed 23 hours after the last dose. The two goats received an actual dose rate of 13 and 12 ppm dry feed (0.51 and 0.49 mg ai/kg bw, respectively). Total recovered radioactivity amounted to 64% of the administered dose in goat 1 and 88% in goat 2. Radioactivity recovered from urine, faeces and cage wash amounted to 61% of the administered dose in goat 1 (24% in urine; 36%

in faeces) and 84% in goat 2 (26% in urine, 58% in faeces). In both animals, radioactivity amounted to 0.15–0.19% of the applied dose in milk and 0.05% in edible tissues and organs.

The total radioactive residues (TRR) in tissues and milk were 0.10 mg/kg eq (liver), 0.036 mg/kg eq (kidney), 0.016 mg/kg eq (fat), 0.010 mg/kg eq (muscle) and 0.028 mg/kg eq (pooled milk). Radioactivity levels in afternoon milk were higher than residue levels in morning milk (just before the next dosing). Radioactivity levels in milk did not reach a clear plateau, although a flattening of the curve started by day 5–8 (0.026–0.048 mg/kg eq).

Methanol and water extracted 98% TRR for milk, 53% TRR for liver, 63% TRR for kidney, 72% TRR for muscle and 82% TRR for fat. The parent compound was not found in any of the goat commodities. In goat milk, liver, kidney and fat, the metabolite M650F06 (ω-hetarylhexanoic acid) was the most abundant component of the residues (22–47% TRR or 0.006–0.021 mg/kg eq), followed by the metabolite M650F01 (ω-hetarylbutanoic acid, 14–26% TRR or 0.003–0.014 mg/kg eq) and the metabolite M650F09 (ω-hetaryloctanoic acid, 7.7–9.4% TRR or 0.002–0.003 mg/kg eq; not detected in liver). A total of 91% (milk), 36% (liver), 46% (kidney), and 57% (fat) of the TRR could be identified in the initial extracts. No metabolite was identified in muscle (total extractable residues: 0.002 mg/kg eq). The solids remaining after initial extraction in liver and kidney were treated with protease and microwave, resulting in a release of most of the radioactivity (38% TRR in liver and 30% TRR in kidney). This radioactivity could not be attributed to any of the known metabolites.

Nine laying hens, orally treated once daily for 10 consecutive days with [2,7-^{14}C]-ametoctradin, were sacrificed 23 hours after the last dose. Hens were treated at an actual dose rate of 12 ppm dry feed (equivalent to 0.81 mg ai/kg bw). The total recovery of the applied dose was 93%. Radioactivity from the excreta and cage wash amounted to 92.4% of the administered dose, while 0.03% was found in liver, 0.06% in muscle, 0.00% in fat and 0.09% in eggs.

Concentrations in eggs increased within the first 6 application days and reached a plateau from day 6 onwards (0.037–0.040 mg/kg eq). The highest radioactivity concentrations in edible tissues were found in liver (0.11 mg/kg eq), followed by muscle (0.026 mg/kg eq) and fat (0.014 mg/kg eq).

Radioactivity was characterized in liver, muscle, fat and eggs. Methanol and water extracted 82% TRR for eggs, 52% TRR for liver, 44% TRR for muscle, and 66% TRR for fat. In hens, only low levels of residues were identified (each compound < 0.01 mg/kg eq). The major compounds were metabolite M650F01 (ω-hetarylbutanoic acid) with 28%, 8.7%, 1.9% TRR in fat, liver, muscle, respectively and parent compound with 22%, 11% TRR in eggs and fat, respectively. Metabolite M650F06 (ω-hetarylhexanoic acid) was only identified in liver and muscle at trace amounts (1.1–1.3% TRR) and metabolite M650F09 was not detected. A total of 22% (eggs), 10% (liver), 39% (fat) and 3.0% (muscle) of the TRR could be identified in the initial extracts. Other peaks and fractions individually ranged up to 12% TRR and 0.0083 mg/kg eq. All individual identified or characterized residues were at low levels (< 0.01 mg/kg eq). The solids remaining after initial extraction from eggs, liver, muscle and fat were subjected to sequential solubilisation procedures, resulting in a release of most of the radioactivity in eggs, liver and muscle (16%, 47%, 55% TRR respectively). This radioactivity could not be attributed to any of the known metabolites. In fat 33% TRR could not be solubilized.

Metabolism of ametoctradin in livestock involves oxidation of the aliphatic side chain to the respective terminal carboxylic acid (forming metabolite M650F09, ω-hetaryloctanoic acid) and subsequent stepwise oxidative cleavage of the side chain (loss of C_2H_4-units) analogous to the β-oxidation of fatty acids to form the metabolites M650F06 (ω-hetarylhexanoic acid) and M650F01 (ω-hetarylbutanoic acid). In goats metabolite M650F06 was the major metabolite found (22–47% TRR) in all tissues and milk, followed by M650F01 (14–26% TRR) and M650F09 (7.7–9.4% TRR). No parent compound was detected in goat tissues and milk. In hens, only low levels of residues were found (each < 0.01 mg/kg eq). The major compounds were metabolite M650F01 (1.9–28% TRR in liver, fat, muscle) and parent compound (22% in eggs and 11% in fat). Metabolite M650F06 was only identified in liver and muscle in trace amounts (1.1–1.3% TRR) and metabolite M650F09 was not detected.

The metabolic pathway in livestock is identical to the metabolic pathway in rats, although in rats more conjugation products are found.

Plant metabolism

The Meeting received plant metabolism studies for ametoctradin in/on fruits (tomatoes), leafy crops (lettuce) and root and tuber vegetables (potato) after foliar treatment.

Uptake and translocation studies with ^{14}C-labelled ametoctradin on leaves from tomato plants showed low uptake (5% TRR) and essentially no translocation of ametoctradin.

Uptake and translocation studies with tomato plants in nutrient solutions containing ^{14}C-labelled M650F03 or ^{14}C-labelled M650F04 soil metabolites showed that both soil metabolites are taken up by tomato plants via the root system concurrently with the stream of water. Both soil metabolites are equally distributed over the whole plants.

Indoor grown tomato plants were sprayed three times with an SC formulation of 2,7-^{14}C-radio labelled ametoctradin at an actual application rate of 3 × 0.30 kg ai/ha. Tomato plants were sampled at maturity 1 day after the last application (1DAT) and separated into leaves and fruit. Total radioactive residues (TRR) in tomato fruit and leaves at 1DAT were 0.36 mg/kg eq and 9.2 mg/kg eq. Residues could be extracted with methanol (99% TRR). The parent compound ametoctradin accounted for 99% TRR (0.036 mg/kg eq) in fruits and 99% TRR (9.0 mg/kg eq) in leaves. No other compounds were detected. The Meeting noted that since the plants were sampled only 1 day after the last application, it is to be expected that parent compound dominates the residue.

Indoor grown lettuce was sprayed three times with an SC formulation of 2,7-^{14}C-labelled ametoctradin at a concentration of 3 × 0.22 kg ai/ha. Plants were sampled at maturity at 7DAT. TRR in lettuce leaves were 8.5 mg/kg eq. Residues could be extracted with methanol (99% TRR). The parent compound accounted for 99% TRR (8.4 mg/kg eq). No other compounds were detected.

Indoor grown potato plants were sprayed three times with an SC formulation of 2,7-^{14}C-radio labelled ametoctradin at an actual concentration of 3 × 0.44 kg ai/ha. Immature plants were taken 14 days prior to the second application and mature plants 7 day after the last application. Plants were separated in tubers and leaves.

TRR in immature and mature tubers was 0.025 and 0.041 mg/kg eq, respectively. Residues in the tubers could be extracted with methanol (81–83% TRR) and water (4.1–7.7%) with 1.0–11% TRR remaining as solids. Ametoctradin was the main compound in immature tubers (0.017 mg/kg eq, 67% TRR), but represented only 3.6% TRR (0.001 mg/kg eq) in mature tubers. Identified metabolites were M650F03 (hetarylacetic acid, 13% and 40% TRR in immature and mature tubers, respectively, 0.003 and 0.016 mg/kg eq) and M650F04 (hetarylcarboxylic acid, 27% TRR in mature tubers only, 0.011 mg/kg eq).

TRR in immature and mature leaves was, respectively, 22 and 45 mg/kg eq. Residues in the leaves could be extracted with methanol (98% TRR). The parent ametoctradin was the main compound (95 and 85% TRR in immature and mature leaves, respectively). All metabolites detected were each ≤ 0.81 mg/kg eq (≤ 1.9% TRR) and in total < 5.0% TRR and thus of minor importance.

From these data it is concluded that in leafy vegetables and fruits parent ametoctradin is the only residue identified at significant quantities (99% TRR). In root and tuber vegetables (potatoes) considerable amounts of residues are found in/on leaves (22 or 45 mg/kg eq), while only low amounts of residues are found in the tubers (0.025 or 0.041 mg/kg eq). Parent compound is the major compound found in/on leaves (85–95% TRR), while varying amounts of parent compound are found in the tubers (67% in immature tubers and 3.6% TRR in mature tubers). In potato tubers two major metabolites are identified: M650F03 (13% and 40% TRR, respectively in immature and mature tubers) and M650F04 (27% TRR in mature tubers only).

Ametoctradin is hardly taken up, is not translocated via the leaves or fruits of plants and is hardly metabolized when sprayed on the leaves of fruits of plants. Since parent compound is found in potato tubers, it seems likely that the parent compound is taken up and translocated via the roots of the

plants. The presence of small amounts of metabolites in potato leaves (total < 5.0% TRR) indicates that once the parent compound is inside the plant it can be metabolized.

The two major metabolites found in potato tubers, M650F03 and M650F04, were not found in rat or in livestock. Metabolites M650F03 and M650F04 were identified in soil degradation studies of ametoctradin, and were the only metabolites taken up by rotational crops (see environmental fate in soil). Metabolites M650F03 and M650F04 were also seen in a variety of supervised field trials after foliar application. In most instances these levels were too low to quantitate but in some supervised field trials, the residues exceeded the LOQ and were reported. Since the ametoctradin formulation was applied 3–4 times with intervals of 5–14 days in the supervised field trials, it seems likely that the spray from the early application(s) reached the soil because of incomplete soil coverage by the plants. It is likely that parent present in these early applications is degraded in the soil to the metabolites M650F03 and M650F04 and these metabolites are taken up by the plants in low levels and can be detected at harvest (7–35 days after the first application). Therefore it seems likely that metabolites M650F03 and M650F04 are the result of uptake from soil via the roots and translocation within the plant, although small amounts may be formed by degradation of the parent compound within the plant. However, since the contribution of the total identified metabolites in leaves is very low (total 3.3–4.4% TRR) and identified residue levels in potato tubers are very low (0.020–0.026 mg/kg eq), uptake from soil and subsequent metabolism within the plant is considered of minor importance in primary crops.

Environmental fate in soil

The Meeting received information on aerobic degradation in soil, soil photolysis and fate in rotational crops.

The half life for 2,7-^{14}C-labelled-ametoctradin ranged from 1.5 to 3.2 days at 20 °C and 6.3 days at 10 °C in a study where three soils (sandy loam and loamy sand) were treated at 1.1 mg ai/kg dry soil (0.40 kg ai/ha). In a second study the half-life was 1.3 days in one sandy loam soil, treated at 1.9 mg ai/kg dry soil (0.72 kg ai/ha). The major metabolites in both studies were M650F01 (max. 54% TAR on day 10), M650F02 (max. 13% TAR on day 3), M650F03 (max. 57% TAR on day 10) and M650F04 (max. 55% TAR on day 120). A number of other degradation products were formed, but all < 5.5% TAR.

Using the data from these two soil degradation studies, the half-lives for the metabolites were estimated at 1–10 days for M650F01, 5–22 days for M650F02, 28–88 days for M650F03 and > 226 days for M650F04. Additional soil studies were performed with metabolites M650F03 and M650F04.

The half life for [pyrimidine-5-^{14}C]-M650F03 ranged from 29–43 days at 20 °C in a study where three soils (loamy sand, sandy loam, sand) were treated at 0.51–0.55 mg ai/kg dry soil (0.20 kg ai/ha). The amount of the major metabolite M650F04 continuously increased in the course of the study with 31–44% TAR present at 120 days. A number of other degradation products were formed, but all < 6.1% TAR.

The half life for [pyrimidine-5-^{14}C]-M650F04 was 228 days at 20 °C in a study where loamy sand was treated at an equivalent rate of 0.20 kg ai/ha. Two minor degradation products were formed (total < 7.0% TAR).

The half life for [2,7-^{14}C] ametoctradin in non-sterile sandy loam soil treated with 2.7 mg ai/kg dry soil (0.40 kg ai/ha) during a 15 days exposure to artificial sunlight (DT_{50} 23 days) was longer than in the dark control (DT_{50} 7 days). Despite the expectation that photolysis contributes to the degradation of ametoctradin based on its significant absorption at 295 nm and moderate photolysis in sterile water, the study results showed that light has no effect on the degradation of ametoctradin in soil, probably because the degradation in aerobic soil is already very fast.

In a confined rotational crop study, [2,7-^{14}C]-ametoctradin was sprayed on a loamy sand soil at a rate of 1.44 kg ai/ha under greenhouse conditions. Rotational crops were sown 30, 120 and 365 days after application, representing first, second and third rotations. Total radioactivity was 0.080–1.2–0.030 mg/kg eq in immature lettuce leaves after first-second-third rotations, 0.060–0.064–

0.016 mg/kg eq in mature lettuce leaves, 2.4–0.28–0.062 mg/kg eq in radish tops, 0.66–0.062–0.018 mg/kg eq in radish roots, 6.0–3.8–1.2 mg/kg in wheat straw, 5.2–2.7–1.7 mg/kg eq in wheat hay, and 1.8–1.2–0.84 mg/kg eq in wheat grain. Total radioactivity in wheat forage was only determined after second and third rotation, being 1.7 an 0.36 mg/kg eq. Except for radish root (second rotation) and mature lettuce leaves (first rotation) no significant amount of parent compound was detected in the various crop samples. Metabolite M650F03 was the major compound in lettuce leaves (30–42% TRR) at first rotation. At second and third rotation the major compound in lettuce leaves was the metabolite M650F04 (26–32% TRR). In radish roots and tops metabolite M650F03 remained the major compound (100 and 96% TRR, respectively) after first rotation followed by 67%TRR and 46% TRR, respectively at second and 39%TRR and 23% TRR, respectively at third rotation. Apart from wheat straw, where the metabolite M650F03 was the major compound (43% TRR) at first rotation, the metabolite M650F04 was the major component in all the wheat samples, 25% TRR in straw at first rotation and 44–98% TRR in all other fractions and different plant back intervals.

In a field rotational crop study at four different locations in Europe significant residues were found in rotational crop wheat after a single treatment of the bare soil with 0.96 kg ai/ha and a plant back interval of 120 days. The parent ametoctradin was not detected in wheat commodities. Metabolite M650F03 was detected between < 0.01 and 0.092 mg/kg and M650F04 between < 0.01 and 0.30 mg/kg in wheat forage and grain. Relatively high metabolite residues were found in wheat straw: 0.016–0.14 mg/kg for M650F03 and 0.040–1.0 mg/kg for M650F04.

In a second field rotational crop study at four different locations in Europe significant residues were found in rotational crops wheat, carrot, cauliflower and head lettuce after a single treatment of the bare ground with 0.96 kg ai/ha at plant back intervals of 30, 120 and 365 days. The parent compound ametoctradin was found in only two samples at the 30 day plant back interval; 0.038 mg/kg in wheat straw and 0.020 mg/kg in cauliflower inflorescence. The two soil metabolites M650F03 and M650F04 formed the majority of the residues in rotational crops. Residues were highest in the animal feed commodities immature carrot plants, carrot tops, wheat forage and wheat straw, moderate in the edible food commodities wheat grain and carrot root and low in lettuce and cauliflower inflorescence. Residues were highest after a plant back interval of approximately 30 days and decreased at longer plant back intervals.

- After a plant back interval of 30 days, metabolite M650F03 and M650F04 were found between < 0.01–0.92 mg/kg eq and < 0.01–0.35 mg/kg eq in animal feedstuff and between < 0.01–0.056 mg/kg eq and < 0.01–0.12 mg/kg eq in wheat grain, carrot roots, cauliflower inflorescence and head lettuce, respectively. Residues were found in all commodities.
- After a plant back interval of 120 days, metabolites M650F03 and M650F04 were found between < 0.01 – 0.054 mg/kg eq and < 0.01 mg/kg eq in animal feedstuff, respectively. No residues above the LOQ were found in wheat grain, carrot roots, cauliflower inflorescence and head lettuce.
- After a plant back interval of 365 days, metabolites M650F03 and M650F04 were found between < 0.01–0.038 mg/kg eq and < 0.01–0.056 mg/kg eq in animal feedstuff and between < 0.01–0.015 mg/kg eq and < 0.01–0.016 mg/kg eq in wheat grain and cauliflower inflorescence, respectively. No residues above the LOQ were found in carrot roots and head lettuce.

In a third field rotational crop study undertaken at two different locations in the USA, bare soil was treated with ametoctradin at a rate of 3 × 0.30 kg ai/ha with a 5 (± 1) day interval . Radish, lettuce and winter wheat were planted at 4 different plant back intervals (PBI: 1, 2, 3 and 4 months). No quantifiable residues (< 0.01 mg/kg) of the parent ametoctradin were observed in any of the rotational crops. Quantifiable residues of the metabolite M650F03 were observed in all rotational crops planted up to 4 months after the last application, with the exception of radish root, where M650F03 residues (0.01 mg/kg) were last observed at the 3 month PBI and lettuce leaves, with non–quantifiable residues at all PBIs. In wheat samples from the 4 month PBI maximum residue levels of M650F03 were 0.07 mg/kg (forage), 0.07 mg/kg (hay), 0.02 mg/kg (grain) and 0.33 mg/kg (straw). Quantifiable residues of the metabolite M650F04 were also observed in all rotational crops planted up to 4 months after the last application, with the exception of radish root and lettuce leaves, were

M650F03 residues were non-quantifiable throughout the study. In wheat samples from the 4 month PBI, the maximum M650F04 residues were 0.13 mg/kg (forage), 0.12 mg/kg (hay), 0.19 mg/kg (grain) and 0.29 mg/kg (straw).

From these data it is concluded that the aerobic degradation in soil proceeds primarily via stepwise oxidative cleavage of the n-octyl side chain. Ametoctradin is transformed to M650F01 (ω-hetarylbutanoic acid), M650F02 (ω-hetarylpropanoic acid) and subsequently to M650F03 (hetarylacetic acid) and M650F04 (hetarylcarboxylic acid) by oxidation. Metabolites underwent further metabolisation by mineralisation to CO_2 or incorporation in humins, humic acids or fulvic acids. Metabolite M650F04 has a very long dissipation time in soil and metabolites M650F03 and M650F04 can be taken up by primary crops and rotational crops.

Methods of residue analysis

The Meeting received description and validation data for analytical methods of ametoctradin, M650F03 and M650F04 in plant commodities or ametoctradin, M650F01 and M650F06 in animal commodities.

As ametoctradin and its soil metabolites M650F03 and M650F04 were shown not to be compatible with existing GC or HPLC-fluorescence multiresidue methods, only single residue methods were submitted to the Meeting. Three single residue analytical methods were proposed to the Meeting.

Macerated samples were extracted with methanol/water. The extract was cleaned up by solvent partition and/or solid phase extraction, if necessary. The final residue could then be determined by HPLC-MS-MS. The Meeting considers validation sufficient for commodities with high water, high acid content, high starch content, dried hops and animal commodities. LOQs were in the 0.01–0.1 mg/kg range for parent and its metabolites in plant and animal commodities.

Methanol/water extraction on samples with incurred radioactive residues from metabolism studies on goat (liver and kidney), wheat (forage, grain), potato leaves and tomato fruits showed that the methanol/water mixture extracted similar amounts of total radioactive residues as the combined methanol extracts in the metabolism studies and resulted in comparable HPLC patterns. Therefore the extraction solvent used in the HPLC-MS-MS methods is sufficiently able to extract the analytes defined.

Stability of pesticide residues in stored analytical samples

The Meeting received information on the stability of ametoctradin, M650F03 and M650F04 in plant commodities as well as ametoctradin, M650F01 and M650F06 in animal commodities stored frozen.

Storage stability studies in plant commodities had variable results. In a study where plant commodities were fortified with a mixture of parent, M650F03 and M650F04 and samples were stored at -20 °C, degradation of parent was found for some commodities (tomatoes) but not in others. In a second study where tomatoes and lettuce with incurred residues from a metabolism study were stored at -18 °C it was shown that parent was stable for at least 3 and 2 years.

Considering both storage stability studies on plant commodities, the Meeting considers parent, M650F03, M650F04 stable for at least 2 years in all plant commodities investigated: commodities with high water content, high acid content, high starch content, high protein content and straw.

Based on storage stability studies at -18 °C in fortified milk samples, the Meeting considers parent and metabolite M650F01 stable for at least 41 days and M650F06 for at least 34 days in milk. Milk samples within the feeding study were analysed within this period. Storage stability studies in animal tissues are not available. Since the tissue samples from the animal feeding study were analysed within 30 days after slaughter and ametoctradin and its metabolites were shown to be stable in various other commodities, storage stability studies are not considered necessary for the purpose of this evaluation.

Definition of the residue

The parent compound ametoctradin was only present in egg and fat of hen (22% and 11% TRR or 0.008 and 0.001 mg/kg eq), and in hen fat it was the only compound identified. Metabolites M650F01, M650F06 were found in significant quantities in other animal tissues in varying amounts. In goat milk, liver, kidney and fat, the metabolite M650F06 (ω-hetarylhexanoic acid) is the most abundant component of the residues (22–47% TRR or 0.006–0.021 mg/kg eq), followed by the metabolite M650F01 (ω-hetarylbutanoic acid, 14%–26% TRR or 0.003–0.014 mg/kg eq). The major metabolite in hens was M650F01 (ω-hetarylbutanoic acid) with 28%, 8.7%, 1.9% TRR in fat, liver, muscle, respectively. For this reason, parent and the metabolites M650F01 and M650F06 are the candidate compounds for inclusion in the residue definition for animal commodities.

Metabolites M650F01 and M650F06 are found in the rat and are therefore covered by the toxicity studies on parent. Since M650F01 and M650F06 are major components of the residue and valid analytical methods are available to quantitate parent and its metabolites in animal commodities, the Meeting decided to include the metabolites M650F01 and M650F06 in the residue definition for animal commodities.

Fat solubility of the parent compound is indicated by the log Kow of 4.18–4.40 and its presence in hen fat and eggs only. Metabolites M650F01 and M650F06 are amphoteric compounds and they are not fat-soluble. Since the metabolites M650F01 and M650F06 are the major components of the residue, the sum of parent, M650F01 and M650F06 is considered not fat-soluble.

In primary crops, parent compound ametoctradin is the only compound found in significant quantities (> 95% TRR). Therefore parent should be included in the residue definition for plant commodities. However, in rotational crops uptake of residues proceeds via the soil and the main metabolites taken up from the soil are M650F03 (30–100% TRR) and M650F04 (26–98% TRR), while parent is found in trace amounts. The level of the metabolites found in the various rotational crops is significant, even after a plant back interval of 365 days (up to 0.056 mg/kg eq for M650F04).

Metabolites M650F03 and M650F04 are not found in the rat. In 90 day dietary toxicity studies, no adverse effects were observed after exposure to either M650F03 or M650F04 up to the limit dose (about 1000 mg/kg bw per day). This is comparable to ametoctradin which showed no adverse effects were observed at or near the limit dose of approximately 1000 mg/kg bw per day in an extensive set of repeated-dose toxicity studies.

The Meeting noted that the parent compound alone is a good marker for compliance with GAP. Although metabolites M650F03 and M650F04 have a similar lack of toxicity and the same core structure as the parent compound, the metabolites M650F03 and M650F04 are only found at significant levels in rotational crops. Since an ADI or ARfD is not considered necessary for the parent compound, there is no dietary intake concern for parent compound or metabolites M650F03 and M650F04. For these reasons, the Meeting decided not to include the metabolites M650F03 and M650F04 in the residue definition and to refrain from setting a residue definition for estimation of the dietary intake of ametoctradin.

The Meeting recommended the following residue definition for ametoctradin:

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

Definition of the residue for compliance with the MRL for animal commodities: *sum of ametoctradin, ω-hetarylbutanoic acid (M650F01) and ω-hetarylhexanoic acid (M650F06), expressed as ametoctradin.*

The Meeting considers the residue is not fat-soluble

Results of supervised residue trials on crops

The Meeting received supervised trials data for ametoctradin on grapes, bulb onions, green onions, broccoli, head cabbage, cucumbers, melons, pumpkins, summer squash, sweet peppers, chili peppers, tomatoes, head lettuce, leaf lettuce, mustard greens, spinach, potatoes, celery, and dried hops.

All plant commodities from supervised residue trials were analysed within 4–24 months, although storage temperatures varied. Since ametoctradin, M650F03 and M650F04 are shown to be stable for a long period of time, trials where samples were stored for a few days at +5 °C before being frozen and trials where temperatures of frozen samples increased to -1 °C were considered acceptable.

Trials conducted at the same location and at the same kg ai/ha dose rate, where only the spray concentration was different, were not considered as independent trials. Trials conducted at the same location, where only the crop variety was different, were not considered as independent trials. The maximum value from each location was selected for maximum residue level recommendations.

As an ADI and ARfD were considered not necessary, no STMR and no HR values are reported as a long and short term exposure assessment is not needed.

The OECD MRL calculator was used as a tool in the estimation of the maximum residue level from the selected residue data set obtained from supervised field trials conducted according to the critical GAP. For those trials where the outcome of the OECD MRL calculator was different from the recommendation made by the Meeting, a rationale is provided for this deviation.

Grapes

Field trials involving grapes were performed in Canada, USA, Germany, France, Spain, Italy and Greece.

Critical GAP for grapes in the USA is for 4 foliar spray applications (interval 7 days) at 0.31 kg ai/ha and PHI 14 days with adjuvant recommended. Trials from USA and Canada (4 × 0.29–0.32 kg ai/ha, interval 6–8 days, PHI 14–15 days, adjuvant added) matched this GAP. Trials from USA and Canada were conducted at two spray concentrations per location (0.0086–0.032 and 0.042–0.065 kg ai/hL); both far lower than indicated in the GAP (0.13–0.16 kg ai/hL). The highest residue value from each location was selected: 0.21, 0.33, 0.34, 0.87, 0.89, 0.92, 0.97, 1.3, 1.4, 1.4, 1.9 and 2.2 mg/kg (n=12).

Critical GAP for grapes in the Former Yugoslav Republic of Macedonia is for 3 foliar spray applications (interval 10 days) at 0.075 kg ai/hL and PHI 35 days. In trials performed in Southern France, Spain, Italy and Greece (3 × 0.060 kg ai/hL, interval 10 days, PHI 34–36 days) matching this GAP parent residues were: 0.15, 0.22, 0.37, 0.72, 1.1, 1.1, 2.7 and 3.1 (n=8).

The Meeting noted that the US and Southern European dataset for grapes resulted in similar residues (Mann-Whitney U test). However, since the GAPs are different, the Meeting agreed that the Southern European dataset for grapes matching Former Yugoslav Republic of Macedonia GAP could be used to support a grape maximum residue level recommendation and estimated a maximum residue level of 6 mg/kg on grapes. For the purpose of livestock dietary burden calculation, the Meeting estimated an STMR of 0.605 mg/kg.

Bulb vegetables

Field trials involving bulb onions were performed in Canada and the USA.

Critical GAP for bulb vegetables (includes bulb onions) in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In trials from USA and Canada (3 × 0.29–0.34 kg ai/ha, interval 4–8 days, PHI 0 days, adjuvant added) matching this GAP parent residues were: 0.095, 0.095, 0.14, 0.19, 0.21, 0.22, 0.25, 0.43, 0.46, 0.84 mg/kg (n=10).

The Meeting agreed that the USA and Canadian datasets for bulb onions matching USA GAP could be used to support a bulb onion maximum residue level recommendation and estimated a maximum residue level of 1.5 mg/kg on bulb onions and decided to extrapolate the recommendation for bulb onions to garlic and shallots.

Field trials involving spring onions were performed in the USA.

Critical GAP for bulb vegetables (includes green onions) in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from USA and Canada (3 × 0.29–0.34 kg ai/ha, interval 4–8 days, PHI 0 days, adjuvant added) matching this GAP parent residues were: 3.4, 4.3, 9.1 mg/kg (n=3).

The Meeting agreed that the USA and Canadian datasets for spring onions matching USA GAP could be used to support a green onion maximum residue level recommendation and estimated a maximum residue level of 20 mg/kg on spring onions.

Brassica vegetables

Field trials involving broccoli were performed in Canada and the USA.

Critical GAP for brassica vegetables (includes broccoli) in the USA is for 3 foliar spray applications (interval 7 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from USA and Canada (3 × 0.29–0.31 kg ai/ha, interval 6–9 days, PHI 0 days, adjuvant added) matching this GAP parent residues in broccoli heads and stems were 1.2, 1.2, 1.3, 1.6, 1.7, 2.5, 2.9, 3.2 mg/kg (n=8).

Field trials involving head cabbage were performed in Canada and USA.

Critical GAP for brassica vegetables (includes head cabbage) in the USA is for 3 foliar spray applications (interval 7 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from USA and Canada (3 × 0.29–0.31 kg ai/ha, interval 5–9 days, PHI 0 days, adjuvant added) matching this GAP parent residues in head cabbage with wrapper leaves (as marketed) were 0.35, 1.1, 1.4, 1.6, 1.8, 2.2, 3.1, 3.2, 3.3, 7.5 mg/kg (n=10).

The Meeting noted that the datasets for broccoli and head cabbage resulted in similar residues (Mann-Whitney U test). Since the GAPs are the same and there is a GAP for brassica vegetables, the Meeting agreed to combine the data to propose a group maximum residue level for brassicas. This resulting in the following residues: 0.35, 1.1, 1.2, 1.2, 1.3, 1.4, 1.6, 1.6, 1.7, 1.8, 2.2, 2.5, 2.9, 3.1, 3.2, 3.2, 3.3, 7.5 (n=18).

The Meeting agreed that the combined datasets for broccoli and head cabbage matching USA GAP could be used to support a maximum residue level recommendation for brassica (cole or cabbage) vegetables, Head cabbages, Flowerhead cabbages and estimated a maximum residue level of 9 mg/kg. For the purpose of livestock dietary burden calculations the Meeting estimated a highest residue of 7.5 mg/kg for brassicas.

Fruiting vegetables, Cucurbits

Supervised residue trials on outdoor and indoor grown cucumbers were conducted in Canada, USA, the UK, the Netherlands, France, Greece and Spain.

Critical GAP for fruiting vegetables, cucurbits in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and a PHI of 0 days, with adjuvant recommended. In field trials from USA and Canada (3 × 0.29–0.30 kg ai/ha, interval 6–8 days, PHI 0 days, adjuvant added) matching this GAP parent residues in cucumber were: 0.060, 0.08, 0.090, 0.12, 0.12, 0.16, 0.16, 0.24 mg/kg (n=8).

Critical GAP for cucumbers in the Former Yugoslav Republic of Macedonia (Southern Europe) is for 4 foliar spray applications (interval 10 days) at 0.30 kg ai/ha and PHI 1 days. In field trials from Southern France and Greece and greenhouse trials from Europe (3 × 0.24 kg ai/ha, interval 7 days, PHI 1 day) matching this GAP parent residues in cucumber were 0.038, 0.09, 0.11 and 0.17 mg/kg (n=4) for field trials and 0.024, 0.037, 0.15 and 0.18 mg/kg (n=4) for greenhouse trials. As the datasets for outdoor and indoor grown cucumbers resulted in similar residues (Mann-Whitney U test), the datasets were combined: 0.024, 0.037, 0.038, 0.09, 0.11, 0.15, 0.17 and 0.18 mg/kg (n=8) for outdoor and indoor grown cucumbers.

The Meeting noted that datasets for USA and Former Yugoslav Republic of Macedonia resulted in similar datasets (Mann-Whitney U test). However, since the GAPs are different, the Meeting decided to take only the USA dataset into account in making estimations.

Field trials involving melons were performed in the USA.

Critical GAP for fruiting vegetables, cucurbits in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from USA (3 × 0.29–0.31 kg ai/ha, interval 6–8 days, PHI 0 days, adjuvant added) matching this GAP, parent residues in melons with peel were: 0.18, 0.49, 0.59, 0.60, 0.72, 0.80, 1.3, 1.7 mg/kg (n=8).

Field trials involving pumpkins were performed in the USA.

Critical GAP for fruiting vegetables cucurbits in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from the USA (3 × 0.29–0.30 kg ai/ha, interval 7 days, PHI 0 days, adjuvant added) matching this GAP, parent residues in pumpkins with peel were: 0.10, 0.14, 0.34, 0.47, 1.3 mg/kg (n=5).

Field trials involving summer squash (i.e., courgette/zucchini) were performed in the USA.

Critical GAP for fruiting vegetables cucurbits in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from the USA (3 × 0.28–0.31 kg ai/ha, interval 6–8 days, PHI 0 days, adjuvant added) matching this GAP, parent residues in summer squash with peel were: 0.13, 0.22, 0.36, 0.98, 1.1 mg/kg (n=5).

The Meeting noted that the datasets for melons, pumpkins and summer squash were similar (Kruskal-Wallis test). Since the GAP is the same for each of these commodities, the Meeting agreed to propose a group maximum residue level for cucurbits, except cucumbers, based on the combined residue data for melons, pumpkins and summer squash and agreed to propose a separate maximum residue level for cucumbers.

The combined dataset for melons, pumpkins and summer squash resulted in the following residues: 0.10, 0.13, 0.14, 0.18, 0.22, 0.34, 0.36, 0.47, 0.49, 0.59, 0.60, 0.72, 0.80, 0.98, 1.1, 1.3, 1.3, 1.7 mg/kg (n=18). The Meeting agreed that the combined dataset matching the GAP of the USA could be used to support a maximum residue level recommendation for cucurbits, except cucumber, and estimated a maximum residue level of 3 mg/kg in/on cucurbits, except cucumber, based on the combined data.

The Meeting agreed that the dataset for cucumbers matching the US GAP could be used to support a maximum residue level recommendation for cucumbers, and estimated a maximum residue level of 0.4 mg/kg for cucumbers.

Fruiting vegetables other than cucurbits

Field trials involving sweet peppers were performed in Canada, the USA, Greece, Italy, Spain, France, Germany, the Netherlands and Belgium.

Critical GAP for fruiting vegetables other than cucurbits (includes sweet peppers) in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 4 days with adjuvant recommended. In field trials from the USA and Canada (3 × 0.29–0.32 kg ai/ha, interval 6–8 days, PHI 4 days, adjuvant added) matching this GAP, parent residues in sweet peppers were: 0.050, 0.080, 0.085, 0.14, 0.16, 0.22, 0.84 mg/kg (n=7).

The GAP for peppers in Former Yugoslav Republic of Macedonia (Southern Europe) is for 4 foliar spray applications (interval 10 days) at 0.30 kg ai/ha and a 1 day PHI. In greenhouse trials from Europe (3 × 0.23–0.25 kg ai/ha, interval 7 days, PHI 1 day) matching this GAP, parent residues in sweet peppers were: 0.20, 0.21, 0.28, 0.34, 0.37, 0.47, 0.79, 0.90 mg/kg (n=8).

Field trials involving chili peppers were performed in USA.

The GAP for fruiting vegetables, other than cucurbits (includes chili peppers), in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 4 days with adjuvant

recommended. In field trials from the USA (3 × 0.29–0.31 kg ai/ha, interval 7 days, PHI 4 days, adjuvant added) matching this GAP none of the residue values could be selected, as the laboratory was unable to show adequate performance of the analytical method.

Field trials involving tomatoes were performed in Canada and the USA.

Critical GAP for fruiting vegetables other than cucurbits (includes tomatoes) in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 4 days with adjuvant recommended. In field trials from the USA and Canada (3 × 0.28–0.32 kg ai/ha, interval 6–8 days, PHI 4 days, adjuvant added) matching this GAP, parent residues in tomatoes (including two trials on cherry tomatoes) were: 0.050, 0.10, 0.11, 0.12, 0.15, 0.16, 0.16, 0.18, 0.20, 0.20, 0.22, 0.22, 0.25, 0.32, 0.60, 0.70, 0.76 mg/kg (n=17).

The Meeting noted that the sweet pepper dataset corresponding to the GAP of the Former Yugoslav Republic of Macedonian (3 × 0.30 kg ai/ha PHI 1 days) resulted in higher residues than the dataset corresponding to the US GAP (3 × 0.30 kg ai/ha, PHI 4 days) (Mann-Whitney U test). However, both datasets would result in the same maximum residue level recommendation (1.5 mg/kg). The sweet pepper dataset, matching USA GAP, resulted in similar residues as the tomato dataset, matching USA GAP, (Mann-Whitney U test). The Meeting concluded that these datasets could be combined to allow a commodity group recommendation for fruiting vegetables other than cucurbits. This resulted in the following dataset: 0.050, 0.050, 0.080, 0.085, 0.10, 0.11, 0.12, 0.14, 0.15, 0.16, 0.16, 0.16, 0.18, 0.20, 0.20, 0.22, 0.22, 0.22, 0.25, 0.32, 0.60, 0.70, 0.76 and 0.84 mg/kg (n=24).

The Meeting estimated a maximum residue level of 1.5 mg/kg fruiting vegetables other than cucurbits, except sweet corn and mushrooms, based on the combined dataset. For the purpose of livestock dietary burden calculations, the Meeting estimated an STMR of 0.16 mg/kg in/on fruiting vegetables other than cucurbits.

The FAO Manual (section 6.9.2) describes how a generic concentration factor may be used for conversion of HR residue values from fresh peppers to dried chili peppers. A concentration factor of 10 is used for the estimation of parent residue levels of pesticides in dried chili peppers.

The Meeting agreed to apply the concentration factor of 10 for dried chili peppers to the maximum residue level for sweet peppers (1.5 mg/kg) and estimated a maximum residue level in peppers, chili, dried of 15 mg/kg.

Leafy vegetables

Field trials involving head lettuce were performed in Canada and the USA.

Critical GAP for leafy vegetables (includes head lettuce) in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from the USA and Canada (3 × 0.29–0.32 kg ai/ha, interval 4–7 days, PHI 0 days, adjuvant added) matching this GAP none of the residue values could be selected as the analytical laboratory could not demonstrate adequate performance of the analytical method.

Field trials involving leaf lettuce were performed in Canada and the USA.

Critical GAP for leafy vegetables (includes leaf lettuce) in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from the USA and Canada (3 × 0.30–0.31 kg ai/ha, interval 4–6 days, PHI 0 days, adjuvant added) matching this GAP none of the residue values could be selected as the laboratory could not demonstrate adequate performance of the analytical method.

Field trials involving mustard greens were performed in USA.

Critical GAP for leafy vegetables (includes mustard greens) in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from the USA (3 × 0.28–0.31 kg ai/ha, interval 6–8 days, PHI 0 days, adjuvant added) matching this GAP, parent residues in mustard greens were: 9.2, 13, 13, 16, 19, 24, 28 mg/kg (n=7).

Field trials involving spinach were performed in Canada and the USA.

Critical GAP for leafy vegetables (includes spinach) in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from USA and Canada (3 × 0.29–0.33 kg ai/ha, interval 4–8 days, PHI 0 days, adjuvant added) matching this GAP in spinach were: 6.0, 11, 12, 13, 13, 20, 21, 35 mg/kg (n=8).

The Meeting noted that the datasets for mustard greens and spinach are similar (Mann-Whitney U-test), confirming the experience of the JMPR that residues in leafy vegetables at DAT=0 are similar. Since the GAPs are the same for mustard greens and spinach, the Meeting agreed to combine the data to propose a group maximum residue level for leafy vegetables, based on the combined residue dataset for mustard greens and spinach. The combination of the two datasets resulted in the following residues: 6.0, 9.2, 11, 12, 13, 13, 13, 13, 16, 19, 20, 21, 24, 28 and 35 mg/kg (n=15).

The Meeting agreed that the combined dataset for mustard greens and spinach, matching US GAP, could be used to support a maximum residue level recommendation for leafy vegetables and estimated a maximum residue level of 50 mg/kg in/on leafy vegetables based on the combined dataset. For the purpose of livestock dietary burden calculations, the Meeting estimated a highest residue of 35 mg/kg for leafy vegetables, based on the combined residue dataset.

Potatoes

Field trials involving potatoes were performed in Canada and the USA.

Critical GAP for root and tuber vegetables (includes potatoes) in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 4 days with adjuvant recommended. In field trials from the USA (3–4 × 0.28–0.31 kg ai/ha, interval 4–8 days, PHI 4 days, adjuvant added) matching this GAP parent residues in potato tubers were: < 0.01 (12), 0.010 (5), 0.020, 0.025 (2), 0.035 mg/kg (n=21).

The Meeting agreed that the dataset for potatoes matching US GAP could be used to support a maximum residue level recommendation for potatoes, and estimated a maximum residue level of 0.05 mg/kg in/on potatoes. For the purpose of livestock dietary burden calculations the Meeting estimated a highest residue of 0.035 mg/kg for potatoes.

Celery

Field trials involving celery were performed in Canada and the USA.

Critical GAP for leafy vegetables (includes celery) in the USA is for 3 foliar spray applications (interval 5 days) at 0.31 kg ai/ha and PHI 0 days with adjuvant recommended. In field trials from the USA and Canada (3 × 0.29–0.32 kg ai/ha, interval 4–6 days, PHI 0 days, adjuvant added) matching this GAP were: 4.2, 4.7, 5.1, 5.5, 6.2, 6.7, 7.0, 11 mg/kg (n=8).

The Meeting agreed that the dataset for celery matching US GAP could be used to support a maximum residue level recommendation for celery, and estimated a maximum residue level of 20 mg/kg in/on celery.

Hops, dry

Field trials involving dried hops were performed in Germany and the USA.

Critical GAP for hops in the USA is for 3 foliar spray applications (interval 7 days) at 0.31 kg ai/ha and PHI 7 days with adjuvant recommended. Trials from USA (3x 0.30–0.31 kg ai/ha, interval 10–11 days, PHI 7 days, adjuvant added) matched this GAP. Trials from the USA were conducted at two spray concentrations per location (0.015–0.021 and 0.037–0.043 kg ai/hL); both far lower than indicated in the GAP (0.13–0.16 kg ai/hL). The highest residue from each location could be selected: 0.96, 2.4, 6.7 mg/kg (n=3). The Meeting agreed that 3 trials were insufficient to estimate a maximum residue level recommendation for dried hops.

Additional trials performed in the USA at higher dose rate (0.53–0.54 kg ai/ha, interval 10–11 days, PHI 6–8 days) could be matched to the USA GAP by using the proportionality approach by multiplying by 0.31/0.54=0.57. Parent residues in dried hops were 0.57 × (9.3, 18, 29) mg/kg (n=3). After applying the proportionality factor this results in the following dataset: 5.3, 10, 17 mg/kg (n=3). When combining the two datasets this resulted in the following dataset: 0.96, 2.4, 5.3, 6.7, 10, 17 mg/kg (n=6).

The Meeting agreed that the normal and scaled dataset for dried hops matching US GAP could be used to support a maximum residue level recommendation for dried hops, and estimated a maximum residue level of 30 mg/kg in/on dried hops.

Residues from rotational crops

Parent residues above 0.01 mg/kg are not expected in rotational crops.

Fate of residues during processing

Information on the fate of residues during processing by radioactivity studies showed that ametoctradin is stable (96–109%) under standard conditions used to simulate food processing operations (pH 4 and 90 °C, pH 5 and 100 °C, pH 6 and 120 °C).

Processing studies with ametoctradin were undertaken for grapes, bulb onions, gherkins, tomatoes and hops. Since no long or short term exposure assessments are considered necessary, only the processing factors that lead to maximum residue level proposals or the processing factors that are needed for livestock dietary burden calculations are listed in the table below.

Using the $STMR_{RAC}$ obtained from ametoctradin use, the Meeting estimated STMR-Ps for processed commodities as listed below. The Meeting considered the appropriate STMR-P to be used in the livestock dietary burden calculation or dietary intake calculation.

Commodity	Processing factors (parent only)	Processing factor (median or best estimate) (parent only)	STMR-P = $STMR_{RAC}$xPF mg/kg
grape raisin	1.9, 2.0, 4.8, 6.2 (n=4)	3.4	not necessary
grape wet pomace	1.8, 2.5, 2.7, 2.9, 3.9, 4.2, 4.8, 5.1 (n=8)	3.4	0.605 × 3.4 = 2.1
tomato wet pomace	1.1, 1.2, 1.4, 1.4 (n=4)	1.3	0.16 × 1.3=0.21 (based on fruiting vegetables other than cucurbits)

Based on a maximum residue level of 6 mg/kg for grapes and a processing factor of 3.4, the Meeting estimated a maximum residue level of 20 mg/kg for raisins.

Residues in animal commodities

The Meeting estimated the dietary burden of ametoctradin residues on the basis of the livestock diets listed in the FAO manual Appendix IX (OECD feedstuff table). Calculation from highest residue, STMR (some bulk commodities) and STMR-P values provides the levels in feed suitable for estimating MRLs. Since no long or acute dietary exposure assessment is needed, STMR values for animal commodities are not needed and therefore no mean dietary burden is calculated.

All plant commodities used in the dietary burden calculation are listed below. Residues in plant commodities were based on parent only.

Crop	Feedstuff	Highest Residue	STMR or STMR-P	DM (%)
Forages				

Crop	Feedstuff	Highest Residue	STMR or STMR-P	DM (%)
Cabbage	heads, leaves	7.5	not needed	15
Kale	leaves	35	not needed	15
Rape	forage	35	not needed	30
Roots & Tubers				
Potato	culls	0.035	not needed	20
Byproducts				
Grape	pomace, wet		2.1	15
Tomato	pomace, wet		0.21	20

Dietary burden calculations for beef cattle, dairy cattle, broilers and laying poultry are provided in Annex 6. A mean and maximum dietary burden for livestock, based on ametoctradin use, is shown in the table below.

Animal dietary burden for ametoctradin parent, expressed as ppm of dry matter diet

	US	EU	AU	JP	overall
	max	max	max	max	max
beef cattle	0.053	46.72	116.7	-	116.7 [a]
dairy cattle	11.68	46.72	96.15	-	96.15 [b]
poultry broiler		0.018			0.018
poultry layer		17.52			17.52 [c d]

[a] Highest maximum beef or dairy cattle dietary burden suitable for maximum residue level for mammalian meat.

[b] Highest maximum dairy cattle dietary burden suitable for maximum residue level for milk.

[c] Highest maximum poultry broiler or poultry layer dietary burden suitable for maximum residue level for poultry meat.

[d] Highest maximum poultry layer suitable for maximum residue level for eggs.

Livestock feeding studies

The Meeting received a feeding study on lactating cows.

Four groups of three lactating Holstein-Friesian cows were dosed once daily via capsules at levels of 0.0, 2.5, 7.5 and 25 ppm parent compound in dry weight feed for 28 consecutive days. Milk was collected throughout the study and tissues were collected on day 28 within 25 hours after the last dose. Parent was not found in milk or any of the tissues (< 0.01 mg/kg). Metabolites M650F01 and M650F06 were only found in liver and kidney samples. Mean and maximum total residues (parent + 1.10 × M650F01 + 0.993 × M650F06), expressed as parent equivalents, are shown in the table below.

Animal commodity	Dose level (ppm feed)	Mean Residue (mg/kg)	Highest Residue (mg/kg)
Liver	2.5	< 0.031	< 0.031
	7.5	0.033	0.036
	25	0.073	0.096
Kidney	2.5	< 0.031	< 0.031
	7.5	< 0.031	< 0.031
	25	0.039	0.048
Fat	2.5	< 0.031	< 0.031
	7.5	< 0.031	< 0.031
	25	< 0.031	< 0.031
Muscle	2.5	< 0.031	< 0.031
	7.5	< 0.031	< 0.031
	25	< 0.031	< 0.031
Milk	2.5	< 0.031	< 0.031
	7.5	< 0.031	< 0.031
	25	< 0.031	< 0.031

Residues in animal commodities

In a feeding study where lactating cows were dosed at 25 ppm ametoctradin in the dry feed, total residues (sum of parent, M650F01 and M650F06) were 0.073–0.096 mg/kg eq in liver and 0.039–0.048 mg/kg eq in kidney. No residues were found in muscle, fat and milk (each < 0.031 mg/kg eq). However, since the estimated maximum dietary burden in ruminants is much higher (116.7 ppm in beef cattle and 96.15 ppm in dairy cattle, based on parent only in feed commodities), the feeding study cannot be used to estimate residues in ruminant commodities. Therefore the data are insufficient to propose maximum residue levels in ruminants.

No feeding study is available for poultry. In a metabolism study, where laying hens were dosed at 12 ppm ametoctradin in the dry feed, total residues were 0.0088 mg/kg eq in eggs (22% × 0.040 mg/kg), 0.0055 mg/kg eq in fat (11% + 28% × 0.014 mg/kg), in 0.011 mg/kg eq in liver (8.7% + 1.3% × 0.11 mg/kg) and 0.00078 mg/kg in muscle (1.9% + 1.1% × 0.026 mg/kg). Since the estimated maximum dietary burden in poultry is in the same order of magnitude (17.52 ppm in poultry, based on parent only in feed commodities), the metabolism study can be used to estimate residues in poultry commodities. After extrapolation to a dietary burden of 17.52 ppm, residues in poultry commodities all lie below the limit of quantification of 0.031 mg/kg eq (total residues) of the available analytical method.

The Meeting was unable to estimate maximum residue levels in ruminant commodities, because of insufficient data. The Meeting estimated a maximum residue level for ametoctradin total residues of 0.03* mg/kg for eggs, poultry meat and poultry edible offal. The total residue in animal commodities is not considered fat-soluble.

DIETARY RISK ASSESSMENT

Since no ADI and no ARfD is considered necessary, no long-term or short-term intake assessment is considered necessary. However, to get an impression of the margins of exposure, the International Estimated Daily Intake (IEDI) for ametoctradin was calculated. The results are shown in Annex 3 of the 2012 report of the JMPR.

As a conservative approach, the crop with the highest residues (leafy vegetables) was used to estimate the total median residue of ametoctradin of individual crops. When the highest median residue for leafy vegetables from the presented field trials (i.e., 13 mg/kg) is used for all possible plant commodities and the highest median residue for animal commodities from the presented feed studies (i.e., 0.031 mg/kg for poultry commodities) is used for all possible animal commodities, the IEDI was in the range of 0.232–0.477 mg/kg bw/d. This IEDI also accommodates possible contributions from metabolites M650F03 and M650F04 in rotational crops. Considering the absence of adverse effects at or near the limit dose of approximately 1000 mg/kg bw/day in an extensive set of repeated-dose toxicity studies, the margins of exposure ranged between 2100–4300.

When the highest maximum residue level proposed for plant commodities (i.e., 50 mg/kg for leafy vegetables) is used for all possible plant commodities and the highest maximum residue level proposed for animal commodities (i.e., 0.031 mg/kg for poultry commodities), the IEDI was in the range of 0.893–1.836 mg/kg bw/d. The margins of exposure ranged between 540–1100.

The Meeting concluded that the long-term and short-term intake of residues of ametoctradin from uses considered by the Meeting, or from possible future uses is unlikely to present a public health concern.