ACIBENZOLAR-*S*-METHYL (288)

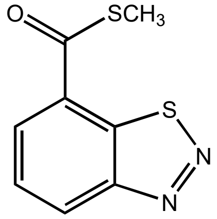
First draft prepared by Dr Dugald MacLachlan, Department of Agriculture and Water Resources, Australia

EXPLANATION

Acibenzolar-S-methyl is a plant activator that stimulates the natural, inherent defence mechanisms of plants. Through this activation, control of *Erysiphe graminis* (powdery mildew of cereals) and *Mycosphaerella musci* (black Sigatoka of banana) is achieved. This activation also results in partial control of *Septoria* spp. (side effect only) and *Puccinia* spp. (side effect only) in cereals. At the 47th Session of the CCPR (2015), it was scheduled for the evaluation as a new compound by 2016 JMPR.

The Meeting received information on the metabolism of acibenzolar-S-methyl in lactating goats and laying hens, cotton, lettuce, sorghum, sunflower, rice, tomato, tobacco and wheat, follow crops, methods of residue analysis, freezer storage stability, GAP information, supervised residue trials on citrus (oranges, grapefruit and lemons), pome fruit (apples and pears), stone fruit (apricots and peaches), strawberries, bananas, kiwifruit, onions, Brassica vegetables (cabbage, broccoli and mustard greens), cucurbits (cucumbers, melons and squash), tomatoes, leafy vegetables (lettuce and spinach), celery, potatoes and wheat as well as a livestock transfer study (lactating cow).

IDENTITY

Common name:	Acibenzolar-S-methyl
Chemical name:	
IUPAC:	*S*-methyl benzo[1,2,3]thiadiazole-7-carbothioate
CAS:	*S*-methyl 1,2,3-benzothiadiazole-7-carbothioate
Manufacturer's code numbers:	CGA245704
CAS number:	135158-54-2
CIPAC Code:	597
Molecular formula:	$C_8H_6N_2OS_2$
Molecular mass:	210.3 g/mole
Structural formula.	

Specifications:

Specifications for acibenzolar-S-methyl have not been developed by the FAO.

Physical and chemical properties (pure acibenzolar-S-methyl 99.6%

Property	Result	Reference
Appearance	Odourless, white fine crystalline powder	Das 1994 0064
Melting point	132.9 ± 0.1 °C	Das 1994 0063

Property	Result	Reference
Boiling point	*ca.* 267 °C	Das 1994 0176
Relative density	1.54 g/cm³ at 22 °C	Füldner 1994 0070
Surface tension	The surface tension of pure acibenzolar-S-methyl in water was determined to be 70.7 mN/m at 20.5 °C (90% of saturation concentration). Acibenzolar-S-methyl is not surface active.	Kuhne 2011 10047
Dissociation constant	The active substance has no dissociation constant in an accessible pH range.	Jäkel 1993 0074
Vapour pressure	4.6×10^{-4} Pa at 25 °C	Geoffroy 1994 0073
Volatility	Calculated Henry's law constant: 1.3×10^{-2} Pa m^3/mol	Burkhard 1995 0129
Solubility in water	7.7 mg/L at 25 °C	Stulz 1993 0061
Solubility in organic solvents (at 25 °C)	*n*-hexane 1.3 g/L methanol 4.2 g/L *n*-octanol 5.4 g/L ethyl acetate 25 g/L acetone 28 g/L toluene 36 g/L dichloromethane 160 g/L	Stulz 1993 0060
Partition coefficient *n*-octanol/water	log K_{ow} = 3.1 at 25 °C	Stulz 1993 0062
Hydrolysis under sterile conditions	pH 5; DT_{50}: stable pH 7; DT_{50}: 162 d pH 9; DT_{50}: 19 h	Adam 1994 0050
UV spectra	Molecular extinction coefficient (ε) at: 253.2 nm 14700 L/mol/cm 287.6 nm 5400 L/mol/cm 323.8 nm 7200 L/mol/cm No absorption maximum was observed between 400 and 750 nm.	Müller 1995 0205
Photolysis	At a concentration of 1.91 mg/kg in acetate buffer (pH 5.12) the half-life at 25 °C was 0.9 hours (12 h light/12 h dark). No photolysis product > 10%	Phaff 1996 0247
Quantum yield	The quantum yield (in water) of direct photolysis was found to be: 0.0356 at 289 nm 0.0208 at 327 nm Calculated mean rate constants and half-lives were: 1.46/h and 0.47 hours at 289 nm 0.802/h and 0.86 hours at 327 nm	Phaff 1996 0247

Technical material (97.9%)

Property	Result	Reference
Appearance	Beige fine powder with a weak, burnt-like odour	Das 1995 0120
Surface tension	The surface tension (determined using the Wilhelmy plate method) of technical acibenzolar-S-methyl was in the range of 71.8–73.4 mN/m at 20 ± 0.2 °C (filtrates of 0.1 g/L suspensions).	Ryser 1995 0143

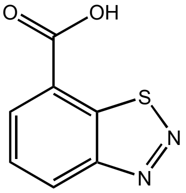
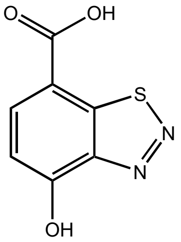
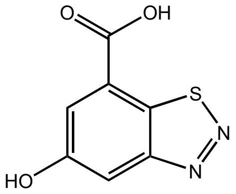
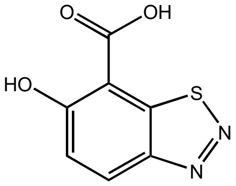
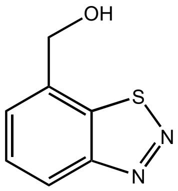
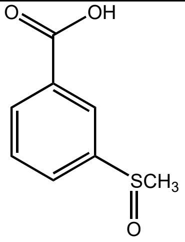
Acibenzolar-S-methyl is available in a water dispersible granule (WG) formulation.

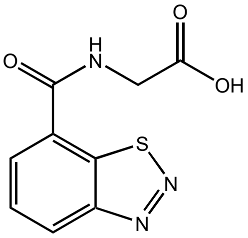
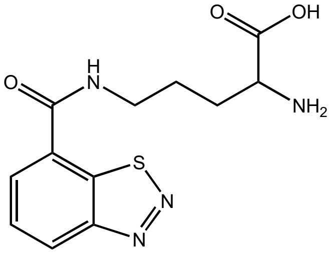
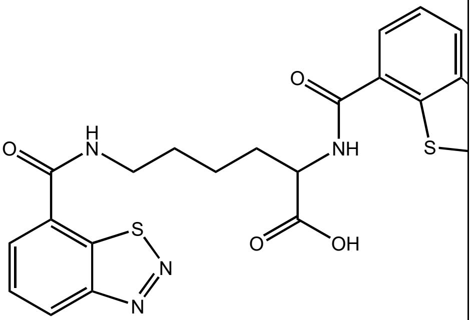
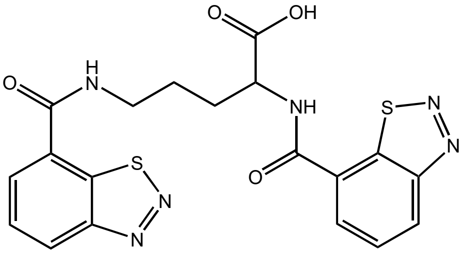
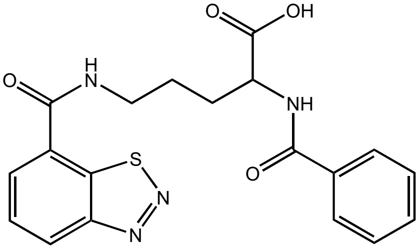
Formulations	Active ingredient content
WG	500 g/kg

METABOLISM AND ENVIRONMENTAL FATE

The metabolite summary table provides a reference for the numbering scheme used in the current evaluation.

Degradation compounds from metabolism of acibenzolar-S-methyl in plants, animals and the environment:

Compound code (other names)	Chemical structure	Found in
CGA210007 1,2,3-Benzothiadiazole-7-carboxylic acid acibenzolar acid		Plants: Wheat, tomato, tobacco, lettuce, rice Animals: Rat, hen, goat Environment: Water, soil
CGA323060 1,2,3-Benzothiadiazole-4-hydroxy-7-carboxylic acid 4-OH acibenzolar acid		Plants: Tomato, tobacco, lettuce, rat
CGA324041 1,2,3-Benzothiadiazole-5-hydroxy-7-carboxylic acid 5-OH acibenzolar acid		Plants: Wheat, tomato, tobacco, lettuce Animals: Goat, rat
SYN546642 1,2,3-Benzothiadiazole-6-hydroxy-7-carboxylic acid 6-OH acibenzolar acid		Environment: Soil
CGA243093 Benzo[1,2,3]thiadiazole-7-yl-methanol		Plants: Lettuce, rat
CGA379019 3-Methanesulfinyl-benzoic acid		Plants: Rice

Compound code (other names)	Chemical structure	Found in
Met 2U		Animals: Rat, goat
EX1		Animals: Hen
EX8a		Animals: Hen
EX8b		Animals: Hen
EX9a		Animals: Hen

The Meeting received studies on the metabolism of acibenzolar-S-methyl in plants (sunflower, sorghum, cotton, wheat, tomato, tobacco, lettuce and rice), laboratory animals (rats), as well as lactating goats and laying hens. The metabolism of acibenzolar-S-methyl in plants and animals was investigated using [^{14}C-U-phenyl]-acibenzolar-S-methyl. The structural formula and the positions of the ^{14}C label are shown below. The studies on rats were evaluated by the WHO Core Assessment Group of the 2016 JMPR.

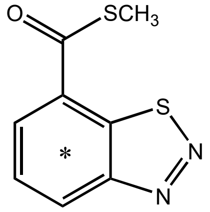


Figure 1 Label positions of acibenzolar-S-methyl: marked as * to indicate uniform labelling of the six carbons in the phenyl ring

The identification of residue components in the animal and plant metabolism studies was achieved using, where available, authentic standards of the compounds involved (Lettuce: CGA 210007, CGA 245704, CGA324041, CGA323060, CGA329834, CGA 337304, CGA379018, CGA379019, CGA379789; Wheat: CGA210007, CGA245704, CGA324041; Tomato: CGA21007, CGA254704, CGA324041, CGA323060; Tobacco: CGA21007, CGA254704, CGA324041, CGA323060; Rice: CGA 210007, CGA 245704, CGA324041, CGA323060, CGA329834, CGA 337304, CGA176315, CGA329832, CGA329833, CGA206485; Goat and chicken: CGA210007, CGA324041, MET2U) and in some cases, also by ^{1}H-NMR and MS-spectroscopy. Additional techniques such as hydrolysis, derivatization, and enzymatic degradation were used in many cases to aid in characterizing metabolites.

Plant metabolism

Acibenzolar-S-methyl is typically used for four different situations:

- as a seed treatment
- as a foliar application
- as a soil treatment for rice at transplanting
- as an application to the soil beneath a growing crop.

The Meeting received plant metabolism studies with acibenzolar-S-methyl on four different crop groups using seed, foliar and granular application treatments. The crops studied are representative of oilseeds (sunflower, sorghum and cotton—seed treatment application), cereals (wheat—foliar application; rice—granular application), fruiting vegetables (tomatoes—foliar application) and leafy crops (lettuce and tobacco—foliar application).

Seed treatments

Cotton

Findak (2009 50007) conducted a metabolism study with [^{14}C]acibenzolar-S-methyl on cotton maintained outdoors. Cottonseeds were pre-coated with an SC blank formulation followed by application of [^{14}C]acibenzolar-S-methyl to each seed at 0.0006 mg/seed (1× rate) or 0.001 mg/seed (1.7× rate) prior to planting. Treated seeds were planted in outdoor above-ground plots in a sandy loam soil (pH 7.6; 0.4% organic matter). Whole plant samples were collected 78 days after planting at BBCH 51 (25% maturity) and cottonseeds, fibres and stalks with leaves were collected at maturity, 190 days after planting at BBCH 89. Samples were homogenised; aliquots were combusted and radio-assayed by LSC to determine the TRR.

Only low levels of radioactivity were detected in cotton commodities at harvest (Table 1).

Table 1 TRRs in cotton commodities after growing cottonseed treated with [^{14}C]acibenzolar-S-methyl

	TRR (mg equiv/kg)	
Commodity	0.0006 mg ai/seed (1×)	0.001 mg ai/seed (1.7×)
Whole plant (25% maturity)	0.0006	0.0013
Stalks (maturity)	0.0003	0.0003
Fibres	0.0002	0.0002
Seed	0.0002	0.0002

The levels of radioactivity were too low to enable further characterisation and identification of metabolites.

Residues above the LOQ of typical analytical methods are not expected at harvest following seed treatment of cotton.

Sunflower

Quistad *et al.* (2011a 50167) studied the metabolism of [^{14}C]acibenzolar-S-methyl in sunflowers grown in outdoor plots. Sunflower seeds (var 8N358CLDM) were pre-coated with WG formulation inerts followed by application of [^{14}C]acibenzolar-S-methyl to each seed at a rate of 0.048 mg ai/seed. Treated seeds were planted in outdoor above-ground plots. The soil was a sandy loam soil (pH 6.9, 0.8% organic matter). Plants were grown to maturity and whole sunflower seeds harvested, 98 days after planting.

Harvested mature whole seeds (meat in shell) were homogenised. Analysis was by extraction (hexane) and combustion. TRR in seeds at harvest were 0.002 mg equiv/kg while those in seeds from the untreated control were < 0.001 mg equiv/kg. The levels of radioactivity were too low to enable characterisation and identification of metabolites.

Residues above the LOQ of typical analytical methods are not expected at harvest following seed treatment of sunflowers.

Sorghum

In a separate study, Quistad *et al.* (2011b 50168), investigated the metabolism of [^{14}C]acibenzolar-S-methyl on outdoor grown sorghum following application as a seed treatment. Sorghum seeds (var NK300) were pre-coated with WG formulation inerts followed by application of [^{14}C]acibenzolar-S-methyl to each seed at a rate of 0.0065 mg ai/seed. Treated seeds were planted in outdoor above-ground plots. Forage samples were collected at BBCH 85 (99 days after planting) and grain and stover at BBCH 99 (133 days from planting). The hulls were removed from the grain. Samples were homogenised; aliquots were extracted with hexane (grain only), and/or combusted, then radio-assayed to determine the TRR.

TRRs in forage were < 0.001 mg equiv/kg, stover 0.002 mg equiv/kg, and grain 0.001 mg equiv/kg. The levels of radioactivity were too low to enable characterisation and identification of metabolites. Commodities obtained from crops grown using untreated seed contained no detectable residue (< 0.001 mg equiv/kg).

Residues above the LOQ of typical analytical methods are not expected at harvest following seed treatment of sorghum.

Foliar applications

Tomato

Nicollier (1996a 0299) conducted a metabolism study with [^{14}C]acibenzolar-S-methyl on tomato plants (variety Mont Favet) maintained indoors (greenhouse). Tomato plants (growth stage 69) were sprayed with a WG formulation of [^{14}C]acibenzolar-S-methyl at a rate of 15.2 g ai/hL. The application was repeated 14 and 28 days after the first application. The total amount applied was equivalent to the recommended rate of seven applications with 6.5 g ai/hL (273 g ai/ha, 3 plants/m^2). Tomato fruit (one tomato including stalk per plant) and foliage samples (one branch with leaves per plant) were collected for analysis 0 days after the first application and 0, 7 and 30 days after the third application.

Tomato samples were washed with CH_3CN/H_2O (50:50, v/v) to determine the surface radioactivity. Washed tomatoes and foliage samples were homogenised under liquid nitrogen and extracted with CH_3CN/H_2O (80:20, v/v). The presence of conjugates was examined by treatment of the solvent extracts with cellulase (from *Aspergillus niger*), hydrolysis with 0.1 N NaOH (1 hour at room temperature) or 2 N NaOH (2 hours at 75 °C).

Total radioactive residues found at harvest were 0.312 mg equiv/kg (parent content: 0.003 mg/kg) in tomatoes and 0.719 mg equiv/kg in foliage (parent < LOD). The surface ^{14}C on tomatoes decreased from 34.8% TRR (one hour after third application) to 1.4% TRR (one month after third application). The unextracted radioactivity at maturity was low accounting for 3.4% TRR in tomatoes and 7.4% TRR in foliage (Table 2).

Extractability of ^{14}C residues with the solvent system (CH_3CN/H_2O) used was good at 97% (surface wash + extracted) of the TRR for tomato fruit at maturity.

Table 2 Distribution of ^{14}C in tomato plants maintained indoors after foliar treatment with [^{14}C]acibenzolar-S-methyl (Nicollier 1996a 0299)

DAA	Sample	TRR	Acibenzolar-S-methyl	Surface wash	Extracted	Unextracted	Total
		(mg equiv/kg)	(mg/kg)	(%TRR)	(%TRR)	(%TRR)	(%TRR)
0 (1 h after 1st)	Fruit	1.401	1.321	95.9	4.1	0.2	100.2
	Foliage	6.834	6.187	NA	99.6	0.4	100.0
0 (1 h after 3rd)	Fruit	0.759	0.256	34.8	64.2	2.3	101.3
	Foliage	3.486	2.589	NA	99.4	3.2	102.6
7 (after 3rd)	Fruit	0.689	0.101	12.9	77.3	4.8	95.0
	Foliage	2.987	1.374	NA	83.2	5.5	88.7
30 (after 3rd)	Fruit	0.312	0.003	1.4	95.6	3.4	100.4
60 (after 3rd)	Foliage	0.719	ND	NA	94.8	7.4	102.2

NA = not applicable

ND = not detected

The TLC pattern of the extracted ^{14}C from tomato fruit and foliage samples collected at maturity consisted of seven and eight fractions, respectively. Three metabolites could be characterised as 5-OH acibenzolar acid (CGA324041), 4-OH acibenzolar acid (CGA323060) and acibenzolar acid (CGA210007). 5-OH Acibenzolar acid (CGA324041) accounted for 6.5 and 1.5% TRR, 4-OH acibenzolar acid (CGA323060) for 0.4 and 6.1% TRR and acibenzolar acid (CGA210007) for 8.1 and 19.2% TRR in fruit (sum of surface and penetrated) and foliage respectively.

The presence of conjugates was investigated. After treatment of the initial solvent extract of fruit with cellulase, the relative amount of 4-OH acibenzolar acid (CGA323060) and acibenzolar acid (CGA210007) increased significantly from 0.4 to 8.2% TRR and from 8.1 to 34.2% TRR, respectively. Complete hydrolysis of TLC fractions I1a–I4 in favour of acibenzolar acid (CGA210007) could be achieved after treatment with 2 N NaOH for two hours at increased temperature (75 °C). After this treatment, acibenzolar acid (CGA210007) accounted for 64.3% TRR (Table 3).

Nearly complete hydrolysis of TLC fractions I1a–I4 to 5-OH acibenzolar acid (CGA324041), 4-OH acibenzolar acid (CGA323060) and acibenzolar acid (CGA210007) could also be achieved under mild conditions with 0.1 N NaOH (1 hour at room temperature). The remaining diffuse fraction I1b could be then cleaved quantitatively with cellulase to 4-OH acibenzolar acid (CGA323060). This behaviour suggests that the TLC fractions I1a–I4 consists of sugar conjugates of 4-OH acibenzolar acid (CGA323060) and acibenzolar acid (CGA210007). 4-OH Acibenzolar acid (CGA323060) is bound as a glycoside and acibenzolar acid (CGA210007) as an easy cleavable ester.

Table 3 Characterisation and identification of ^{14}C in mature tomato fruit and foliage after foliar application with [^{14}C]acibenzolar-S-methyl (Nicollier 1996a 0299)

	Foliage	Fruit	
		Extract	after cellulase + 2 N NaOH (2 h at 75 °C)
TRR (mg equiv/kg)	0.719	0.312	
		%TRR	
Extracted (CH_3CN/H_2O) + Surface wash	94.8	97.0	85.1
acibenzolar-S-methyl	ND	0.8	0.8
CGA210007	19.2	8.1	64.3
CGA324041	1.5	6.5	7.9
CGA323060	6.1	0.4	6.8
Fractions hydrolysable to CGA210007 and in small amounts to CGA324041 and CGA323060	66.3 (6 max 44.7%)	78.6 (6 max 49.4%)	ND
Unresolved	0.8	2.4	4.9
Origin	0.9	0.4	0.4
Unextracted	7.4	3.4	3.4
Total	102.3	100.4	88.5

NA = not applicable

ND = not detected

The metabolism of acibenzolar-S-methyl in tomatoes proceeds via hydrolysis of acibenzolar-S-methyl to acibenzolar acid (CGA210007). Subsequent oxidation of the phenyl ring leads to CGA324041 and 4-OH acibenzolar acid (CGA323060). CGA323060 and acibenzolar acid (CGA210007) form conjugates, 4-OH acibenzolar acid (CGA323060) was conjugated as a glycoside and acibenzolar acid (CGA210007) as an ester. The major component of the ^{14}C residues in tomatoes following foliar application is acibenzolar acid (CGA210007) in both free and conjugated forms.

Lettuce

The metabolism of [^{14}C]acibenzolar-S-methyl in lettuce was studied by Kiffe (1998 0460). Applications of a WG formulation were made to two sets of plants at rates of 35 g ai/ha and 105 g ai/ha, equivalent to seasonal rates of 140 g ai/ha per season (1×) and 420 g ai/ha per season (3×). Spray volumes were equivalent to 500 L/ha. The first application was at the 7–9 leaf stage (BBCH 17–19) and subsequent applications at one week intervals. Plants were maintained indoors in a greenhouse. Surface washes were performed using CH_3CN/H_2O (50:50 v/v). Solvent extraction of ^{14}C residues was performed on homogenised washed samples using CH_3CN/H_2O (80:20 v/v). For microwave extraction, the residues remaining in the solids after the initial solvent extraction were further extracted using 1-propanol/H_2O (80:20 v/v) under argon in a microwave oven (5 minutes 100 °C, 10 minutes 120 °C and 20 minutes 150 °C).

TRR in lettuce plants harvested one week after final application were 1.014 mg equiv/kg (1× rate) and 3.668 mg equiv/kg (3× rate) (Table 4). In these samples, parent acibenzolar-S-methyl residues were 0.167 and 0.708 mg/kg, respectively. The residue contained in surface rinses amounted to 20.2% (1×) and 23.0% (3×) of TRR, with penetrated residue (sum of extract after surface wash and unextracted radioactivity) amounting to 83.2% (1×) and 74.2% (3×) of the TRR found at harvest.

The solvent extracted ^{14}C at harvest was 99.0% (1×) and 81.4% (3×) of the TRR. To investigate the presence of conjugates, the solvent extracts were sequentially treated with sodium hydroxide (0.1 N) and cellulase (from *Aspergillus niger*). For the 1× lettuce samples, hydrolysis led to an increase in acibenzolar acid (CGA210007) from 4.4 to 24.5% TRR and for 4-OH acibenzolar acid (CGA323060) from 1.0 to 20.0% TRR confirming the presence of conjugates.

Table 4 Characterisation and identification of ^{14}C in lettuce leaves after four applications of [^{14}C]acibenzolar-S-methyl to lettuce plants (Kiffe 1998 0460)

	Lettuce (1×)		Lettuce (3×)	
		after 2 N NaOH + cellulase for 2 h at 75 °C		after 2 N NaOH + cellulase for 2 h at 75 °C
TRR (mg equiv/kg)	1.014		3.668	
		%TRR		
Surface wash	20.2		23.0	
acibenzolar-S-methyl	16.5		19.3	
CGA210007	0.6		0.6	
CGA324041	0.1		0.1	
CGA323060	0.1		0.1	
Unidentified fractions	2.3 (6 max 0.7)		2.7 (6 max 0.9)	
Extracted (Soxhlet)	78.8 (inc 6.6 MWE)	69.3	59.4	54.0
acibenzolar-S-methyl	0.4	ND	ND	ND
CGA210007	4.4	24.5	1.3	12.8
CGA324041	0.9	0.5	0.5	ND
CGA323060	1.0	20.0	0.8	22.3
CGA243093	0.7	4.7	ND	2.1
Unidentified fractions	61.6 (6 max 25.2)	18.4 (6 max 6.3)	47.8 (6 max 16.1)	10.8 (max 5.1)
Unresolved	9.8	1.2	6.0	6.0
Unextracted	4.6		14.9	
Total	103.4		97.2	

ND = not detected

MWE = microwave extract

For lettuce, metabolism of acibenzolar-S-methyl proceeds via hydrolysis of acibenzolar-S-methyl to acibenzolar acid (CGA210007) followed by ester conjugation with sugars. Subsequent oxidation of the phenyl ring led to 5-OH acibenzolar acid (CGA324041), benzo[1,2,3]thiadiazole-7-yl-methanol (CGA243093) and to 4-OH acibenzolar acid (CGA323060), the latter forming sugar conjugates as O-glycosides.

Tobacco

Nicollier (1996b 0266) studied the uptake, penetration, distribution and degradation of [^{14}C]acibenzolar-S-methyl in greenhouse grown tobacco plants (variety Xanthi). Nine week-old tobacco plants were sprayed three times with [^{14}C]acibenzolar-S-methyl, formulated as water dispersible granules at a total rate of 170 g ai/ha. The first application (20 g ai/ha) was made at the 7-leaf stage to 12 tobacco plants, the second application (50 g ai/ha) 21 days later, on 10 tobacco plants, and the third application (100 g ai/ha) was made 34 days after the first application to the 10 tobacco plants. Two tobacco plants were harvested approximately 1 hour after the first application. Just before the second application, one old leaf and one new grown leaf per plant were collected (21 days after 1st application). One hour after the third application, two leaves per plant were collected. Mature leaves, sorted into lower and upper leaves, were collected 17, 27, 35, 45 and 52 days after the third application. Part of the collected leaves from the various harvest times were kept for the curing process. Mature tobacco leaves were cured at 23 °C and 60% humidity. Tobacco leaves were hung in the chamber from their bases. After two months, the stems and leaves became pliable, indicating the completion of the curing process.

Solvent extraction of ^{14}C residues was performed on homogenised samples using CH_3CN/H_2O (80:20 v/v). For samples where the initial extracts contained < 90%TRR, the residues remaining after the solids after the initial solvent extraction were further extracted using microwave extraction with CH_3CN/H_2O (80:20 v/v) at 150 °C.

Translocation of the radioactivity into new grown leaves was low (Table 5). Residues found 21 days after the first application were 0.596 and 0.031 mg equiv/kg in treated and new grown leaves with parent contents of 0.053 mg/kg and 0.001 mg/kg, respectively. At maturity, TRRs in tobacco plants were 1.388 mg equiv/kg in lower leaves, 0.434 mg equiv/kg in upper leaves and 0.022 mg equiv/kg in stems.

Parent acibenzolar-S-methyl accounted for 0.079 mg/kg, 0.026 mg/kg and 0.002 mg/kg in lower leaves, upper leaves and stems, respectively.

The ^{14}C extraction efficiency of the solvent system used (CH_3CN/H_2O) was good with > 88% TRR recovered from leaves. The unextracted radioactivity (after cold and microwave extraction) accounted for 5.0 and 9.4% TRR in lower leaves (uncured and cured), and 4.5 and 7.3% TRR in upper leaves (uncured and cured), respectively.

Table 5 Distribution of ^{14}C in tobacco after foliar application with [^{14}C]acibenzolar-S-methyl (Nicollier 1996b 0266)

DAA (application number)	0 (1st 1 hr)	21 (1st)		0 (3rd 1 hr)		10 (3rd)	17–45 (3rd)	52 (3rd)	
Sample	Whole tops	New growth	Old leaves	Whole tops	Leaves 3 appl	Leaves 2 appl	Lower leaves	Upper leaves	Stems
TRR (mg equiv/kg)	8.849	0.031	0.596	2.139	3.207	1.879	1.388	0.434	0.022
Acibenzolar-S-methyl (mg/kg)	8.636	0.001	0.053	1.501	0.683	0.449	0.079	0.026	0.002
					%TRR				
Extracted	103.1	97.9	90.3	103.0	88.0	94.2	92.5	95.7	83.3
MWE	NA	NA	NA	0.5	3.6	1.5	1.7	2.5	3.2
Unextracted	0.3	3.5	6.3	1.6	10.9	3.5	5.0	4.5	21.2
Total	103.4	101.4	96.6	105.1	102.5	99.2	99.2	102.7	107.7

NA = not applicable

MWE = microwave extracted

The TLC pattern of the extracted ^{14}C found in cured and uncured leaves consisted of approximately seven fractions. Parent acibenzolar-S-methyl was present in low amounts in tobacco leaves (max. 6.1% TRR) (Table 6). The presence of conjugates was investigated. A significant portion (70.4% TRR) of acibenzolar acid (CGA210007) was released from lower leaves (uncured) by alkaline/enzyme treatment; the minor metabolites 5-OH acibenzolar acid (CGA324041) (2.7% TRR) and 4-OH acibenzolar acid (CGA323060) (4.8% TRR) were also characterised in these portions. The results obtained after alkaline/enzyme treatment show that majority of ^{14}C is present in the form of ester conjugates of acibenzolar acid (CGA210007) together with small amounts of sugar conjugates of 5-OH acibenzolar acid (CGA324041) and 4-OH acibenzolar acid (CGA323060). The results for upper leaves were like those for lower leaves, as were those for cured leaves. The proportion of acibenzolar acid (CGA210007) recovered after cellulase treatment, cellulase/2 N NaOH 2 h 75 °C and 0.1 N NaOH 1 h RT was 22.1, 70.4 and 66.7% TRR respectively.

Table 6 Characterisation and identification after three applications of [^{14}C]acibenzolar-S-methyl to tobacco plants (Nicollier 1996b 0266)

	Lower leaves (17–45 DAA)			
		after cellulase + 2 N NaOH for 2 h at 75 °C	Upper leaves (52 DAA)	Stems (52 DAA)
TRR (mg equiv/kg)	1.388	1.388	0.434	0.022
		%TRR		
Extracted (Soxhlet)	93.4	84.5	97.1	83.1
acibenzolar-S-methyl	5.7	ND	6.1	11.2
CGA210007	9.0	70.4	6.4	24.3
CGA324041	1.7	2.7	0.9	nd
CGA323060	1.3	4.8	0.7	nd

	Lower leaves (17–45 DAA)			
		after cellulase + 2 N NaOH for 2 h at 75 °C	Upper leaves (52 DAA)	Stems (52 DAA)
Fractions hydrolysable to CGA210007 and in small amounts to CGA324041 and CGA323060	74.0 (4 max 53.8%)	5.2 (2 max 3.6%)	80.8 (4 max 58.6%)	42.7 (3–4 max 25.2%)
Unresolved	0.9	1.4	1.2	3.2
Origin	0.8	ND	1.0	1.7
Microwave extracted	0.8	0.8	1.3	3.2
Unextracted	5.0	5.0	4.5	21.7
Total	99.2	90.3	102.9	108

ND = not detected

In summary, like results for tomato and wheat, the metabolism of acibenzolar-S-methyl in tobacco proceeds via hydrolysis of acibenzolar-S-methyl to acibenzolar acid (CGA210007) followed by ester conjugation with sugars. Subsequent oxidation of the phenyl ring led to 5-OH acibenzolar acid (CGA324041) and to 4-OH acibenzolar acid (CGA323060) followed by sugar conjugation as O-glycosides.

Wheat

Walser (1995a 0149) conducted a metabolism study with [^{14}C]acibenzolar-S-methyl on spring wheat (variety Besso) maintained either indoors (4-leaf stage) and outdoors (tillering). Wheat plants at the 4-leaf stage (indoor experiment) and at the end of tillering (field experiment) were sprayed with a WG formulation of [^{14}C]acibenzolar-S-methyl at a rate of 50 g ai/ha.

For the indoor experiment, plant (shoots) and soil samples (5 pots/interval) were collected for analysis 0, 1, 3, 7 and 14 days after application. Shoot samples were washed with CH_3CN and the washed shoots homogenised and extracted with CH_3CN.

TRR declined rapidly in wheat shoots through the 14 days after application. Acibenzolar-S-methyl was converted to acibenzolar acid (CGA210007) so that by 14 days after application acibenzolar acid (CGA210007) was present at much higher levels than parent compound (Table 7). The major component of the ^{14}C residue by 14 days after application was unable to be extracted with the solvent system employed.

Table 7 Distribution of radioactivity in shoots of wheat plants maintained indoors after foliar treatment with [^{14}C]acibenzolar-S-methyl (Walser 1995a 0149)

		Surface wash (%TRR)		Washed plants (%TRR)		
DAA	TRR			Extract		Unextracted
	(mg equiv/kg)	Acibenzolar-S-methyl	CGA210007	Acibenzolar-S-methyl	CGA210007	
0	1.601	85.6	3.0	3.5	0.1	–
1	1.013	55.3	5.6	3.3	14.3	11.6
3	0.514	33.7	2.7	2.9	15.6	21.2
7	0.312	11.7	6.6	2.2	9.4	42.7
14	0.468	4.6	9.3	1.5	6.2	62.7

For the outdoor (field) experiment, approximately 20 shoots were collected for analysis after 0, 14 and 28 DAA. Mature whole plants were collected 75 DAA and separated into straw, husks and grains and at each sampling time three soil cores (5 cm × 30 cm) were collected. Wheat samples were homogenised and extracted with CH_3CN/H_2O (80:20 v/v) followed by Soxhlet extraction using CH_3CN. Additional sub-samples of solvent extracts were treated with base to liberate conjugates and post-extraction solids (PES) were subjected to various treatments to liberate bound residues and conjugates and to characterise radioactivity associated with natural components.

TRRs in mature wheat grown under field conditions were 0.014 mg equiv/kg in grains, 0.233 mg equiv/kg in husks and 0.328 mg equiv/kg in straw (Table 8).

The ^{14}C extraction efficiency at mature harvest using CH_3CN/H_2O was 30% for straw, 39% for husks and 41% for grain. The proportion of ^{14}C unable to be extracted using CH_3CN/H_2O increased with time after application. After cold and Soxhlet extraction, the unextracted radioactivity accounted for 59.7% TRR in grains, 56.6% TRR in husks and 67.9% in straw at maturity.

Table 8 Distribution of radioactivity in wheat plants maintained outdoors after foliar treatment with [^{14}C]acibenzolar-S-methyl (Walser 1995a 0149)

DAA	Sample	TRR	Extracted (%TRR)		Unextracted (%TRR)	Total
		(mg equiv/kg)	Solvent extract	Soxhlet		(%TRR)
0	Shoot	1.847	98	NA	2.0	100.0
14	Shoot	0.290	86.8	1.9	13.0	101.7
28	Stalk	0.227	73.9	2.2	22.3	98.4
28	Ear	0.183	85.0	1.1	16.8	102.9
75	Straw	0.328	30.3	1.2	67.9	99.4
75	Husk	0.233	39.4	0.7	56.6	100.9
75	Grain	0.014	41.2	NA	94.4	102.9

NA = not applicable

Identification of ^{14}C residues is summarized in Tables 9–11. The TLC metabolite pattern of the extracted ^{14}C from wheat commodities consisted of six fractions. Parent acibenzolar-S-methyl was only detected on the day of application. The major component of the ^{14}C identified was free acibenzolar acid (CGA210007) which represented 8.4–14.4% TRR in straw, husks and grain at harvest. The only other metabolite identified was 5-OH acibenzolar acid (CGA324041) but only at relatively low levels (1.7–3.7% TRR).

Table 9 Characterisation and identification of ^{14}C residues in outdoor grown wheat plants (Walser 1995a 0149)

DAA	1 hour	14 days	28 days		75 days		
Commodity	Shoots	Shoots	Stalks	Ears	Straw	Husks	Grain
TRR (mg equiv/kg)	1.847	0.290	0.227	0.183	0.328	0.233	0.014
				%TRR			
Extracted	98.1	89.5	78.0	86.1	29.8	35.7	36.8
Origin	0	1.6	0	ND	ND	0	1.4
Fractions I_1–I_4	ND	67.1	69.5	72.8	11.8	20.3	23
CGA324041	ND	2.6	1.2	2.1	1.7	1.9	3.7
CGA210007	3.4	15.4	4.1	7.1	14.4	12.1	8.4
ASM	92.8	ND	ND	ND	ND	ND	ND
Unresolved	1.9	0.9	1.0	3.0	0.7	0.7	0.3
Soxhlet	NA	1.9	2.2	1.1	1.2	0.7	NA
Unextracted	2.0	13.0	22.3	16.8	67.9	56.6	59.7
Total	100.1	102.5	100.3	102.9	97.7	92.3	96.5

ND = not detected

NA = not analysed

ASM = acibenzolar-S-methyl

The presence of conjugates was investigated using hydrolysis and/or enzyme treatment (cellulase from *Aspergillus niger* and esterase from porcine liver) of the solvent extracts. Hydrolysis resulted in a significant increase in acibenzolar acid (CGA210007) detected in all samples. At maturity, acibenzolar acid (CGA210007) after hydrolysis represented 22.2–23.5% TRR (Table 10).

Table 10 Characterisation and identification of ^{14}C residues in outdoor grown wheat plants, results following treatment with 0.1 N NaOH for one hour at room temperature (Walser 1995a 0149)

DAA	1 hour	14 days	28 days		75 days		
Commodity	Shoots	Shoots	Stalks	Ears	Straw	Husks	Grain
TRR (mg equiv/kg)	1.847	0.290	0.227	0.183	0.328	0.233	0.014
				%TRR			
Extracted		79.9	70.5	83.4	28.8	34.2	36.3
Origin		0	1.2	2.2	0.3	0.3	1.0
Fractions I_1–I_4		16.8	17.8 [a]	18.9 [b]	3.5	7.2	5.6
CGA324041		2.4	2.7	4.9	1.3	2.0	4.8
CGA210007		57.9	45.5	56.3	22.2	23.5	23.5
ASM	92.8	ND	ND	ND	ND	ND	ND
Unresolved		0.9	1.1	1.3	0.3	0.5	1.4
Soxhlet		1.9	2.2	1.1	1.2	0.7	NA
Unextracted		13.0	22.3	16.8	67.9	56.6	59.7
Total		92.9	92.8	100.2	96.7	90.8	96.0

ND = not detected

NA = not analysed

ASM = acibenzolar-S-methyl

[a] Following treatment with 6 N HCl is reduced by 6.8% TRR with a corresponding increase in CGA210007

[b] Following treatment with 6 N HCl is reduced by 6.5% TRR with a corresponding increase in CGA210007

Sequential hydrolysis (husks, straw: boiling water; 10% NaOH reflux 16 h; CH_2Cl_2; grain: 0.05 N NaOH 6 h RT; 1 N HCl 17 h, reflux) of the ^{14}C unextracted by solvent in straw, husks and grain released additional radioactivity that was characterised as acibenzolar acid (CGA210007). A large proportion of the ^{14}C unextracted by the solvent system used was associated with natural products such as cellulose, lignin, protein, water soluble polysaccharides and starch; 36.2% TRR for straw, 29.1% TRR for husks and 21.1% TRR for grain (Table 11).

Table 11 Characterisation and identification of ^{14}C in PES of wheat straw, husks and grain (Walser 1995a 0149)

	Straw	Husks	Grain
TRR (mg equiv/kg)	0.328	0.233	0.014
		%TRR	
Extracted (Soxhlet)	27.6	35.0	36.8
acibenzolar-S-methyl	–	–	–
CGA210007	14.4	12.1	8.4
CGA324041	1.7	1.9	3.7
Fractions hydrolysable to CGA210007	11.8 (5, max 4.3%)	20.3 (5 max 10%)	23.0 (5 max 16.7%)
Unresolved	0.7	0.7	0.3
Origin	–	–	1.4
Microwave extracted	1.2	0.7	NA
Unextracted	67.9	56.6	59.7
Water soluble polysaccharides	2.9	2.9	–
Cellulose	2.2	2.3	8.5
Lignin	31.1	23.9	
Starch			5.8
Protein			8.7
Not characterised			6.9
Fractions hydrolysable to CGA210007	23.1	17.4	15.8
Polar unknowns			13.1
Total	97.7	92.3	96.5

NA = not applicable

Radioactivity found in field soil was also investigated and was strongly adsorbed in the upper layer (0–5 cm). The ^{14}C levels found in deeper layers were never higher than 0.001 mg/kg. TRR in the 0–5 cm layer decreased from 0.041 mg equiv/kg at 0 DAA to 0.013 mg equiv/kg at 75 DAA, of which 94.4% TRR remained unextracted after soil processing.

In summary, acibenzolar acid (CGA210007) free and conjugated is the major component of the ^{14}C residue in wheat commodities following foliar application. A significant proportion of the ^{14}C was incorporated into natural products.

Soil treatment at transplanting

Rice

Krause (1996 0370) studied the uptake, penetration, distribution and degradation of [^{14}C]acibenzolar-S-methyl in greenhouse grown rice plants. Three-week-old rice plants were treated in a seedling box at the 3-leaf stage (BBCH 13) with a granule formulation of [^{14}C]acibenzolar-S-methyl at a rate of 1 g ai/seedling box, equivalent to 200 g ai/ha. One day after application, rice plants together with soil were transplanted into containers filled with soil and flooded with water. Samples of water and plants were collected 11, 50 and 78 DAA and mature plants 119 DAA (118 days after transplantation). Solvent extraction of residues was performed on homogenised samples using CH_3CN/H_2O (80:20 v/v). The residues remaining in the solids after initial solvent extraction were further extracted using CH_3CN/H_2O (80:20 v/v) in a microwave oven (10 minutes 100 °C, 10 minutes 120 °C and 20 minutes 140 °C) = microwave extraction.

The ^{14}C residues in paddy water were measured at various intervals after application (Figure 2). Residues in paddy water reached a maximum of 25% AR at 1–2 weeks after soil application and flooding, declining to 15% AR by 40 DAA. Thereafter the ^{14}C residues in paddy water declined rapidly to reach 0.4% AR by 60 DAA. Acibenzolar acid (CGA210007) was the major metabolite present in paddy water representing 32.5% TRR at 11 DAA, 45.6% TRR at 50 DAA and 56.6% TRR at 78 DAA.

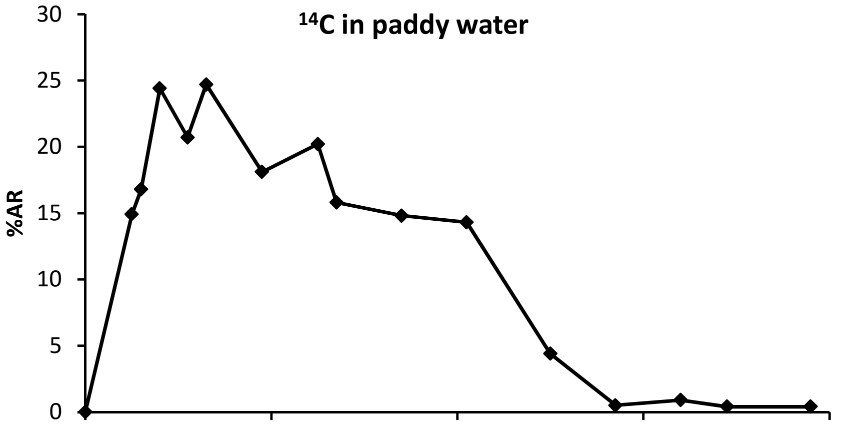


Figure 2 Temporal change in proportion of applied radioactivity in paddy water

One DAA, rice seedlings had taken up 1.3% AR, 2.0% AR by 11 DAA, 5.1% AR at 50 DAA, 7.2% AR at 78 DAA and 11.1% AR at harvest, 119 DAA. At maturity, 0.2% AR was in grain, 0.2% AR in husks and 10.7% AR in straw while 80.7% AR remained in the soil. Approximately 8% AR was not accounted for, presumably lost by volatilisation or transpiration.

The extraction efficiency for ^{14}C with the solvent system employed (CH_3CN/H_2O) was poor for rice commodities at harvest, at 6.4% TRR for grain, 33% TRR for husks and 41% TRR for straw (Tables 12 and 13). Unextracted ^{14}C in plant samples was high and represented 91.6, 59.2, 54.8 and 39.2% TRR in grain, soil, husks and straw, respectively. Acibenzolar-S-methyl was only detected in samples of leaves collected 1 DAA. The major metabolite identified in leaves collected 11–78 DAA was acibenzolar acid (CGA210007) at 6.7–11.1% TRR in leaves. Acibenzolar acid (CGA210007) was present at 1.7–10.2% TRR in rice commodities (grain, husks, straw) at harvest, 119 DAA.

Table 12 Characterisation and identification of ^{14}C in leaves of rice plants treated with acibenzolar-S-methyl (Krause 1996 0370)

	Leaves (1 DAA)	Leaves (11 DAA)	Leaves (50 DAA)	Leaves (78 DAA)
TRR (mg equiv/kg)	14.754	22.257	1.286	0.425
		%TRR		
Extracted (Soxhlet)	97.9	69.9	62.3	50.0
acibenzolar-S-methyl	1.7	–	–	–
CGA210007	86.1	11.1	4.9	3.7
CGA379019		1.7	–	–
Unidentified fractions		55.1	54.8	43.6
Unresolved	10.2	1.8	2.6	2.7
Microwave extracted	NA	NA	9.8	17.3
acibenzolar-S-methyl			–	–
CGA210007			1.8	6.9
CGA379019			0.3	0.6
Unidentified fractions			6.8	7.5
Unresolved			1.0	2.2
Unextracted	2.1	30.4	30.7	32.0
Total	100.1	100.1	102.8	99.3

NA = not analysed

Table 13 Characterisation and identification of ^{14}C in soil and rice commodities collected at plant maturity (119 DAA) (Krause 1996 0370)

	Grain	Husks	Straw	Soil
TRR (mg equiv/kg)	0.085	0.159	1.992	0.136
		%TRR		
Extracted (Soxhlet)	6.4	33.1	41.2	35.1
acibenzolar-S-methyl	–	–	–	–
CGA210007	1.7	1.4	3.1	17.8
CGA379019	0.6	3.7	1.0	–
Unidentified fractions	3.6	25.1	35.7	16.0
Unresolved	0.5	2.9	1.2	1.3
Microwave extracted	2.0	11.4	21.3	6.0
acibenzolar-S-methyl		–	–	–
CGA210007		2.3	7.1	1.2
CGA379019		–	–	–
Unidentified fractions		6.6	11.0	4.2
Unresolved		2.5	3.2	0.6
Unextracted	91.6	54.8	39.2	59.2
Total	100	99.3	101.5	100.3

The presence of conjugated residues in the solvent extracts was investigated. Following hydrolysis, the proportion of unidentified ^{14}C decreased and there was a concomitant increase in the proportion of acibenzolar acid (CGA210007) and to a lesser extent 3-methanesulfinyl-benzoic acid (CGA379019) (Tables 14 and 15). On hydrolysis acibenzolar acid (CGA210007) increased to account for 56.2, 16.8 and 5.6% TRR in straw, husks and grain, respectively. 3-Methanesulfinyl-benzoic acid (CGA379019) was also significant at 8.8, 7.9 and 18.8% TRR in straw, husks and grain, respectively.

Table 14 Characterisation and identification of ^{14}C in leaves of rice plants treated with acibenzolar-S-methyl after mild hydrolysis (0.1 N NaOH 1 h RT) or Mazerozym/cellulase treatment of solvent extracts (Krause 1996 0370)

	Leaves (1 DAA)	Leaves (11 DAA)	Leaves (50 DAA)	Leaves (78 DAA)
TRR (mg equiv/kg)	14.754	22.257	1.286	0.425
		%TRR		
Extracted (Soxhlet)	NA	69.6	62.3	50.2
acibenzolar-S-methyl		–	–	–
CGA210007		57.0	43.8	35.0
CGA379019		3.2	10.3	6.9
Unidentified fractions		7.2	1.5	4.4
Unresolved		2.2	6.7	3.9
Microwave extracted	NA	NA	9.8	17.3
acibenzolar-S-methyl			–	–
CGA210007			6.5	12.4
CGA379019			1.7	2.3
Unidentified fractions			0.5	1.3
Unresolved			1.1	1.3
Unextracted	NA	30.4	30.7	32.0
Total	NA	100.0	102.8	99.5

NA = not analysed

Table 15 Characterisation and identification of ^{14}C in rice commodities collected at plant maturity (119 DAA) after mild hydrolysis (0.1 N NaOH 1 h RT) or Mazerozym/cellulase treatment of solvent extracts (Krause 1996 0370)

	Grain	Husks		Straw	
			Mazerozym/ cellulase + 0.1 N NaOH 1 h RT		Mazerozym/ cellulase + 0.1 N NaOH 1 h RT
TRR (mg equiv/kg)	0.085	0.159		1.992	
			%TRR		
Extracted (Soxhlet)	6.5	33.1	32.5	41.1	41.2
acibenzolar-S-methyl	–	–	–	–	–
CGA210007	3.7	12.3	5.5	33.9	27.9
CGA379019	1.3	5.3	4.7	3.1	5.6
Unidentified fractions	0.9	11.4	18.9	2.2	4.8
Unresolved	0.6	4.1	3.4	1.9	2.9
Microwave extracted	2.0	11.4	11.4	21.3	21.3
acibenzolar-S-methyl		–		–	
CGA210007		2.7		14.2	
CGA379019		1.5		3.2	
Unidentified fractions		5.3		2.4	
Unresolved		1.8		1.5	
Unextracted	91.6	54.8	54.8	39.2	39.2
Total	100.1	99.3	98.7	101.6	101.7

As the unextracted ^{14}C in plant samples (i.e. following cold and microwave extraction) was high, the PES were subject to harsh alkaline and acid conditions to assist further characterisation (1 N HCl 100 °C 14 h; 6 N HCl 100 °C 14 h; 0.1 N NaOH, room temperature 1 h; cellulase/Mazerozym treatment; cellulase/Mazerozym treatment and 0.1 N NaOH 1 h room temperature; pepsin). Following hydrolysis of PES under harsh alkaline and acid conditions, the unextracted residues remaining in grain were reduced from 91.6% TRR to 39.3% TRR. Small amounts of additional acibenzolar acid

(CGA21007) were liberated (1.8–8.1% TRR). CGA37019 was the major component liberated by the harsh treatment and accounted for 17.5% TRR in grain. Further investigation also revealed incorporation of ^{14}C into natural products, 24.4% TRR for grain, 31.3% TRR for husks and 17.2% TRR for straw.

Table 16 Characterisation of ^{14}C in post-extraction solids of rice commodities (Krause 1996 0370)

	Grain (%TRR)	Husks (%TRR)	Straw (%TRR)
Solubilised on harsh hydrolysis	51.2	45.6	32.1
Unidentified fractions (II_0, II_1–II_{2a}, II_{11H})	3.5	1.2	1.7
CGA379019	17.5	1.1	2.5
CGA379789 (II_4)			0.7
CGA324041 (II_{5a})		0.2	
CGA210007	1.9	1.8	8.1
CGA379018 (II_{7H})	3.8		0.1
Sugar [c]	9.3	–	–
Cellulose	3.3	14.4	1.0
Water-soluble polysaccharides	8.9 [a]	4.2	5.4
Protein	2.9	–	–
Lignin	–	12.6	10.8
Unresolved	0.1	10.1 [b]	1.8
Unextracted after harsh hydrolysis	39.3	3.1	0.5
Total	90.5	48.7	32.6

[a] Hemicellulose fraction

[b] Unresolved fraction contains 7.7% water phase material

[c] Analysed as glucosazone

In summary, for rice, metabolism of acibenzolar-S-methyl proceeds via hydrolysis to acibenzolar acid (CGA210007) followed by ester conjugation with sugars. Subsequent oxidation and ring opening of the thiadiazole ring led to 3-methanesulfinyl-benzoic acid (CGA379019) which is likely bound to natural products.

The metabolism on lettuce, rice, tobacco, tomato, and wheat is consistent with less exhaustive studies reported in the literature on the translocation and uptake of acibenzolar-S-methyl employing unlabelled-parent compound. These studies supplement the information obtained in the metabolism studies utilising [^{14}C]acibenzolar-S-methyl and are reported for completeness.

Foliar

Following foliar application to tomatoes (Scarponi *et al.* 2001) or pepper leaves (Buonaurio *et al.* 2002), residues of acibenzolar-S-methyl in treated (basal) leaves decreased rapidly with interval after application. Translocation to untreated (apical) leaves was also rapid amounting, in tomato plants, to 30.5% of total amount of acibenzolar-S-methyl at 2 hours after application. Acibenzolar acid (CGA210007) was only detected in tomato leaves and only found in treated (basal) leaves suggesting on foliar application there is limited translocation of this metabolite. No investigation of possible conjugates was undertaken.

Soil

Following soil application, the translocation of residues from root system to above ground parts was rapid since residues of acibenzolar-S-methyl and acibenzolar acid (CGA210007) were detected within 1 h after treatment (Myresiotis *et al.* 2014). With increasing intervals after application, the residue levels of acibenzolar-S-methyl in tomato parts were gradually reduced, while the concentration of acibenzolar acid (CGA210007) increased.

In conclusion, the metabolism of acibenzolar-S-methyl in plants has been investigated in representative crops of four crop groups: sunflower and cotton (oilseeds), wheat, sorghum and rice (cereals), tomatoes (fruiting vegetables), and lettuce and tobacco (leafy crops). The TRRs observed in oilseeds following seed treatment application were too low to permit characterisation and therefore the nature of residues in these crops was not investigated further.

The nature of residues in cereals, fruiting vegetables and leafy crops were shown to be similar and mainly comprised of acibenzolar acid (CGA210007). Other than on the day of application, parent acibenzolar-S-methyl occurred only at low levels and then only at short intervals after application. Metabolites 4-OH acibenzolar acid (CGA323060), benzo[1,2,3]thiadiazole-7-yl-methanol (CGA243093) and 5-OH acibenzolar acid (CGA324041) were also identified, albeit generally at low levels (i.e. < 10% TRR or < 0.05 mg/kg). 4-OH Acibenzolar acid (CGA323060) was more significant in lettuce where it occurred at similar levels to acibenzolar acid (CGA210007), or was the major component (20–22%). In rice grown in paddies and treated by granule application, 3-methanesulfinyl-benzoic acid (CGA379019) was the most significant component of the residue (19%) in grain, whereas acibenzolar acid (CGA210007) was the most abundant component of the residue in husks and straw (17% and 56%, respectively).

Table 17 Residues of acibenzolar-S-methyl and its metabolites in various plant commodities

Crop		Tomato	Tobacco	Lettuce	Wheat			Rice		
DALA		30	17–45	7	75	75	75	119	119	119
Matrix		Fruit	Leaf	Leaf	Grain	Husks	Straw	Grain	Husks	Straw
Acibenzolar-S-methyl		0.8	6.1	16.9–19.3						
Acibenzolar acid	Free	8.1	6.4–9.0	< 5	8.4	12.1	14.4	1.7	3.7	10.2
	Conj	56.2	61.4	11.5–20.1	15.1	11.4	7.8	2.0	11.3	37.9
	Bound				15.8	17.4	23.1	1.9	1.8	8.1
4-OH acibenzolar acid	Free			0.9–1.1						
	Conj			19–21.5						
3-methanesulfinyl benzoic acid	Free							0.6	3.7	5.3
	Conj							0.7	3.1	5.3
	Bound							17.5	1.1	2.5

The metabolism of acibenzolar-S-methyl in crops proceeds via hydrolysis, hydroxylation and subsequent conjugation with plant material. The metabolites of acibenzolar-S-methyl in crops is shown in Figure 3.

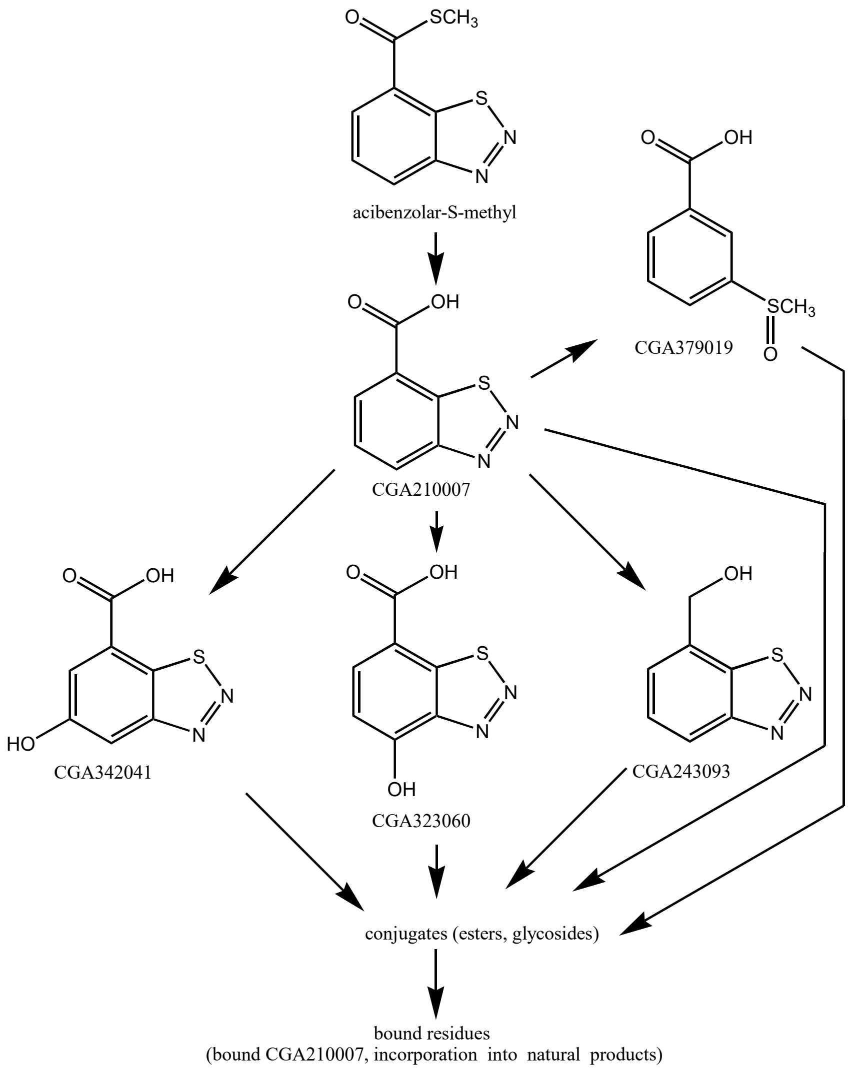


Figure 3 Plant metabolites of acibenzolar-S-methyl

Animal metabolism

Laboratory animal studies

Metabolism of acibenzolar-S-methyl in rats was evaluated by the WHO Core Assessment Group of the 2016 JMPR.

Lactating goats

Rümbeli (1995 0183) studied the metabolism of [^{14}C]acibenzolar-S-methyl in lactating goats (Gemsfarbige Gebirgsziege, 45–51 kg bw). Two goats were orally administered [^{14}C]acibenzolar-S-methyl at 10 mg/dose, twice daily for a period of four consecutive days. Feed consumption during the dosing period was 1.7 kg/d for one goat and 1.4 kg/d for the other, with animals offered 0.6 kg concentrate, 1 kg maize cubes and 0.1 kg hay per day. The doses were equivalent to 11.9 ppm in the diet (0.36 mg/kg bw). Milk production averaged 1.9 and 2.4 kg/d for the two goats. During the treatment period, milk, urine, and faeces were collected daily from both goats. Approximately 6 hours after the final dose, the goats were sacrificed and tissues were collected. Samples were analysed within 6 months of collection.

Most the ^{14}C residues were recovered in the excreta; 75.1% of the administered dose. Urine contained 63.5% of the administered dose while faeces contained 11.6%.

For tissues, ^{14}C residues were highest in kidney, (0.283 mg equiv/kg), followed by the liver (0.041 mg equiv/kg) (Table 18). In general, levels of radioactivity were lowest in fat (0.002–0.003 mg equiv/kg) and muscle (0.003 mg equiv/kg).

Table 18 Distribution of ^{14}C following administration of [^{14}C]acibenzolar-S-methyl for four days (Rümbeli 1995 0183)

	%AD	mg equiv/kg
Tissues		
Liver	0.047	0.041
Kidney	0.059	0.283
Perirenal fat	0.005	0.003
Omental fat	0.003	0.002
Leg muscle	0.019	0.003
Tenderloin	0.001	0.003
Milk	0.118	Mean 0.012 (range 0.009–0.086)
Excreta		
Faeces	11.6	
Urine	63.5	
Other		
GIT and contents	10.2	
Blood	0.096	0.032
Bile	0.003	0.128
Total	89.54	

Extraction with CH_3CN followed by $CH_3CN:H_2O$ (80:20 v/v) extracted 89.1% TRR for liver, 98.3% TRR for kidney, 83.6% TRR for muscle, and 93.7% TRR for fat + skin (extraction of the residue remaining after an initial hexane extraction) and 99.3% TRR for milk (Table 19). Selected extracts were also subjected to hydrolysis using 6 N HCl at 100 °C to investigate the presence of conjugates.

Parent acibenzolar-S-methyl was not detected in any tissue or in milk. The major component of the ^{14}C was acibenzolar acid (CGA210007) (37.2–89.8% TRR) together with its conjugates (2.3–22.1%TRR).

Table 19 Characterisation and identification of ^{14}C residues in solvent extracts from goat milk, kidney and liver and excreta (Rümbeli 1995 0183)

	milk	kidney	liver	muscle		Urine	Faeces
					+ 6 N HCl 100 °C 16 h		
TRR (mg equiv/kg)	0.012	0.283	0.041	0.003	0.003		
				%TRR			
Analysed solvent extract	99.3	98.3	93.1 [a]	86.3	86.3	100	98.3
acibenzolar-S-methyl	–	–	–	–		–	0.8
CGA210007	70.3	89.8	77.9	37.2	64.6	96.8	68.9
CGA324041	–	–	–	–	–	–	1.6
Glycine conj of CGA210007 (Met 2U)	22.1	2.3	2.6	4.4	4.4	–	–
Unidentified conj of CGA210007 (L3)			1.4				
Unidentified conj of CGA210007 (L5)			1.4				
unresolved	5	4.7	8.2	8.0	8.0	2.4	2.8
Origin	2.0	1.5	1.6	36.7	9.3	0.8	1.3
Unextracted	0.7	1.7	6.9	13.7	13.7	–	1.7

[a] Includes CH_3OH/H_2O extract which accounted for 4% TRR

The metabolites of acibenzolar-S-methyl in goats are shown in Figure 4. In goats acibenzolar-S-methyl is hydrolysed to acibenzolar acid (CGA210007) which is the major metabolite in excreta, milk and tissues. Acibenzolar acid (CGA210007) undergoes conjugation with glycine to [(benzo[1,2,3]thiadiazole-7-carbonyl)-amino]acetic acid (MET 2U) which was found in significant amounts in milk and in minor amounts in tissues. Acibenzolar acid (CGA210007) also undergoes conjugation with unidentified endocons present in liver and muscle which quantitatively released acibenzolar acid upon hydrolysis in 6 M HCl at 100 °C. Hydroxylation of acibenzolar acid gives 5-OH acibenzolar acid (CGA324041) which was eliminated via faeces.

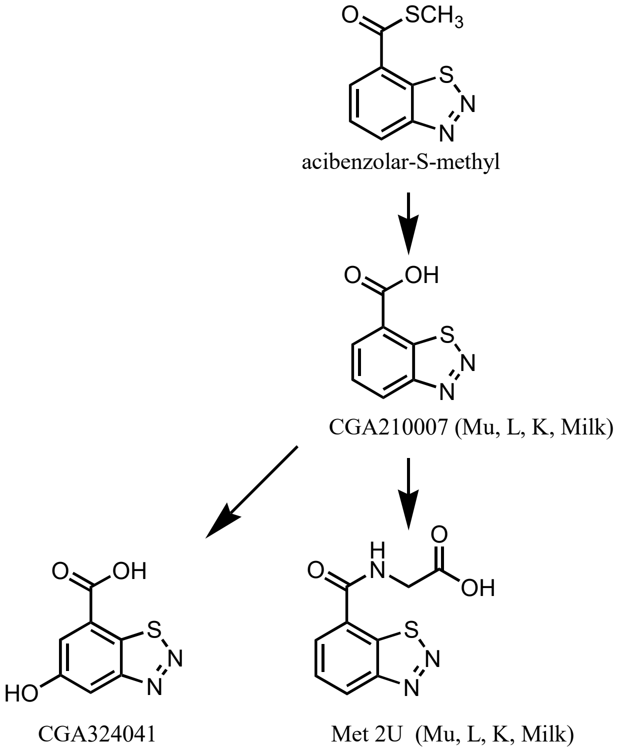


Figure 4 Metabolites of acibenzolar-S-methyl identified in tissues and milk of lactating goats (Mu = muscle, L = liver, K = kidney).

Laying hen

Rümbeli (1996 0349) studied the metabolism of acibenzolar-S-methyl in laying hens. Leghorn LSL blanches hens (22 wks old, 1.50–1.59 kg bw) were dosed orally *via* capsules, once a day for four consecutive days, with [^{14}C]acibenzolar-S-methyl at doses equivalent to 19.1 ppm in the diet (1.41 mg/kg bw). Feed consumption was 116 g/d. Laying efficiency during the dosing period was 100%. Egg samples were collected twice a day; excreta samples were also collected twice daily. Eggs were separated into egg whites and yolks. Hens were sacrificed 6 hours after the final dose, and samples of skin with subcutaneous fat and peritoneal fat, thigh and breast muscle, kidneys, liver, and gastrointestinal tract and contents were collected. Eggs, tissues, and excreta were extracted and analysed within six months of sampling. Total radioactivity was determined by liquid scintillation counting. Characterisation and identification of metabolites was by 2D-TLC, HPLC and high voltage electrophoresis.

Recovery of the administered dose was 91.0% after the dosing period based on the eggs, tissues, and excreta with the majority found in the excreta (87.4% AD). Gizzard (2.37% AD), eggs (0.003% AD) and edible tissues (muscle, liver, fat, and skin; 0.047–0.12% AD) accounted for relatively minor amounts of the dose (Table 20). Radioactivity in eggs increased with duration of dosing with concentrations in yolk higher than in white (at 4 days 0.006 mg equiv/kg in yolk and 0.001 mg equiv/kg in white). Mean TRR levels in edible tissues were 0.903 mg equiv/kg in kidney, 0.332 mg equiv/kg in liver, 0.013 mg equiv/kg in breast muscle, 0.013 mg equiv/kg in peritoneal fat, and 0.045 mg equiv/kg in skin plus subcutaneous fat.

Table 20 Recovery of administered dose in eggs, tissues, and excreta of laying hens following dosing for four consecutive days with [^{14}C]acibenzolar-S-methyl (Rümbeli 1996 0349)

Sample	% AD	Residue (mg equiv/kg)
Tissues		
Liver	0.124	0.332
Kidneys	0.095	0.903
Fat + skin	0.052	0.045 skin/sub cut 0.013 peritoneal
Lean meat	0.047	0.013 breast
Eggs	0.003	0.001 (white 0.001, yolk 0.002)
Excreta	87.45	
Other		
Cage wash/debris	0.88	
Gizzard	2.317	
Blood	0.048	0.138
Total	91.02	

Sequential extraction with CH_3CN followed by $CH_3CN:H_2O$ (80:20 v/v) extracted 92.0% TRR for liver, 92.7% TRR for muscle, and 93.7% TRR for fat + skin (extraction of the residue remaining after an initial hexane extraction) and 82.9% TRR for egg white. Free acibenzolar acid (CGA210007) was the major component of the ^{14}C residues in all tissues and eggs (50.0–77.7% TRR) and was also present in small amounts in conjugated forms (3.6–9.4% TRR) (Table 21). Parent compound was not detected in any tissue or in eggs.

Table 21 Characterisation and distribution of ^{14}C residues in eggs and tissues from laying hens dosed with [^{14}C]-acibenzolar-S-methyl (mean values) (Rümbeli 1996 0349)

	Egg white		Liver	Muscle	Skin and Fat
		+ hydrolysis			
TRR (mg equiv/kg)	0.001	0.001	0.332	0.013	0.038
			%TRR		
Extract	82.9		92.0	92.7	93.7
acibenzolar-S-methyl	–	–	–	–	–
CGA210007	50.0	68.1	77.2	73.2	76.8
CGA210007 (δ) conjugate of ornithine (EX1)	–	–	–	–	2.6
CGA210007 (δ) conjugate of α-benzoyl-ornithine (EX9a)	–	–	3.6	6.7	6.8
Unresolved/unidentified	19.0 (7)	4.7	5.1	8.7	8.0
Origin	14.0	10.1	6.1	4.1	2.6
Unextracted	17.1	17.1	8.0	7.3	3.2

For egg white the seven unidentified metabolites converted to CGA210007 on hydrolysis in 6 N HCl at 100 °C for 16 h.

The extraction of excreta with CH_3CN followed by $CH_3CN:H_2O$ was nearly quantitative at 96.4%. The metabolites identified in excreta were EX1 (17.8%), EX2 (12.6%), EX3 (2.1%), EX4 (4.8%), EX5 (6.2%), EX6 (4.0%), EX7 (2.9%), EX8a (1.0%), EX8b (11.0%), EX9a (6.7%), EX9b (2.8%), EX9c (0.9%), CGA210007 (18.7%) and acibenzolar-S-methyl (1.6%). EX1 was identified as the CGA210007 (δ) conjugate of ornithine, EX8a was identified as the bis-CGA 210007 (α,ε) conjugate of lysine, EX8b was identified as the bis-CGA210007 (α,δ) conjugate of ornithine and EX9a was identified as the CGA210007 (δ) conjugate of α-benzoyl-ornithine.

The metabolites of acibenzolar-S-methyl identified in hens are shown in Figure 5. Metabolism of acibenzolar-S-methyl proceeded via hydrolysis to form acibenzolar acid (CGA210007) as the major metabolite in excreta, eggs and tissues. Followed by conjugation of acibenzolar acid with ornithine and elimination via excreta, conjugation of acibenzolar acid with α-benzoyl-ornithine also occurred with this compound found in minor amounts in tissues and excreta. Several other conjugates were found in excreta; bis-conjugation of acibenzolar acid with ornithine and lysine and probably conjugation with other amino acids.

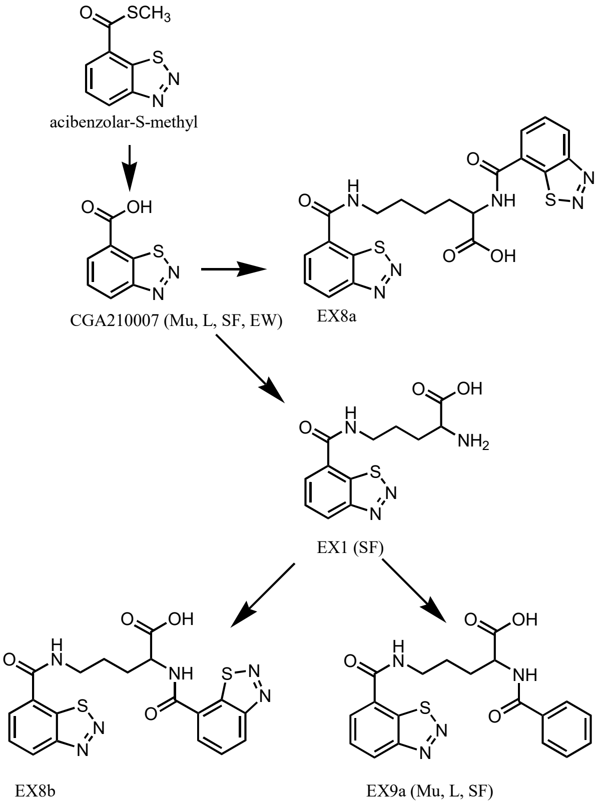


Figure 5 Metabolites of acibenzolar-S-methyl identified in tissues and eggs of laying hens (Mu = muscle, L = liver, SF = skin/fat, EW = egg white).

The nature of residues in goats and hens were shown to be similar and consisted mainly of acibenzolar acid (CGA210007) and its conjugates. In the goat, acibenzolar acid (CGA210007) underwent hydroxylation to give 5-OH acibenzolar acid (CGA324041) which was observed at a level of 1.6% AD in faeces.

Parent acibenzolar-S-methyl was only observed in excreta at levels of 1.6% and 20.6% AD in hens and goats, respectively. The metabolism of acibenzolar-S-methyl in livestock proceeds via hydrolysis, hydroxylation and subsequent conjugation. The metabolic profile in goats and hens was substantially equivalent to that seen in the rat. The proposed metabolites of acibenzolar-S-methyl in animals are shown in Figure 6.

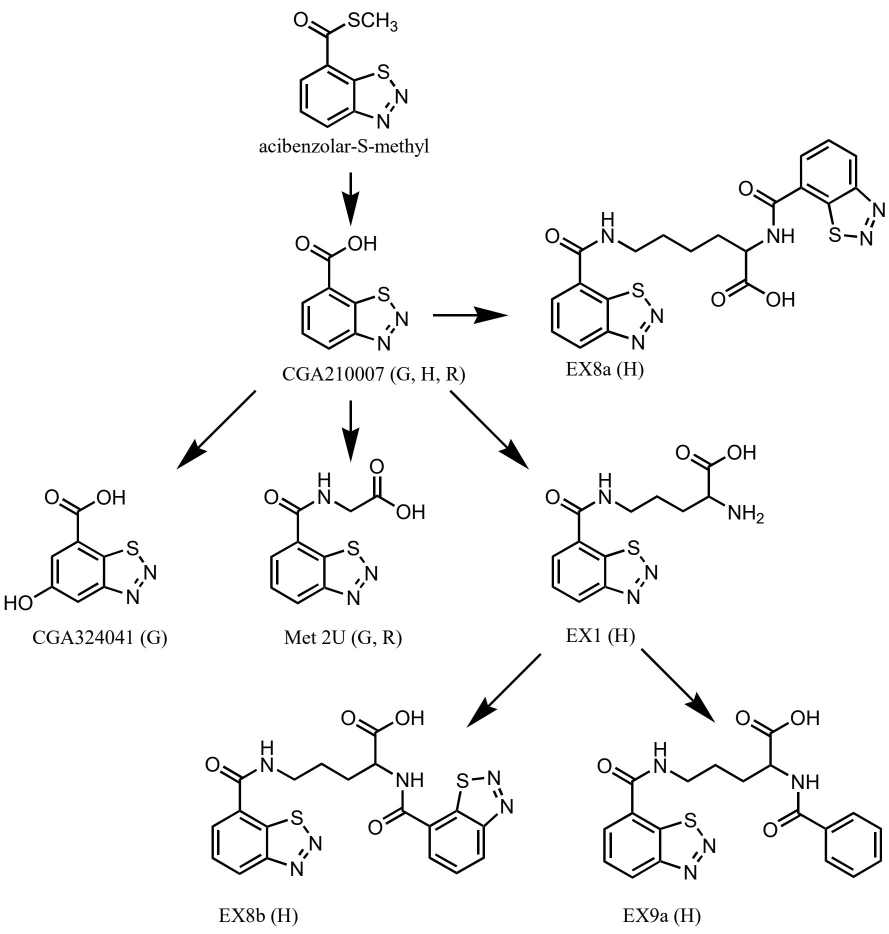


Figure 6 Metabolites of acibenzolar-S-methyl identified in livestock tissues, eggs and milk

ENVIRONMENTAL FATE

The FAO Manual on the Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue Levels in Food and Feed (2009) explains the data requirements for studies of environmental fate. The focus should be on those aspects that are most relevant to MRL setting. For acibenzolar-S-methyl, supervised residue trials data are available for numerous crops. Aerobic degradation in soil is relevant, as well as the normal requirements for hydrolysis, photolysis and rotational crop studies.

The Meeting received information on soil aerobic metabolism, hydrolysis and photolysis properties of acibenzolar-S-methyl. Studies were also received on the behaviour of [^{14}C]acibenzolar-S-methyl in a confined rotational crop situation.

Acibenzolar-S-methyl residues are not persistent in soils and it is unlikely that acibenzolar-S-methyl residues in soils resulting from recommended uses make a significant contribution to the residues in succeeding crops.

Route of degradation in soil

Aerobic degradation in soil

Several studies have investigated the aerobic degradation of [^{14}C]acibenzolar-S-methyl in soil.

Miner (2012 10086) studied the rate and route of degradation of [^{14}C]acibenzolar-S-methyl in four different soils: UK 18 Acres sandy loam, Gardner, ND (USA) sandy loam, Swiss Collombey sandy loam and Swiss Gartenacker silt loam (Table 22). [^{14}C]acibenzolar-S-methyl was applied at a nominal dose rate of 0.147 mg/kg dry weight soil, equivalent to a single field application rate of 112 g ai/ha (assuming an incorporation depth of 5 cm and a bulk density of 1.5 g/cm^3). The soils were incubated under aerobic conditions in the laboratory and maintained at a soil moisture of pF2 and dark conditions at 20 °C for up to 189 days. At various intervals after application, samples were extracted and analysed for parent compound, degradation products and unextracted residues. Extractions included 0.01 M $CaCl_2$ followed by CH_3CN/0.05 M sodium hexametaphosphate (SHMP) (80:20 v/v) twice, followed by a single extraction with CH_3CN/0.5 M formic acid (aqueous) (70:30 v/v) at 60 ºC for 2 hours. Any volatile radioactivity was continuously flushed from the vessels and volatile collection traps were radio-assayed.

The mean recovery from all soils was 98% AR. The amount of extracted ^{14}C in 18 Acres and Gardner soil samples decreased from 95–102%AR at 0 DAA to 19–21%AR by 189 DAA. In the Collombey and Gartenacker soils, the amount of extracted ^{14}C decreased, from 94–98% at 0 DAA to 24–41%AR at 125 DAA. At all sampling intervals and with all soils, the %AR extracted using $CaCl_2$ was $\leq$ 48%AR. In 18 Acres, Gardner, Collombey and Gartenacker soils, acibenzolar acid (CGA210007) was then major soil degradate and reached a maximum of 95% AR in the period 4 hours to 2 DAA and then declined.

Subsequent ring hydroxylation of acibenzolar acid (CGA210007) (ortho to the carboxyl group) led to the formation of the other major soil degradate, 6-OH acibenzolar acid (SYN546642). 6-OH Acibenzolar acid (SYN546642) first appeared at 1 or 2 DAA for 18 Acres, Collombey and Gartenacker soils, and at 14 DAA for Gardner soil. Levels of 6-OH acibenzolar acid (SYN546642) increased to a maximum of 40%AR at 92 DAA in the Gartenacker soil then declined to 24%AR at 125 DAA. For the Collombey soil 6-OH acibenzolar acid (SYN546642) was still increasing at 125 DAA. A slight decrease in %AR was observed for 18 Acres and Gardner soils from their maximum %AR when the collection time was extended to 189 DAA.

In addition, a discrete unknown metabolite was also observed in 18 Acres and Gardner soils, which did not exceed 2% of applied activity.

Mineralisation was significant. Carbon dioxide was a major degradation product in all soils, reaching a maximum 55, 62, 50 and 46% AR by the end of the incubation for 18 Acres, Gardner, Collombey and Gartenacker soils, respectively.

Unextracted residues (bound residues) increased slowly, reaching a maximum of 34, 34, and 27%AR at 58 DAA and 22%AR at 92 DAA, for 18 Acres, Gardner, Gartenacker and Collombey soils, respectively and thereafter remained at these levels or declined slightly. Organic matter fractionation (OMF) was conducted on bound residues > 10%AR for selected soils samples. Fractionation demonstrated ^{14}C was associated with predominantly the humin fraction and to a lesser extent with the humic and fulvic acid fractions.

Table 22 Properties of soils used in the aerobic degradation study of Miner (2012 10086)

Name	18 Acres	Gardner	Collombey	Gartenacker
Particle size (% w/w):				
Clay (< 2 µm)	13	15	3	11
Silt (50–2 µm)	32	15	40	55
Sand (2000–50 µm)	55	70	57	34
Texture (USDA)	Sandy Loam	Sandy Loam	Sandy Loam	Silt Loam
pH (water) 1:1 (soil to water weight ratio)	6.5	7.6	7.6	7.6
pH (water) 1:2.5 (soil to water weight ratio)	6.5	7.6	7.7	7.6
pH (0.01 M $CaCl_2$) 1:1	6.1	7.3	7.4	7.3
pH (0.01 M $CaCl_2$) 1:2.5	6.1	7.3	7.4	7.4
Organic matter [a] (%)	4.1	4.4	5.4	3.4
Organic carbon [a] (%)	2.4	2.6	3.1	2.0
CEC (meq/100 g soil)	15.8	17.4	10.8	8.6
Moisture at pF2.0 (0.1 bar, w/w %)	26.6	35.6	27.1	41.4

Name	18 Acres	Gardner	Collombey	Gartenacker
Particle size (% w/w):				
Moisture at pF2.5 (0.33 bar, w/w %)	21.1	21.5	24.9	27.0
Microbial Biomass by Fumigant Method (mg carbon/100 g soil)				
Prior to Initial	NA	NA	NA	25.64
Initial (start of study)	42.82	13.89	32.04	26.01
Final (end of study)	39.95	14.01	31.28	23.31

NA = not applicable

CEC = cation exchange capacity

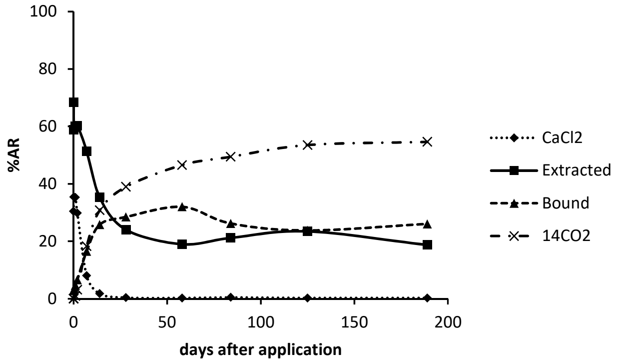
AEC = anion exchange capacity.

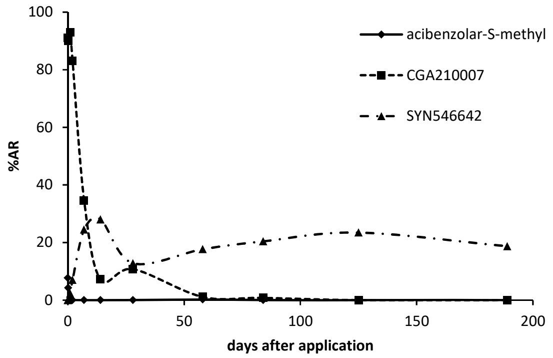
[a] Organic carbon (OC) % = organic matter (OM) %/1.724.

The total recoveries and distribution of radioactivity from each soil are shown in detail in Figure 7.

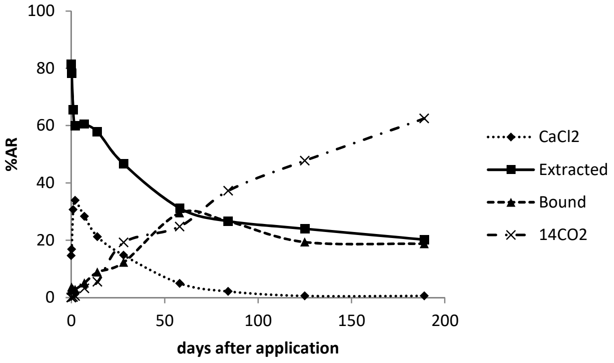
Acibenzolar-S-methyl was rapidly degraded in all four soils, with no parent present in any soil after 7 days. In all soils, hydrolysis of the parent ester was the predominant route of degradation yielding acibenzolar acid (CGA210007) (the primary degradate). Acibenzolar acid (CGA210007) was further oxidized to a hydroxylated phenyl product, 6-OH acibenzolar acid (SYN546642), which was also a significant degradate in all soils (Figure 7). In all soils, mineralization was a major route of degradation; CO_2 accounted for 46–62%AR, while OMF of bound residues demonstrated up to 18%AR associated with the humin fraction, and up to 7 and 11%AR with humic and fulvic acid fractions, respectively.

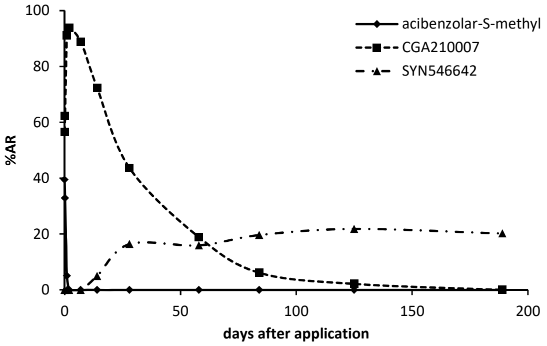
18 Acres sandy loam



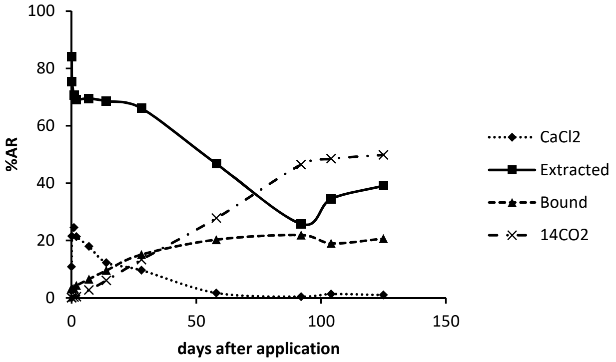


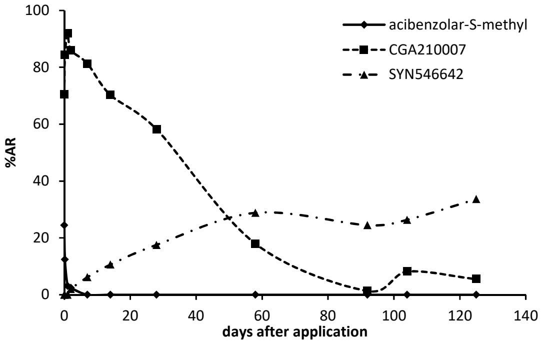
Gardner sandy loam





Collombey loamy sand





Gartenacker silty loam

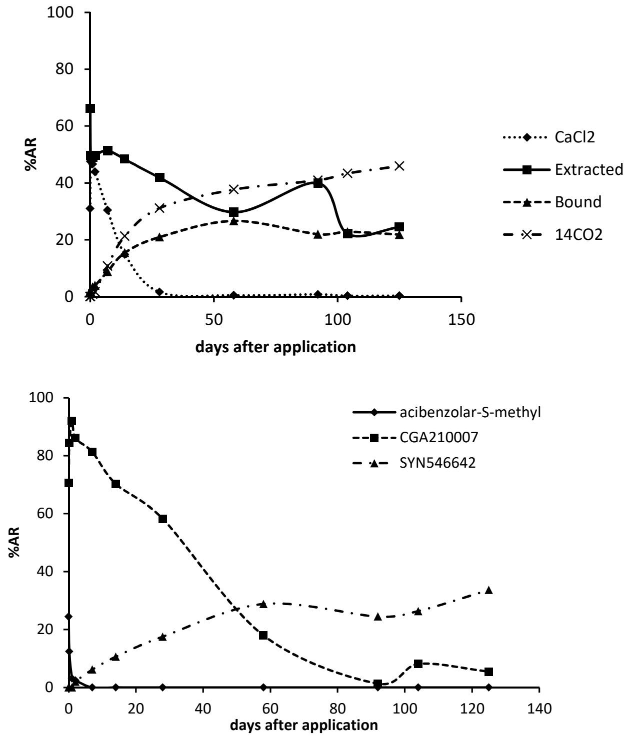


Figure 7 Temporal evolution of ^{14}C residues in four soils

The degradation rate (DT_{50}) of the parent was determined using non-linear regression and a single first order kinetic model. SFO kinetics describes the degradation of acibenzolar-S-methyl well with a Chi-square (χ^2) value less than 14 in all cases and r^2 values were > 0.86. The results are presented in Table 23.

Table 23 DT_{50} and DT_{90} values for acibenzolar-S-methyl in various soils (Miner 2012 10086)

Soil	Calculated Kinetic Values (SFO) for Parent with Associated Statistics						
	Number of data points used (Sampling Interval Range DAA)	DT_{50} (days)	DT_{90} (days)	K	χ^2	r^2	Prob > t
18 Acres	6 (0–1 days)	0.19	0.63	0.46	2.65	0.863	0.4814
Gardner	8 (0–2 days)	0.38	1.3	0.45	7.68	0.981	0.00022
Collombey	8 (0–2 days)	0.19	0.64	0.47	13.65	0.976	0.0025
Gartenacker	6 (0–1 days)	0.073	0.24	0.20	8.58	0.986	0.0017

Note: Values from harsh extracts were excluded in parent half-life calculations.

SFO: single first order kinetics (non-linear method) calculated using CAKE version 1.3 (Release) running on R version 2.12.2 (25/02/2011).

DT_{50}: Calculated degradation half-life of parent.

K: rate constant.

χ^2: chi-square statistical value.

r^2: linear regression coefficient.

Prob > t: statistical probability value related to a statistical t-test.

The SFO (non-linear) half-life range for acibenzolar-S-methyl was approximately 0.1–0.4 days.

Rate of aerobic degradation in soil

Kind (2011 10062) presented the kinetic evaluation of the degradation rate of acibenzolar-S-methyl and its metabolites in aerobic laboratory studies with twelve soils; the data from Miner (2012 10086) were refit by Mackenzie (2012 10099). Kinetic models were applied in a stepwise approach as proposed by FOCUS (2006). Model fits were evaluated with χ^2-tests and a visual assessment. The estimation of model parameters was evaluated by a *t*-test (FOCUS, 2006). Some soil incubations were conducted at reference conditions of 20 °C, moisture at pF2. All other trials were normalised to these reference conditions.

The analysis resulted in an estimated DT$_{50}$ for acibenzolar-S-methyl of 0.131 days (geometric mean, n = 8, maximum: 0.441 days). An overview of the results is given in Table 24 below.

Table 24 DT$_{50}$ and DT$_{90}$ values obtained by modelling of the aerobic degradation of acibenzolar-S-methyl in soil

Soil	Kinetics showing best fit	χ^2	DT$_{50}$ (days)	DT$_{90}$ (days)	Normalisation factor (fmoist × ftemp)	Normalised DT$_{50}$ (days)	Normalised DT$_{90}$ (days)	Reference
Loam (Les Evouettes)	SFO	8.52	0.190	0.631	0.818 × 1[a]	0.155	0.516	Kind 2011
Loamy sand (Collombey)	SFO	1.58	0.204	0.679	0.768 × 1[a]	0.157	0.521	Kind 2011
Sand (Speyer 2.1)	SFO	0.56	0.516	1.72	0.855 × 1[a]	0.441	1.471	Kind 2011
Sandy loam (Weide)	SFO	0.8	0.368	1.22	0.673 × 1[a]	0.248	0.821	Kind 2011
Silty loam (Gartenacker 1)	SFO	1.08	0.263	0.874	0.699 × 1[a]	0.184	0.611	Kind 2011
Silty loam (Gartenacker 2)	SFO	2.97	0.544	1.81	0.431 × 1[a]	0.234	0.780	Kind 2011
Silty loam (Gartenacker 3)	SFO	5.11	0.981	3.26	0.699 × 0.388	0.266	0.883	Kind 2011
Silty loam (Gartenacker 4)	SFO	3.93	0.260	0.862	0.699 × 1[a]	0.182	0.603	Kind 2011
Silty loam (Gartenacker)	SFO	1.49	0.031	0.102	[b]	0.031	0.102	McKenzie 2012
Geometric mean (Gartenacker n = 5)						0.145	0.482	
Sandy loam (18 Acres)	SFO	0.31	0.037	0.122	[b]	0.037	0.122	McKenzie 2012
Sandy loam (Gardner)	SFO	5.92	0.109	0.361	[b]	0.109	0.361	McKenzie 2012
Sandy loam (Collombey)	SFO	6.23	0.057	0.189	[b]	0.057	0.189	McKenzie 2012
Overall geometric mean (n = 8)						0.131	0.436	

[a] Studies were conducted at 20 °C

[b] Studies were conducted at 20 °C and moisture pF2

The degradate acibenzolar acid (CGA210007) was found in all studies and trials enabling an estimation of soil DT$_{50}$ to be undertaken. The analysis resulted in a DT$_{50}$ for acibenzolar acid (CGA210007) of 19.0 days (geometric mean, n = 8, maximum: 90.6 days). An overview of the results is given in Table 25 below.

Table 25 DT_{50} and DT_{90} values obtained by modelling of the aerobic degradation of acibenzolar acid (CGA210007) in soil

Soil	Kinetics showing best fit	χ^2	DT_{50} (days)	DT_{90} (days)	Normalisation factor (fmoist × ftemp)	Normalised DT_{50} (days)	Normalised DT_{90} (days)	Reference
Loam (Les Evouettes)	SFO	7.01	20.6	68.5	0.818 × 1 [a]	16.9	56.0	Kind 2011
Loamy sand (Collombey)	SFO	8.94	20.3	67.3	0.768 × 1 [a]	15.6	51.7	Kind 2011
Sand (Speyer 2.1)	SFO	1.69	106	353	0.855 × 1 [a]	90.6	302	Kind 2011
Sandy loam (Weide)	SFO	4.24	19.5	64.8	0.673 × 1 [a]	13.1	43.6	Kind 2011
Silty loam (Gartenacker 1)	SFO	7.34	24.1	80.2	0.699 × 1 [a]	16.8	56.1	Kind 2011
Silty loam (Gartenacker 2)	SFO	9.46	108	359	0.431 × 1 [a]	46.5	155	Kind 2011
Silty loam (Gartenacker 3)	SFO	7.57	76.3	253	0.699 × 0.388	20.7	68.5	Kind 2011
Silty loam (Gartenacker 4)	SFO	11.7	12.1	40.2	0.699 × 1 [a]	8.46	28.1	Kind 2011
Silty loam (Gartenacker)	SFO	6.78	9.43	31.3	[b]	9.43	31.3	McKenzie 2012
Geometric mean (Gartenacker, n = 5)						16.7	55.4	
Sandy loam (18 Acres)	SFO	4.15	4.09	13.6	[b]	4.09	13.6	McKenzie 2012
Sandy loam (Gardner)	SFO	6.11	27.2	90.5	[b]	27.2	90.5	McKenzie 2012
Sandy loam (Collombey)	SFO	7.84	29.0	96.3	[b]	29.0	96.3	McKenzie 2012
Overall geometric mean (n = 8)						19.0	63.2	

[a] Studies were conducted at 20 °C

[b] Studies were conducted at 20 °C and moisture pF2

The degradate 6-OH acibenzolar acid (SYN546642) was only identified in one study (McKenzie 2012). An acceptable fit could not be determined in one of the soils, nevertheless all the results are presented in Table 26 below.

Table 26 DT_{50} and DT_{90} values obtained by modelling of the aerobic degradation of 6-OH acibenzolar acid (SYN546642) in soil

Soil	Kinetics showing best fit	χ^2	DT_{50} (days)	DT_{90} (days)	Normalisation factor (fmoist × ftemp)	Normalised DT_{50} (days)	Normalised DT_{90} (days)	Reference
Sandy loam (18 Acres)	SFO	17.4	259	862	[b]	259	862	McKenzie 2012
Sandy loam (Gardner)	SFO [a]	13.0	1000	> 1000	[b]	1000	> 1000	McKenzie 2012
Sandy loam (Collombey)	SFO	13.1	378	> 1000	[b]	378	> 1000	McKenzie 2012
Silty loam l (Gartenacker)	SFO	17.9	130	431	[b]	130	431	McKenzie 2012

[a] Fit visually and statistically not acceptable, conservative DT_{50} of 1000 days assumed

[b] Studies were conducted at 20 °C and moisture pF2

The rate and route of degradation of acibenzolar-S-methyl was also studied in three soils from Brazil (Brandelli 1999 10028). The test substance, [^{14}C]acibenzolar-S-methyl was applied to the soils at a concentration of 0.45 kg ai/ha. The temperature was 20 °C and the soils held at 40% of the maximum water-holding capacity (MWHC).

The recovered radioactivity ranged from 86.2 to 100.1%AR of the applied dose for the three soils, Typic hapludox, Cumulic humaquept and Rhodic hapludox, respectively. After 120 days incubation, the evolution of $^{14}CO_2$ reached 31.8%AR in Typic hapludox, 26.4%AR in Cumulic humaquept and 29.3%AR in Rhodic hapludox soils. No other volatile components were found.

Over the 120 days incubation, the extracted radioactivity decreased steadily from 89.7 to 18.5%AR, 86.8 to 29.5%AR and 89.6 to 18.2%AR in Typic hapludox, Cumulic humaquept and Rhodic. hapludox soils, respectively.

The unextracted radioactivity increased up to 56 DAA in Typic hapludox soil, reaching 47.3%AR, and decreased thereafter to 38.6%AR at 120 DAA. In Cumulic humaquept soil, the unextracted radioactivity increased over the 120-day study duration, reaching 41.9%AR by 120 DAA. In Rhodic hapludox soil, the unextracted radioactivity increased up to 70 DAA, reaching 54.7%AR, and decreased thereafter to 43.7%AR after 120 days.

The decrease in concentration of parent acibenzolar-S-methyl over time was fitted to a first-order kinetic model. The calculated first-order parameters for acibenzolar-S-methyl in these soils are presented in Table 27 below.

Table 27 DT_{50} and DT_{90} values obtained by modelling of acibenzolar-S-methyl degradation in three Brazilian soils

Soil	Calculated kinetic values (SFO) for acibenzolar-S-methyl		
	DT_{50} (days)	DT_{90} (days)	K
Typic hapludox	2.1	7.0	0.326349
Cumulic humaquept	1.7	5.6	0.411892
Rhodic hapludox	1.6	5.2	0.439401

K: rate constant.

Aqueous hydrolysis

Adam (1994 0050) studied the hydrolytic stability of [^{14}C]acibenzolar-S-methyl at 24, 50 and 75 °C for 30 days in dark, sterile, aqueous buffered solutions at pH 1, 5, 7, 9 and 13. The rate of hydrolysis increased with temperature and, with exception of pH 1, also with pH (Table 28). At pH 5, parent acibenzolar-S-methyl exhibited the greatest stability against hydrolysis. The only hydrolysis product observed at all pH conditions was acibenzolar acid (CGA210007), which itself was stable against further hydrolysis.

Table 28 Half-lives of hydrolysis for acibenzolar-S-methyl

pH	Temperature (°C)			
	20 [a]	24	50	75
1	58 days	45 days	19 days	7 days
5	3.8 years	> 99 days	44 days	6 days
7	162 days	76 days	44 hours	4 hours
9	19 hours	10 hours	47 minutes	6 minutes
13	< 5 min	< 5 min	ND	ND

[a] Calculated based on Arrhenius parameters

Acibenzolar-S-methyl undergoes rapid hydrolysis under basic conditions (pH 9 and 13) at moderate temperatures (ca. 20–24 °C).

Aqueous hydrolysis is expected to contribute to the degradation of acibenzolar-S-methyl in the environment but only at basic pHs.

Soil photolysis

A laboratory study on the photolysis or photodegradation of acibenzolar-S-methyl in soil was conducted (Adam 1995 0226). The photolysis of [^{14}C]acibenzolar-S-methyl in wet and dry soil under light and dark test conditions was studied.

Light was shown to enhance the slow degradation of acibenzolar-S-methyl on dry soil. Light may also significantly enhance degradation of the main product (acibenzolar acid CGA210007) which is rapidly formed on wet soil. Under the conditions employed, bound residues were found to be the main final degradation products.

[^{14}C]acibenzolar-S-methyl (> 96% purity) was applied in acetonitrile to 2 mm thick soil layers (silt loam from Les Evouettes: 54.3% silt, 31.8% sand, 13.9% clay, 2.1% OC, pH 7.3, 58.3% FC) at 75% FC (wet soil, 22.2 ppm) or air dried for 1 week (dry soil, 19.8 ppm). Soil layers were kept in the dark or exposed to Xenon arc lamp (Hanau Suntest unit) for 30 days, with intermittent dark/light periods of 12 hours each (spectral cut-off at 290 nm; 35 W/m^2 at 300–400 nm and soil layer level), at 24 °C. Volatiles were trapped in ethylene glycol, sulphuric acid and NaOH. Duplicate samples of wet soil were removed at 0, 1, 2, 3, 4, 168, 360 and 720 hours after treatment and single samples of dry soil at 0, 2, 4, 168, 360 and 720 hours after treatment. Samples were extracted with acetone/water (80:20, v/v) and extracts were analysed by TLC and HPLC. Bound residues were determined by combustion.

The results obtained were related to days Florida summer sunlight equivalents (FSSE), i.e., summer sunlight at latitude 30°N and additionally to summer sunlight at latitude 40°N.

In wet soil, acibenzolar-S-methyl was rapidly hydrolysed in both dark and light conditions to acibenzolar acid (CGA210007) which was more rapidly degraded in the light with bound residues also greater in the irradiated samples (up to 61%AR) (Table 29). In dry soil in the dark, acibenzolar-S-methyl was slowly hydrolysed (80% remaining after 30 days) to acibenzolar acid (CGA210007). Degradation was enhanced in light (46.8% remaining after 30 days), where only small amounts of acibenzolar acid (CGA210007) (4.7%) were detected and considerable bound residues were formed (up to 20.2%).

Table 29 Photodegradation of acibenzolar-S-methyl in wet and dry soil

Condition		% of applied radioactivity (mean of 2 replicates for wet soil)									
HAT		Light					Dark				
		Recovered	Acibenzolar-S-methyl	CGA210007	Bound	CO_2	Recovered	Acibenzolar-S-methyl	CGA210007	Bound	CO_2
Wet	0	103.5	92.2	9.5	1.9	–	90.5	66.6	22.1	1.8	–
	4	92.4	11.5	68.6	12.4	–	99.2	10.2	85.4	3.6	–
	720	75.4	–	4.4	61.0	3.6	92.6	–	55.2	32.8	2.0
Dry	0	97.4	96.3	–	1.1	–	97.4	96.3	–	1.1	–
	4	93.5	76.4	5.1	12.0	–	95.5	85.2	7.3	2.9	–
	720	72.8	46.8	4.7	20.2	1.1	98.1	80.0	10.4	7.8	–

N.B. three unknown photoproducts were detected, each < 4%

HAT = Hours after treatment

Light may enhance the slow degradation of acibenzolar-S-methyl on dry soil. It may also significantly enhance degradation of its main degradate (CGA210007) which is rapidly formed in wet soil.

Confined rotational crop studies

A confined rotational crop study was conducted on a clay soil (6.6% sand, 35.7% silt, 57.7% clay; pH 6.6; 3.0% organic matter; 42.2 meq/100 g CEC) treated with [^{14}C]acibenzolar-S-methyl at 50 g ai/ha

(Walser 1995b 0148). Radish (var Rex), lettuce (var Lattuga Iceberg), winter wheat (var Sardona) and corn (var Deca 250) were sown into the soil at 30, 113, 141 and 337 days after the soil application (DAA). The treated boxes were maintained in a screened enclosure. Lettuce was sampled at 50% maturity and at full maturity (61 and 82 days), radish was sampled at 180 days, wheat samples were taken at 180, 370 and 414 DAA and corn was sampled at 50% and full maturity (404 and 498 DAA).

The uptake of radioactivity in rotational crops following soil application of [^{14}C]acibenzolar-S-methyl was very low for lettuce, winter wheat, corn and radish: all residue levels in the crops were found to be equal or below 0.001 mg equiv/kg. No attempts were made to extract plant samples because of the negligible residues.

In the four rotational crop plots a decrease of the radioactivity levels in the 0–5 cm soil layer was noticed from lettuce planting time (30 DAA) to corn harvest (498 DAA), namely from 0.040 mg equiv/kg to 0.014 mg equiv/kg. Unextracted radioactivity in soil samples for the same time intervals varied from 0.035 mg equiv/kg (86.4% TRR) to 0.013 mg equiv/kg (90.9% TRR). The ^{14}C-levels in the underlying soil layers at harvest of corn were low, ranging from 0.008 mg equiv/kg for the 5–10 cm soil layer to 0.006 mg equiv/kg for the 10–20 cm soil layer and 0.004 mg equiv/kg for the 20–30 cm layer.

Kennedy (1998, 0481) conducted a confined rotational crop study on a sandy loam soil (60% sand, 29% silt, 11% clay; pH 7.7; 0.7% organic matter; 6.9 meq/100 g CEC) treated with [^{14}C]acibenzolar-S-methyl at 421 g ai/ha. Radish (var Cherry Belle), winter wheat (var Yecora Rojo) and mustard (var Florida Broadleaf) were sown into the soil at 30, 60 and 210 days after the soil application (DAA). The treated boxes were maintained in a screened enclosure.

TRR in crops planted 30 days after bare soil treatment were low, ranging from 0.013 to 0.107 mg equiv/kg. TRRs in crops planted 60 days after bare soil treatment were similar ranging from 0.013 to 0.157 mg equiv/kg. TRRs in crops planted 210 days after bare soil treatment were lower, ranging from 0.004 to 0.026 mg equiv/kg.

Soil samples taken post application contained radioactivity levels of 0.171, 0.203 and 0.092 mg equiv/kg for the containers used for plantings at 30, 60 and 210 days after application, respectively. Soil residues generally decreased over the duration of the study, with corresponding soil TRR values of 0.109, 0.079 and 0.080 mg equiv/kg at the time of planting and 0.031, 0.044 and 0.133 mg equiv/kg at the time of cereal harvest.

Extracted residues in all harvested crops ranged from 9.6% TRR (mature wheat grain, 60 days PBI) to 82.2% TRR (25% maturity wheat forage, 210 days PBI). After conventional extraction, selected samples were treated with cellulase plus NaOH to investigate conjugation. Metabolites in these extracts were identified by co-chromatography with known reference compounds. The results showed that the metabolism of acibenzolar-S-methyl in rotated crops was similar for all crop types and proceeded by hydrolysis of the S-methyl ester leading to formation of the major metabolite, acibenzolar acid (CGA210007) which existed as polar conjugates that could be cleaved by treatment with cellulase plus NaOH. Subsequent hydroxylation of the phenyl ring gave metabolites 5-OH acibenzolar acid (CGA324041) and 4-OH acibenzolar acid (CGA323060). Comparison with primary metabolism studies shows that the pathway in rotational crops is consistent with that in primary crops.

The characterisation of extracted soil samples showed that the major degradate observed at the 30, 60 and 210 day PBI planting occasion was acibenzolar acid (CGA210007) with minor amounts of acibenzolar-S-methyl and 6-OH acibenzolar acid (SYN546642) (Table 30). At the cereal harvest interval for the 30, 60 and 210 day PBI containers, the extracted soil samples showed that the major degradates were acibenzolar acid (CGA210007) and 6-OH acibenzolar acid (SYN546642). The major soil metabolite 6-OH acibenzolar acid (SYN546642) was not found in any of the crop samples which indicates that it is not taken up by rotational crops.

Table 30 Characterisation and identification of ^{14}C in rotational crops following application of [^{14}C]acibenzolar-S-methyl to soil.

Metabolite fraction	Mustard leaves	Radish leaves	Radish roots	25% mature wheat	50% mature wheat	Mature wheat straw	Mature wheat grain
30 day PBI							
TRR (mg equiv/kg)	0.027	0.021	0.013	0.107	0.044	0.059	0.029
%TRR							
A-1	28.3	17.4	32.1	9.6	10.2	9.5	5.8
A-2				8.6	11.2	12.0	3.4
B	5.7	1.3	3.4	3.8			
C	4.5	5.2	2.4	13.8	10.5	5.9	1.4
CGA324041/CGA323060	2.3	7.0	2.1	7.9	8.7	4.3	3.3
E	2.2		0.9	4.1	4.4	8.9	1.8
F	–	–	–	4.7	4.0	–	1.5
CGA210007	4.8	1.3	1.7	6.8	3.5	1.7	1.6
H	–	–	–	–	–	–	–
I	–	1.4	–	–	–	–	8.2
J	–	–	–	–	–	–	
acibenzolar-S-methyl	0.3	0.4	1.9	1.0	3.1	2.9	3.5
Unresolved	2.4	4.8	1.9	1.0	3.1	2.9	3.5
60 day PBI							
TRR (mg equiv/kg)	0.040	0.028	0.013	0.157	0.051	0.083	0.032
%TRR							
A-1	20.2	10.7	28.7	9.5	21.5	5.1	2.9
A-2	12.0	9.5	4.6	7.0	–	6.0	2.3
B	–	1.9		3.9	2.7	12.9	
C	7.6	8.0	2.3	14.8	11.5	7.0	0.7
CGA324041/CGA323060	–		4.2	15.9	10.2	3.9	0.8
E	3.3		1.7	1.4	–	3.1	0.4
F	–	–	–	5.6	3.5	–	–
CGA210007	4.0	1.2	1.9	10.5	2.9	1.5	0.4
H	1.3	0.9	0.9	–	–	–	–
I	2.3	2.8	2.6	–	–	–	0.5
J	–	–	–	–	1.0	–	
acibenzolar-S-methyl	0.3	0.7	0.2	0.1	0.2	1.5	1.0
Unresolved	4.8	6.2	2.7	7.4	2.1	1.5	1.0
210 day PBI							
TRR (mg equiv/kg)	0.013	0.007	0.004	0.026	0.021	0.021	0.008
%TRR							
A-1	54.1			18.2	30.3	8.1	
A-2				16.3		5.2	
B	1.9				2.4	7.1	
C	3.4			6.1	12.3	3.7	
CGA324041/CGA323060	5.3			14.3	11.6	2.0	
E	1.7			7.1	9.6	3.6	
F	–			5.8	4.4	–	
CGA210007	3.1			6.7	5.7	2.2	
H	–			–	–	0.6	
I	–			–	–	–	
J	–			–	–	–	
acibenzolar-S-methyl	0.1			0.3	0.3	0.2	
Unresolved	3.3			5.6	2.9	3.5	

The results show that the metabolism of acibenzolar-S-methyl in rotated crops was similar for all crop types and proceeded by hydrolysis of the S-methyl ester to give the major metabolite acibenzolar acid (CGA210007). Subsequent hydroxylation of the phenyl ring gave metabolites 5-OH acibenzolar acid (CGA324041) and 4-OH acibenzolar acid (CGA323060). Comparison with primary crop metabolism studies shows that the pathway in rotational crops is consistent with that in primary crops. The major soil metabolite 6-OH acibenzolar acid (SYN546642) was not found in any of the crop samples.

Environmental fate summary

Acibenzolar-S-methyl is rapidly degraded in soil under aerobic conditions with half-lives ranging from 0.03 to 0.44 days. Two major degradates that exceeded 10% of applied radioactivity and were identified, acibenzolar acid (CGA210007) and 6-OH acibenzolar acid (SYN546642). Acibenzolar acid (CGA210007) reached maximum proportions of 92–95% of applied radioactivity at one to two days after application, thereafter declining with an average half-life of 19 days (range 4.1–90.6 days). 6-OH Acibenzolar acid (SYN546642) increased as a proportion of the applied radioactivity with time reaching a maximum of 22–39% at 14 to 125 days after application.

Acibenzolar-S-methyl is stable in sterile aqueous solutions at pH 5 and 7 but rapidly hydrolysed to acibenzolar acid (CGA210007) at pH 9 and above. Once formed acibenzolar acid (CGA210007) is stable to hydrolysis.

Only very low levels of acibenzolar-S-methyl residues are taken up by plants from soil following applications to soil. A confined rotational crop study conducted with radish, lettuce, wheat and corn where soil was treated at 50 g ai/ha with planting at various intervals after application resulted in TRRs that were ≤ 0.001 mg equiv/kg at harvest 180 to 498 days after application. Residues are not anticipated in follow crops. An additional study where acibenzolar-S-methyl was applied to soil at an exaggerated rate (412 g ai/ha) enabled the degradation and metabolism in follow crops to be examined. The degradation/metabolism of acibenzolar-S-methyl was similar for the crop studied (radish, wheat and mustard) and proceeded by hydrolysis of the S-methyl ester to give the major metabolite acibenzolar acid (CGA210007). Subsequent hydroxylation of the phenyl ring gave metabolites 5-OH acibenzolar acid (CGA324041) and 4-OH acibenzolar acid (CGA323060). The major soil metabolite 6-OH acibenzolar acid (SYN546642) was not found in any of the crop samples.

METHODS OF RESIDUE ANALYSIS

Analytical methods

The Meeting received descriptions and validation data for analytical methods for residues of acibenzolar-S-methyl and metabolites in animal and plant matrices. The methods are suitable for analysis of acibenzolar-S-methyl and metabolites in plant and animal matrices.

Principle of the method

Most of the methods developed to quantify residues of acibenzolar-S-methyl, acibenzolar acid (CGA210007) and metabolites hydrolysable to acibenzolar acid (CGA210007) in plant and animal commodities. These analytes are quantified in acibenzolar-S-methyl equivalents. The methods all involve homogenisation and base hydrolysis (1 N NaOH, typically 60–65 °C) followed by extraction of the hydrolysed samples with an organic/aqueous solvent mixture, typically CH_3OH. The main differences between methods involve clean-up conditions, instrumentation for quantification (HPLC-UV, HPLC-ECD, LC-MS/MS), and scale. In addition to the methods summarized for quantification of acibenzolar-S-methyl residues in crops, a method is described for the determination of 4-OH acibenzolar acid (CGA303060) in lettuce and spinach. Acibenzolar-S-methyl and acibenzolar acid (CGA210007) were used as reference materials for fortification and method validation.

Residue analytical method REM172.02 (Formica 1993 0038) and modifications: Residue Analytical Method REM172.11 (Formica 1995a 0175, 1996a 0297, Walser 1998a 0550); Residue Analytical Method AG-671/AG-671A (Hayworth 1998a 0464, 1998b 0486, Hughes 1998 0485, Grunenwald 1998 78570)

In summary, crop samples are homogenised in water and hydrolysed with 1 N NaOH at 60 °C to convert acibenzolar-S-methyl and conjugates of acibenzolar acid (CGA210007) to acibenzolar acid. After cooling, samples are extracted with methanol and $CaCl_2$. The sample solution is filtered and subjected to clean-up/partition with water, saturated NaCl solution and 1 N HCl. The analyte is further extracted (2×) with a mixture of hexane and *tert*-butyl methyl ether (solvent mixture A; 7:3 v/v). The organic phase is then filtered and subjected to additional clean-up partitioning (2×) with 1 N NaOH, phosphoric acid (85%) and solvent mixture A. The combined organic phases are reduced to dryness and subjected to final clean-up by silica cartridge prior to final determination by HPLC (column 1: anion exchange; column 2: C_{18}, 5 µm particle size) coupled with UV detection (235 nm). A calibration curve is constructed using solutions containing acibenzolar acid (CGA210007). For calculation of acibenzolar acid (CGA210007) residues as acibenzolar-S-methyl equivalents a molecular weight conversion factor of 1.17 is used.

Recovery and repeatability data for the determination of acibenzolar-S-methyl residues in crops are presented in Table 31. The average recoveries values for acibenzolar-S-methyl ranged from 75 to 107%. The validated LOQ was 0.02 mg/kg for cereal grain and 0.05 mg/kg for cereal plant, cereal straw and tobacco leaves. The %RSD for acibenzolar-S-methyl at different fortification levels in different matrices ranged from 3.3–17.5%. No interfering peaks were detected in the samples analysed. The linearity of the detector response covered a working range of 0.2 to 4 ng acibenzolar acid (CGA210007) (injected) for cereals and 0.002 to 0.16 µg/mL ($n = 4$; $r^2 > 0.99$) for tobacco leaves.

Table 31 Recovery data obtained during validation of REM172 and AG-671, fortification was with acibenzolar-S-methyl (REM172.02 Formica 1993 0038, Enriquez 1998 0461; REM172.11 Formica 1996a 0297, Walser 1998a 0550)

Matrix	Fortification level (mg/kg)	Average recovery (%)	Range of recoveries (%)	%RSD	Number of recoveries (n)
REM172.02					
Wheat grain	0.02	95	90–98	3.7	5
	0.04	91	86–98	5.1	5
	0.2	88	84–94	4.1	5
Wheat plant	0.05	92	75–107	17.5	3
	0.1	91	80–97	10.2	3
	0.5	94	90–98	4.3	3
Wheat straw	0.05	89	78–98	8.2	5
	0.1	84	76–93	7.8	5
	0.5	85	80–93	6.7	5
Barley grain	0.02	97	97–97	0.0	2
	0.04	93	92–93	0.8	2
	0.2	90	89–91	1.6	2
Barley plant	0.05	100	100	–	1
	0.5	93	93	–	1
Barley straw	0.05	95	95	–	1
	0.1	91	91	–	1
	0.5	90	90	–	1
Tobacco leaves (untreated, dried)	0.05	89	80–94	8.8	3
	0.1	98	95–101	3.3	3
	0.15	87	87	–	1
	0.75	80	80	–	1
REM172.11					
Banana peel	0.02	85	78–96	11.1	3
	0.2	92	89–94	2.7	3
Banana pulp	0.02	91	76–98	8.1	8
	0.2	94	82–102	7.6	8
Tomato	0.02	99	92–102	5.9	3
	0.2	89	88–89	0.7	3
Cucumber	0.02	99	96–104	4.7	3
	0.2	91	90–93	1.7	3
Mango	0.02	84	81–92	5.9	5
	0.2	87	78–95	7.6	5
AG-671/AG-671A					
Tobacco leaves (green leaf)	0.02	82	70–106	17.3	5
	0.10	81	74–92	12.2	3

Matrix	Fortification level (mg/kg)	Average recovery (%)	Range of recoveries (%)	%RSD	Number of recoveries (n)
	0.20	87	87	–	1
	0.5	84	76–91	8.8	4
	1.0	86	86	–	1
	1.5	90	89–91	–	2
	3.0	90	90	–	1
Tobacco leaves (dried leaf)	0.02	101	93–108	–	2
	0.5	85	85	–	1
	2.0	89	89	–	1
	4.0	76	76	–	1
	5.0	78	78	–	1
	20	86	86	–	1
Lettuce (open leaf or head)	0.02	94	84–117	14.3	13
	0.1	90	81–93	4.7	8
	0.2	81	81	–	1
	0.5	92	91–93	1.0	4
	1.0	97	97	–	1
	1.5	84	84	–	1
Spinach	0.02	85	73–99	10.3	8
	0.1	92	84–95	5.7	4
	0.5	100	100–100	–	2
	1.5	91	91	–	1
Celery	0.02	88	72–98	13.4	9
	0.1	91	82–95	5.3	6
	0.5	97	97–97	–	2
Tomato	0.02	91	81–96	5.5	4
	0.1	94	94	–	1
	0.2	80	71–87	10.2	3
	0.5	87	73–99	15.1	3
	1.0	88	83–97	6.9	4
Pepper (sweet or chilli)	0.02	96	83–118	16.6	6
	0.2	91	79–97	8.0	6
	1.0	84	80–95	6.8	6

The reproducibility of the method REM172.11 was confirmed by means of an independent laboratory validation (ILV). The ILV was conducted at two separate laboratories by two different analysts and the associated recovery and repeatability data obtained by ILV are presented in Table 32. No interfering peaks were detected in the samples analysed. The linearity of the detector response covered a working range of 0.004 to 0.08 µg/mL (equivalent to 0.2 to 20 ng CGA210007 injected; linear curve).

Table 32 Recovery data obtained during ILV validation of REM172.11 (Formica 1995a 0175, Walser 1998a 0550) fortification with acibenzolar-S-methyl

Matrix	Fortification level (mg/kg)	Individual recoveries (%)	Range of recoveries (%)	Mean recovery (%)	RSD (%)
Banana peel	0.02	102, 114, 109	102–114	108	5.6
	0.2	98, 98, 101	98–101	99	1.7
Banana pulp	0.02	89, 86, 94	86–94	90	4.5
	0.2	96, 92, 92	92–96	93	2.5
Tomato	0.02	112, 95, 95	95–112	101	9.7
	0.2	72, 96, 88	72–96	85	14.3
Cucumber	0.02	95, 102, 83	83–102	93	10.3
	0.2	91, 95, 93	91–95	93	2.2
Mango	0.02	99, 76, 85, 85, 85	85–99	86	9.6
	0.2	96, 67, 91, 97, 87	67–97	88	13.9

The reproducibility of method AG-671 was also confirmed by means of an independent laboratory validation (ILV) (Hughes 1998 0485). The associated recovery and repeatability data obtained by ILV are presented in Table 33. No interfering peaks were detected in the samples analysed. The linearity of the HPLC-UV detector was tested using standard solutions. Standards of acibenzolar acid (CGA210007) at seven different concentrations over a range 0.35 to 25 ng injected were analysed and the response plotted against amount. Straight lines were obtained with a typical r^2 of > 0.999 obtained for acibenzolar acid (CGA210007) (common moiety target of AG-671).

Table 33 Recovery data obtained during ILV validation of AG-671-fotification with acibenzolar-S-methyl

Matrix	Fortification level (mg/kg)	Recovery (%)		RSD (%)	Number of recoveries (n)
		Mean	Range		
Tobacco (dried leaf) Second attempt data	0.02	94.8	88.6–101	9.3	2
	5.0	78.0	77.6–78.4	0.7	2
Spinach First attempt data	0.02	93.5	92.5–94.5	1.5	2
	1.0	82.9	80.6–85.2	3.9	2
Tomato paste [a] Second attempt data	0.02	91.1	87.6–94.5	5.4	2
	2.0	76.0	69.2–82.7	12.6	2

[a] Tomato paste was used in place of tomato RAC

Residue Analytical Method GRM051.01A (Gemrot 2011a 10056) and Residue Analytical Method GRM051.01B (amendment to GRM051.01A, Gemrot 2011b 10043, Class & Bacher 2012 10098)

Residues of acibenzolar-S-methyl (and any metabolites hydrolysable to acibenzolar acid (CGA210007)) are hydrolysed to produce the common moiety acibenzolar acid (CGA210007). Samples are hydrolysed in NaOH (0.1 M) at 65–70 °C, except for tobacco, where a higher temperature is used (97 °C). The hydrolysed samples are extracted by shaking with methanol, and an aliquot of the filtered extract mixed with NaOH (0.01 M) for SPE clean-up. After purification on an OASIS MAX cartridge, the column eluates are evaporated to remove the solvents and then diluted with formic acid (0.2%). Final determination is by HPLC with triple-quadrupole mass-spectrometric detection (LC-MS/MS).

Residues of acibenzolar-S-methyl and acibenzolar acid (CGA210007) are determined as acibenzolar acid (CGA210007) and calculated as acibenzolar-S-methyl equivalents (conversion factor: 1.17).

The LC-MS/MS method monitors two separate ion transitions for the purposes of analyte quantification (m/z 181 → 97) and confirmation (m/z 181 → 65). Recovery and repeatability data for the determination of acibenzolar-S-methyl residues in crops are presented in Table 34. Average recoveries ranged from 79 to 103%. The LOQ was 0.01 mg/kg for all crops investigated. The %RSDs ranged from 0.5 to 13.

Table 34 Recovery of acibenzolar-S-methyl residues in crops using method GRM051.01—fortification with acibenzolar-S-methyl

Matrix	Fortification level (mg/kg)	Average Recovery (%)	Range recoveries (%)	%RSD	Number of recoveries (n)
Quantification m/z 181 → 97					
Tobacco leaves (dried)	0.01	103	90–110	8	5
	10	83	78–86	4	5
	Overall	93	78–110	13	10
Tomato	0.01	83	75–89	6	5
	1	101	91–104	5	5
	Overall	92	75–104	11	10
Apple	0.01	85	83–88	2	5
	0.5	92	92–94	1	5
	Overall	89	83–94	5	10
Banana peel	0.01	87	84–93	4	5
	0.1	98	97–100	1	5
	Overall	93	84–100	7	10

Matrix	Fortification level (mg/kg)	Average Recovery (%)	Range recoveries (%)	%RSD	Number of recoveries (n)
Quantification m/z 181 → 97					
Banana pulp	0.01	97	94–98	2	5
	0.1	103	98–106	3	5
	Overall	100	94–106	4	10
Hazelnut	0.01	93	89–99	5	5
	0.10	100	98–103	2	5
	Overall	97	89–103	5	10
Spinach	0.01	79	77–81	2	5
	0.5	91	89–93	2	5
	Overall	85	77–93	8	10
Peach	0.01	86	83–91	4	5
	0.5	92	91–92	0.5	5
	Overall	89	83–92	4	10
Confirmation m/z 181 → 65					
Tobacco leaves (dried)	0.01	85	76–100	13	5
	10	85	79–93	6	5
	Overall	85	76–100	10	10
Tomato	0.01	85	82–87	2	5
	1	100	93–104	4	5
	Overall	93	82–104	10	10
Apple	0.01	89	86–91	3	5
	0.5	93	91–94	1	5
	Overall	91	86–94	3	10
Banana peel	0.01	92	90–95	2	5
	0.1	99	97–101	2	5
	Overall	95	90–101	4	10
Banana pulp	0.01	98	96–100	2	5
	0.1	102	98–104	2	5
	Overall	100	96–104	3	10
Hazelnut	0.01	95	89–99	4	5
	0.10	101	99–103	2	5
	Overall	98	89–103	4	10
Spinach	0.01	81	77–83	3	5
	0.5	91	88–93	2	5
	Overall	86	77–93	7	10
Peach	0.01	86	83–90	3	5
	0.5	82	90–93	1	5
	Overall	89	83–93	4	10

The reproducibility of the method GRM051.01was confirmed by means of an independent laboratory validation (ILV). The associated recovery and repeatability data obtained by ILV are presented in Table 35 (Class & Bacher 2012 10098).

No significant interferences (> 30% LOQ) arising from the matrices, labware, reagents or solvents have been observed at the retention time of interest for CGA210007, except in hazelnuts [ILV data only] where an apparent residue was observed in the control sample at approximately 0.004 mg/kg, but only in the secondary LC-MS/MS transition. Because the primary transition was unaffected by any interference, the specificity of the method is considered acceptable.

The linearity of the LC-MS/MS detector was tested using standard solutions and matrix matched standard solutions. Linearity was tested in both solvent mixtures used and for both MS/MS transitions. Standards at five different concentrations (1.0 to 30 ng/mL) were injected and the response plotted against concentration. Straight lines with r^2 of >0.999 were obtained for acibenzolar-S-methyl.

Table 35 Recovery of acibenzolar-S-methyl residues in crops—ILV validation data for method GRM051.01—fortification with acibenzolar-S-methyl

Matrix	Fortification level (mg/kg)	Number of recoveries (n)	Mean recovery (%)	RSD (%)	Recovery range (%)
Quantification m/z 181 → 97					
Spinach	0.01	5	100	12	86–120
	0.5	5	96	4	92–103
	Overall	10	98	9	86–120
Hazelnut	0.01	5	92	9	81–102
	0.1	5	86	11	75–97
	Overall	10	89	10	75–102
Tobacco leaves (dried)	0.01	5	89	10	76–98
	10	5	76	7	70–83
	Overall	10	82	12	70–98
Confirmation m/z 181 → 65					
Spinach	0.01	5	101	13	88–121
	0.5	5	95	5	90–102
	Overall	10	98	10	88–121
Hazelnut [a]	0.01	5	101	14	88–122
	0.1	5	80	9	72–88
	Overall	10	90	17	72–122
Tobacco leaves (dried)	0.01	5	88	9	80–98
	10	5	78	5	74–83
	Overall	10	83	10	74–98

[a] After correction for apparent residue in control (0.0037 mg/kg)

Residue Analytical Method KB items 30588 and 30589 (Anonymous 2012a 10325, 2012b 10326, 2012c 10327)

This method is particularly designed for determination of residues in kiwi fruits and kiwi fruit leaves. The method is based on GRM051.01A. Quantification of residues is by use of an internal standard (fluroxypyr).

Residues of acibenzolar-S-methyl (and any metabolites hydrolysable to acibenzolar acid (CGA210007)) are hydrolysed to produce the common moiety acibenzolar acid (CGA210007). A sample aliquot is weighed into an extraction tube. After addition of fluroxypyr at a known concentration, water and 1 N NaOH are added to the sample and hydrolysis performed in a heating block at 70 ± 5 °C for 30 minutes. The hydrolysed sample is extracted by shaking with methanol. An aliquot of the centrifuged extract is neutralized by addition of 2 N HCl and sodium chloride and an aqueous sodium chloride solution (30%) are added. Liquid-liquid partition is performed once into ethyl acetate. The organic phase is separated and reduced to dryness by a gentle stream of nitrogen. The residue is re-dissolved in CH_3CN/H_2O (50:50 v/v) and the sample is transferred to a 20 mg Hybrid / 50 mg C_{18} dSPE tube. Ammonia (0.2%) is added, the tube is centrifuged and residues determined by LC-MS/MS. The LC-MS/MS method monitors two separate ion transitions for the purposes of analyte quantification: acibenzolar acid (CGA210007): Transition for quantification: m/z = 179 → 107, transition for confirmation: m/z = 179 → 135. Fluroxypyr: Transition for quantification: m/z = 253 → 195, transition for confirmation: m/z = 255 → 197.

Recovery and repeatability data for the determination of acibenzolar-S-methyl residues in crops are presented in Table 36.

The average recoveries for kiwi fruit were 103–105% for the fortification level of 0.02 and 0.2 mg/kg. The LOQ was 0.02 mg/kg. The %RSDs of acibenzolar-S-methyl recoveries for kiwi fruit 13 and 20 respectively. No significant interferences (> 30% LOQ) arising from the matrices, labware, reagents or solvents have been observed at the retention time of interest for CGA210007. The linearity of the LC-MS/MS detector response was demonstrated using standard solutions of

CGA210007 in control matrix at seven concentration levels in the range of 0.1–0.1 ng/mL. A regression with a quadratic fit with 1/*x* weighting is used for calibration.

Table 36 Recovery of acibenzolar-S-methyl residues in kiwifruit—Analyte quantification (m/z = 179 → 57), Method KB

Matrix	Analyte	Fortification level (mg/kg)	Number of recoveries (n)	Recovery (%)	RSD (%)
Kiwifruit	Acibenzolar-S-methyl	0.02	9	103	13
		0.2	9	105	20
	Fluroxypyr (internal standard)	0.02	9	98	16
		0.2	9	98	15

Residue Analytical Method GRM051.05A (Lin 2011 50229, Willard 2012a 50026, 2012b 50017)

This method is particularly designed for determination of residues in citrus and processed fractions of citrus, cucumber, cabbage, spinach, tomato and onion bulb. The method is a modification of GRM051.01A and GRM051.01B.

Crop samples

Residues of acibenzolar-S-methyl (and any metabolites hydrolysable to acibenzolar acid (CGA210007)) are hydrolysed to produce the common moiety acibenzolar acid (CGA210007). Samples are adjusted to volume with water considering the natural moisture of the crop. Hydrolysis was performed with NaOH (1 N) at 65–70 °C. The hydrolysed samples are extracted by shaking with methanol. An aliquot of the centrifuged extract is neutralized by addition of 0.4 N HCl and the organic solvent is evaporated.

Crop oil samples

A sample aliquot is transferred into a separating funnel and diluted with hexane. Liquid-liquid partition is performed three times using a mixture of 0.1 N NaOH/CH_3CN (50:50, v/v). The aqueous phases are combined and adjusted to volume. After evaporation of the organic solvent from a sample aliquot, residues are hydrolysed at 65–70 °C. The cooled extract is neutralized by addition of 0.4 N HCl.

SPE clean-up

After purification on an OASIS HLB cartridge, the column eluates are adjusted to volume with aqueous acetic acid (0.4%). Final determination is performed by LC-MS/MS. Residues of acibenzolar-S-methyl and CGA210007 are determined as stated for GRM051.01A. The LC-MS/MS method monitors two separate ion transitions in negative mode for the purposes of analyte quantification (m/z = 179 → 57) and confirmation (m/z = 179 → 107).

Recovery and repeatability data for the determination of acibenzolar-S-methyl residues in crops are presented in Table 37 for the primary transition only. The mean recoveries for orange whole fruit and processed fractions, cucumber, cabbage, spinach, tomato and onion bulb were in the acceptable range of 70–120% for the fortification level of 0.01 mg/kg and 70–110% for the fortification level of 1.0 mg/kg. The RSDs of acibenzolar-S-methyl recoveries for orange whole fruit and processed fractions, cucumber, cabbage, spinach, tomato and onion bulb were all below 20% for fortification level of 0.01 mg/kg and 10% for the fortification level of 1.0 mg/kg. The LOQ was 0.01 mg/kg for all matrices. No significant interferences (> 30% LOQ) arising from the matrices, labware, reagents or solvents have been observed at the retention time of interest for acibenzolar acid (CGA210007). The linearity of the LC-MS/MS detector response was demonstrated using standard solutions of acibenzolar acid (CGA210007) in 0.4% acetic acid/CH_3CN (70:30, v/v) at seven concentration levels in the range of 0.05–20 ng/mL. A linear regression with a least square fit (1/*x* weighting) was used and a correlation coefficient of r = 0.9999 was obtained.

Table 37 Recovery of acibenzolar-S-methyl residues in crop and processed fractions for method GRM051.05—Analyte quantification (m/z = 179 → 57) Fortification with acibenzolar-S-methyl

Matrix	Fortification level (mg/kg)	Number of recoveries (n)	Recovery (%)		RSD (%)
			Range	Mean	
Orange (whole fruit)	0.01	3	87.4–89.3	88.4	1.1
	1.0	3	99.6–101	101	0.83
	Overall	6	87.4–101	94.5	7.1
Orange (dried pulp)	0.01	3	75.5–87.4	81.7	7.3
	1.0	3	88.6–94.6	90.8	3.7
	Overall	6	75.5–94.6	86.2	7.7
Orange (juice)	0.01	3	99.1–109	103	5.6
	1.0	3	94.6–99.3	96.3	2.7
	Overall	6	94.6–109	99.4	5.2
Orange (oil)	0.01	3	80.0–83.4	82.1	2.3
	1.0	3	76.9–87.8	83.4	6.9
	Overall	6	76.9–87.8	82.7	4.7
Cucumber (fruit)	0.01	3	87.0–93.7	91.4	4.2
	1.0	3	93.8–99.5	96.2	3.1
	Overall	6	87.0–99.5	93.8	4.3
Cabbage (head)	0.01	3	91.5–101	97.1	5.2
	1.0	3	91.3–97.7	95.5	3.8
	Overall	6	91.3–101	96.3	4.2
Spinach (leaves)	0.01	3	89.1–93.7	91.3	2.5
	1.0	3	96.6–98.2	97.2	0.9
	Overall	6	89.1–98.2	94.3	3.8
Tomato (fruit)	0.01	3	95.7–104	99.1	4.8
	1.0	3	92.6–107	99.7	7.1
	Overall	6	92.6–107	99.4	5.4
Onion (dry bulb)	0.01	3	84.0–96.8	92.3	7.8
	1.0	3	80.9–97.1	91.5	10
	Overall	6	80.9–97.1	91.9	8.1

Residue Analytical Method POPIT MET.003.Rev01 (Roncato et al. 2003 10305)

The principle of analytical method POPIT MET.003.Rev01 is based on analytical method REM172.11. This method is particularly designed for determination of total residues of acibenzolar-S-methyl and all metabolites that can be converted to acibenzolar acid (CGA210007) in tomato fruit and potato tubers.

An aliquot of the pre-homogenized sample is mixed with 2 N NaOH and heated at 55–60 °C for 30 minutes. After cooling to room temperature, $CaCl_2$ and methanol are added. The suspension is shaken for 30 minutes. After centrifugation, an aliquot of the clear extract is diluted with water and saturated NaCl and acidified by addition of 1 N HCl. Liquid-liquid partition is performed twice into hexane/methyl-t-butyl ether (7:3). The combined organic phases were partitioned into 1 N NaOH. The alkaline aqueous phase is acidified with phosphoric acid and partitioned three times into hexane/methyl-t-butyl ether (7:3). The combined organic phases are evaporated to dryness by a gentle stream of air. Residues are re-dissolved with sulphuric acid in *n*-propanol (2 mol/L) and the sample is derivatised by heating to approximately 85 °C for 30 minutes. After addition of water and internal standard solution (acibenzolar-acid-methyl ester), the determination of residues was performed by GC-ECD.

Recovery and repeatability data for the determination of acibenzolar-S-methyl residues in crops are presented in Table 38. The mean recoveries for tomato and potato were in the acceptable range of 70–120% for the fortification level of 0.1 mg/kg and 70–110% for the fortification level of 1.0 mg/kg. The RSDs of acibenzolar-S-methyl recoveries for tomato and potato were all ≤ 20% for fortification level of 0.1 mg/kg and ≤ 10% for the fortification level of 1.0 mg/kg. The LOQ was 0.1 mg/kg for tomato and potato. No significant interferences (> 30% LOQ) arising from the matrices, labware, reagents or solvents have been observed at the retention time of interest for acibenzolar-acid *n*-propyl ester. The linearity of the GC-ECD response was demonstrated using standard solutions of acibenzolar-acid *n*-propyl ester at six concentration levels in the range of 0.01 to 0.32 µg/mL. A potential regression was used with correlation coefficients (r) in the range of 0.9956 to 0.9991.

Table 38 Recovery of acibenzolar-S-methyl residues in tomato and potato using method POPIT MET.003

Matrix	Fortification level (mg/kg)	Number of recoveries (n)	Recovery (%)		RSD (%)
			Range	Mean	
Tomato (fruit)	0.1	72, 70, 92, 85, 87, 98, 74, 88, 74, 83	70–98	82	11
	1.0	87, 105, 100, 86	86–105	95	10
Potato (tuber)	0.1	77, 99, 93, 94, 90, 103, 99	77–103	94	9.1
	1.0	80, 76, 80, 87	76–87	81	5.7

Residue Analytical Method POPIT MET.081.Rev11 (Maslowski et al. 2010 10303)

This method is particularly designed for determination of acibenzolar-S-methyl and all metabolites that can be converted to acibenzolar acid (CGA210007) in melons, beans, cotton, soya beans, lemons, citrus (fruit and juice), wheat, coffee, papaya (pulp and whole fruit), corn and rice with a LOQ of 0.01 mg/kg.

An aliquot of the pre-homogenized sample is mixed with water and 1 N NaOH and the sample is shaken for 10 minutes on a mechanical shaker. Afterwards, hydrolysis is performed by heating to approximately 60–70 °C for 30 minutes. After cooling to room temperature, methanol is added and the sample is shaken for 30 minutes. After centrifugation, an aliquot of the clear extract is diluted with water and saturated NaCl solution.

Lemon only—The sample is extracted with toluene and hexane and the organic phases are discarded.

All matrices—1 N HCl is added and residues are liquid-liquid partitioned three to four times into toluene. The combined organic phases are reduced to dryness by a gentle stream of air. Residues are re-dissolved in CH_3CN/H_2O (50:50) and filtered. Final determination is performed by LC-MS/MS.

The LC-MS/MS method monitors two separate ion transitions in negative mode for the purposes of analyte quantification in rice ($m/z = 179 \rightarrow 57$, $m/z = 179 \rightarrow 107$ confirmation)) and one ion transition in melons, beans, cotton, soya beans, lemons, citrus (fruit and juice), wheat, coffee, papaya (pulp and whole fruit) and corn ($m/z = 179 \rightarrow 107$).

Recovery and repeatability data for the determination of acibenzolar-S-methyl residues in crops are presented in Table 39. The mean recoveries for acibenzolar-S-methyl from melons, beans, cotton, soya beans, lemons, citrus (fruit and juice), wheat, coffee, papaya (pulp and whole fruit), corn and rice were in the acceptable range of 70–120%. Recovery efficiencies of acibenzolar acid (CGA210007) were generally lower but still in the acceptable ranges except for citrus juice, coffee beans, lemon fruit, soya beans, beans and melons where the mean recoveries on fortification at 0.01 mg/kg were less than 70%. Acceptable recoveries for acibenzolar acid (CGA210007) were also not achieved for lemons fortified at 0.1 mg/kg. Generally, the accuracy of the method has been demonstrated. The RSDs of acibenzolar recoveries for acibenzolar-S-methyl and acibenzolar acid (CGA210007) in melons, beans, cotton, soya beans, lemons, citrus (fruit and juice), wheat, coffee, papaya (pulp and whole fruit), corn and rice were all $\leq 30\%$ for fortification level of 0.01 mg/kg and $\leq 20\%$ for the fortification level of 0.1 mg/kg. The LOQ was 0.01 mg/kg for acibenzolar-S-methyl and acibenzolar acid (CGA210007) in cotton, wheat, papaya (pulp and whole fruit), corn and rice and 0.1 mg/kg for beans, citrus fruit and juice, coffee, melon, soya beans. Acceptable recoveries were not obtained for lemon fruit.

No significant interferences (> 30% LOQ) arising from the matrices, labware, reagents or solvents have been observed at the retention time of acibenzolar acid (CGA210007). The linearity of the LC-MS/MS detector response was demonstrated using standard solutions of acibenzolar acid (CGA210007) in CH_3CN/H_2O (50:50, v/v) at six concentration levels in the range of 0.05–1.7 ng/mL. For quantification, a linear regression with a least square fit was used, with correlation coefficients (r) in the range of 0.9980 to 0.9999.

Table 39 Recovery of residues of acibenzolar-S-methyl and acibenzolar acid (CGA210007) in various crops for method POPIT MET.081

Matrix	Analyte	Fortification level (mg/kg)	Number of recoveries (n)	Recovery (%)		RSD (%)
				Range	Mean	
Melon (whole fruit)	Acibenzolar-S-methyl	0.01	10	94–120	105	6.6
		0.1	6	99–104	102	1.8
		1.0	5	74–84	79	4.5
	CGA210007	0.01	8	61–74	66	7.2
		0.1	6	70–84	78	6.8
		1.0	5	68–78	72	6.0
Beans (beans)	Acibenzolar-S-methyl	0.01	8	102–110	106	2.5
		0.1	6	101–107	105	3.0
		1.0	6	82–86	84	1.8
	CGA210007	0.01	7	62–67	64	3.2
		0.1	5	86–100	93	6.0
		1.0	6	72–90	81	7.3
Cottonseed	Acibenzolar-S-methyl	0.01	8	104–108	106	1.7
		0.1	6	99–105	102	2.0
		1.0	6	83–96	89	5.2
	CGA210007	0.01	7	77–90	85	5.3
		0.1	6	77–90	85	6.1
		1.0	6	84–97	88	5.2
Soya bean (beans)	Acibenzolar-S-methyl	0.01	8	70–92	78	9.2
		0.1	6	79–82	80	1.5
		1.0	6	77–87	81	5.1
	CGA210007	0.01	8	45–56	50	7.0
		0.1	6	68–80	73	5.8
		1.0	6	70–77	75	3.4
Lemon (whole fruit)	Acibenzolar-S-methyl	0.01	8	83–109	98	10
		0.1	6	72–107	87	15
	CGA210007	0.01	8	45–56	50	7.4
		0.1	6	55–61	58	3.4
Citrus (whole fruit)	Acibenzolar-S-methyl	0.01	7	100–119	108	7.1
		0.1	6	91–119	107	8.8
		2.0	6	79–90	85	5.0
	CGA210007	0.01	7	59–85	73	12
		0.1	6	67–87	79	11
		2.0	6	73–105	87	15
Wheat (grain)	Acibenzolar-S-methyl	0.01	9	78–100	91	10
		0.1	6	91–106	99	5.7
	CGA210007	0.01	7	62–83	72	9.1
		0.1	6	90–109	99	7.4
Coffee (beans)	Acibenzolar-S-methyl	0.01	8	75–90	82	7.5
		0.1	6	70–98	83	15
	CGA210007	0.01	8	46–70	60	14
		0.1	6	66–84	72	10
Citrus (juice)	Acibenzolar-S-methyl	0.01	8	90–100	95	3.6
		0.1	6	69–87	80	8.0
	CGA210007	0.01	8	55–65	60	5.8
		0.1	6	66–77	71	5.7
Papaya (pulp)	Acibenzolar-S-methyl	0.01	8	76–90	83	4.7
		0.1	6	85–96	90	4.4
	CGA210007	0.01	7	73–88	78	7.0
		0.1	5	73–80	76	3.6
Papaya (whole fruit)	Acibenzolar-S-methyl	0.01	8	76–89	84	5.4
		0.1	6	80–87	84	3.3

Matrix	Analyte	Fortification level (mg/kg)	Number of recoveries (n)	Recovery (%)		RSD (%)
				Range	Mean	
	CGA210007	0.01	8	64–76	70	5.8
		0.1	5	70–77	74	3.7
Corn (grain)	Acibenzolar-S-methyl	0.01	8	84–95	90	4.2
		0.1	6	85–90	88	2.4
	CGA210007	0.01	8	64–88	78	10
		0.1	6	72–88	82	6.8
Rice (grain)	Acibenzolar-S-methyl	0.01	8	79–92	84	4.9
		0.1	6	86–93	89	2.8
		0.2	6	87–101	93	5.7
	CGA210007	0.01	8	60–81	72	12
		0.1	6	76–95	84	8.9
		0.2	6	86–95	90	4.4

Extractability of residues—radiovalidation

Validation of the extractability and accountability of analytical method REM172.02 for tobacco leaves and wheat, of REM172.11 for tomatoes and AG-671 for tobacco, lettuce and tomato treated with [^{14}C]acibenzolar-S-methyl was performed. The methods employ 1 N NaOH at 60 °C for the hydrolysis step and methanol for the extraction. These results are presented in Tables 40.

There was generally good agreement between the results from the metabolism study (sum of acibenzolar-S-methyl and acibenzolar acid (CGA210007) free and conjugated) with the results from the different analytical methods.

Table 40 Radiovalidation data for [^{14}C]-acibenzolar-S-methyl residues in tobacco leaves and wheat—REM172.02 (Formica 1995b 0154 1995c 0155) and tomato—REM172.11 (Formica 1996a 0297) and for tobacco, head lettuce and tomato AG671/AG-671A (Hayworth 1998b 0486)

Matrix	TRR (mg equiv/kg)	Residue extracted (mg equiv/kg)	Residue extracted (%TRR)	Measured residue (mg/kg)	Metabolism study (mg/kg)	Accountability (%)
REM172.02						
Tobacco leaves (lower leaves, uncured)	1.39	1.27 1.24	91 89	0.92 0.88	1.05[b]	88 84
Tobacco leaves (lower leaves, cured)	11.6	10.6 10.8	91 93	7.45 7.70	7.68[c]	97 100
Wheat grain[a]	0.014	0.011 0.010	75 72	– –	0.003[d]	– –
Wheat straw	0.33	0.18 0.18	56 56	0.12 0.12	0.16[e]	75 75
Wheat husks	0.23	0.14 0.13	58 57	0.093 0.081	0.11[f]	85 74
REM172.11						
Tomato	0.20[i]	0.184 0.183	90 90	0.136 0.143	0.13[h]	105 110
AG-671/AG-671A						
Tobacco (lower leaves, uncured)	1.39 1.39 1.39	1.50 1.54 1.48	108 111 106	1.09 1.04 1.05	1.05[b]	104 100 100
Head lettuce (with wrapper leaves)	0.96 0.96 0.96	0.86 0.85 0.78	90 88 82	0.33 0.30 0.27	0.4[g]	83 75 68
Tomato	0.31 0.31 0.31	0.29 0.32 0.32	92 103 102	0.18 0.21 0.22	0.2[h]	90 104 111

Measured residue = sum of acibenzolar-S-methyl and free and conjugated acibenzolar acid using the analytical method

[a] The accountability was not performed for wheat grain as the expected residue is below the LOQ

[b] Tobacco, sum of acibenzolar-S-methyl, CGA210007 free and conjugated was 75.6% TRR

[c] Tobacco cured, sum of acibenzolar-S-methyl, CGA210007 free and conjugated was 66.2% TRR

[d] Wheat grain, sum of acibenzolar-S-methyl, CGA210007 free and conjugated was 24.2% TRR

[e] Wheat straw, sum of acibenzolar-S-methyl, CGA210007 free and conjugated was 49.3% TRR

[f] Wheat husks, sum of acibenzolar-S-methyl, CGA210007 free and conjugated was 49.8% TRR

[g] Head lettuce, sum of acibenzolar-S-methyl, CGA210007 free and conjugated was 41.6% TRR

[h] Tomato, sum of acibenzolar-S-methyl, CGA210007 free and conjugated was 65.1 % TRR

[i] Different aliquot and analysis of sample in tomato metabolism report with TRR 0.31 mg equiv/kg.

Procedural recoveries for method REM172.02 were 77% for uncured and 83% for cured tobacco leaves fortified at 1 mg/kg and 98 and 80% for wheat straw fortified at 0.05 and 0.5 mg/kg respectively. Procedural recoveries for REM172.11 were 93 and 90% for tomatoes fortified at 0.02 and 0.2 mg/kg respectively.

Residue Analytical Method REM172.14 (Walser 1999a 0609, 1999b 0572)

Analytical method REM172.14 has been developed for the determination of 4-OH acibenzolar acid (CGA323060) (metabolite of acibenzolar-S-methyl) residues in lettuce and spinach.

Homogenised crop samples are extracted with a mixture of $CH_3CN/H_2O/0.1$ M HCl (80:19:1 v/v) for 1 hour at room temperature and filtered to remove plant material. An aliquot of the filtered extract is reduced under pressure, redissolved in acetate buffer (pH 4.65) and treated with cellulase to cleave conjugates of 4-OH acibenzolar acid (CGA323060). After cellulase cleavage, 1 N HCl is added and the extract is applied to the SPE cartridge (RP $_{18}$) for clean-up prior to final determination by HPLC (column 1: Spherisorb 80-5 SAX, 5 µm particle size; column 2: Nucleosil 100-5, C_{18}, 5 µm particle size) coupled with UV detection (252 nm).

Recovery and repeatability data for the determination of 4-OH acibenzolar acid (CGA323060) residues in crops are presented in Table 41. Recoveries ranged from 75–107%. The LOQ was 0.02 mg/kg for both lettuce and spinach. The individual relative standard deviations (RSDs) were below 20%.

Table 41 Recovery data obtained for 4-OH acibenzolar acid (CGA323060) residues in crops during validation of REM172.14 (Walser 1999a 0609)

Matrix	Fortification level (mg/kg)	Individual recoveries (%)	Range of recoveries (%)	Mean recovery (%)	RSD (%)
Lettuce	0.02	93, 104, 107, 104, 86	86–107	99	9.0
	0.2	96, 99, 101, 90, 99	90–101	97	4.4
Spinach	0.02	75, 79, 75, 75, 79	75–79	77	2.8
	0.2	83, 83, 81, 85, 86	81–86	84	2.3

The reproducibility of the method was confirmed by means of an independent laboratory validation (ILV). The ILV was conducted at two separate laboratories by two different analysts and the associated recovery and repeatability data obtained by ILV are presented in Table 42.

Table 42 Recovery of 4-OH acibenzolar acid (CGA323060) residues in crops using method REM172.14—ILV validation data (Walser 1999b 0572)

Matrix	Fortification level (mg/kg)	Individual recoveries (%)	Range of recoveries (%)	Mean recovery (%)	RSD (%)
Lettuce (heads)	0.02	93, 91, 105, 108, 111	91–111	102	8.9
	0.2	100, 98, 100, 102, 94	94–102	99	3.1
Spinach	0.02	83, 71, 62, 63, 73	62–83	70	12.2
	0.2	74, 69, 76, 72, 69	69–76	72	4.3

No interfering peaks were detected in the samples analysed. The linearity of the detector response covered a working range of 0.008 to 0.08 µg/mL (equivalent to 1.6 to 16 ng CGA323060 injected; linear curve).

Residues in food and feedstuffs of animal origin

Residue Analytical Method REM172.11—Milk (Formica 1995a 0175)

Analytical method REM172.11 has been developed for the determination of acibenzolar-S-methyl residues in milk.

Milk samples are hydrolysed with 1 N NaOH at 60 °C to convert acibenzolar-S-methyl to acibenzolar acid (CGA210007). After cooling, samples are extracted with methanol and $CaCl_2$. The sample solution is filtered and subjected to acid clean-up/partition with water, saturated NaCl solution and 1 N HCl. The analyte is extracted (twice) with a mixture of hexane and *tert*-butyl methyl ether (7:3 v/v) prior to final determination by HPLC (column 1: anion exchange; column 2: C_{18} reversed phase) coupled with UV detection (235 nm). For calculation of acibenzolar acid (CGA210007) residues as acibenzolar-S-methyl equivalents a molecular weight conversion factor of 1.17 is used.

Recovery and repeatability data for the determination of acibenzolar-S-methyl residues in milk are presented in Table 43. Average recovery values were 85 and 95% for the two fortification levels studied while the %RSD were 0.6 and 6.1. The LOQ was 0.005 mg/L for milk. No interfering peaks were detected in the samples analysed. The linearity of the detector response covered a working range of 0.004 to 0.08 µg/mL (equivalent to 0.2 to 4 ng CGA210007 injected; linear curve).

Table 43 Recovery of acibenzolar-S-methyl residues in milk using method REM172.11—Primary validation data. Fortification with acibenzolar-S-methyl

Matrix	Fortification level (mg/L)	Individual recoveries (%)	Range of recoveries (%)	Mean Recovery (%)	RSD (%)
Milk	0.005	91, 82, 82	82–91	85	6.1
	0.05	96, 95, 95	95–96	95	0.6

The reproducibility of the method was confirmed by means of an independent laboratory validation (ILV). The ILV was conducted at two separate laboratories by two different analysts and the associated recovery and repeatability data obtained by ILV are presented in Table 44.

Table 44 Recovery of acibenzolar-S-methyl residues in milk using method REM172.11—ILV validation data. Fortification with acibenzolar-S-methyl (Formica 1995a 0175)

Matrix	Fortification level (mg/L)	Individual recoveries (%)	Range of recoveries (%)	Mean Recovery (%)	RSD (%)
Milk	0.005	103, 95, 94, 95, 95, 95	94–103	96	3.5
	0.05	97, 93, 95, 98, 92, 92	92–98	95	2.7

Residue Analytical Method REM172.12–Tissues and eggs (Formica 1995d 0199, Walser 1998c 0466)

Analytical method REM172.12 has been developed for the determination of acibenzolar-S-methyl residues in tissues and eggs.

Meat, liver, kidney and eggs

Samples are hydrolysed at 60 °C with 2 N NaOH solution in the case of egg samples or methanolic 2 N NaOH for meat, liver and kidney samples to convert acibenzolar-S-methyl to acibenzolar acid (CGA210007). Saturated NaCl solution, HCl (32%) and a mixture of *n*-hexane and *tert*-butyl methyl ether (solvent mixture A; 7:3 v/v) are added and the extract is shaken prior to separation by centrifugation. The extract solution is then subjected to sequential clean-up by alkaline/acid partition using 1 N NaOH, solvent mixture A and phosphoric acid (85%) prior to final determination by HPLC (column 1: Nucleosil SB, 5 µm particle size; column 2: C_{18}, 5 µm particle size) coupled with UV detection (235 nm). For calculation of acibenzolar acid (CGA210007) residues as acibenzolar-S-methyl equivalents, a molecular weight conversion factor of 1.17 is used.

Fat

Samples are extracted with a mixture of *n*-hexane/CH_3CN (60:40 v/v) and the CH_3CN phase is filtered through glass wool. The *n*-hexane phase is extracted (twice) with CH_3CN and the CH_3CN extracts are combined and reduced to dryness. The residue is redissolved in methanol and hydrolysed with 2 N NaOH at 60 °C to convert acibenzolar-S-methyl to CGA 210007. Saturated NaCl solution, HCl (32%) and a mixture of *n*-hexane and *tert*-butyl methyl ether (solvent mixture A; 7:3 v/v) are added and the extract is shaken for 25 minutes and reduced to dryness prior to final determination by HPLC (column 1: Nucleosil SB, 5 µm particle size; column 2: C_{18}, 5 µm particle size) coupled with UV detection (235 nm). For calculation of acibenzolar acid (CGA210007) residues as acibenzolar-S-methyl equivalents, a molecular weight conversion factor of 1.17 is used.

Recovery and repeatability data for the determination of acibenzolar-S-methyl residues in tissues are presented in Table 45. Mean recovery values were in the range of 70–110%. The individual relative standard deviations (RSDs) were below 20%. The validated LOQ was 0.02 mg/kg for meat, eggs, fat, liver and kidney. No interfering peaks were detected in the samples analysed. The linearity of the detector response covered a working range of 0.004 to 0.08 µg/mL (equivalent to 0.2 to 6.4 ng CGA210007 injected). Straight lines were obtained with a typical r^2 value of 0.9998 were obtained for CGA210007 (common moiety target of REM172.12).

Table 45 Recovery of acibenzolar-S-methyl residues in tissues and eggs using method REM172.12—Primary validation data. Fortification with acibenzolar-S-methyl

Matrix	Fortification level (mg/kg)	Individual recoveries (%)	Range of recoveries (%)	Mean Recovery (%)	RSD (%)
Meat	0.02	85, 87, 79, 77, 82, 80, 85, 79	77–87	82	4.4
	0.2	88, 84, 83, 85, 83, 84, 85, 84	83–88	85	1.9
Eggs	0.02	88, 90, 93	88–93	90	2.8
	0.2	82, 87, 87	82–87	85	3.4
Fat	0.02	62, 92, 67, 77, 82, 82, 107, 82	62–107	81	17.2
	0.2	100, 101, 104, 76, 92, 92, 93, 88	76–104	93	9.5
Liver	0.02	87, 102, 87, 87, 87, 92, 93, 92	87–102	91	5.7
	0.2	89, 90, 90, 89, 93, 90, 90, 88	88–93	90	1.6
Kidney	0.02	94, 75, 85, 86, 94, 94, 94, 90	75–94	89	7.6
	0.2	87, 85, 88, 85, 86, 84, 88, 86	84–88	86	1.7

The reproducibility of the method was confirmed by means of an independent laboratory validation (ILV). The ILV was conducted at two separate laboratories by two different analysts and the associated recovery and repeatability data obtained by ILV are presented in Table 46.

Table 46 Recovery of acibenzolar-S-methyl residues in tissues and eggs using method REM172.12—ILV validation data. Fortification with acibenzolar-S-methyl

Matrix	Fortification level (mg/kg)	Individual recoveries (%)	Range of recoveries (%)	Mean Recovery (%)	RSD (%)
Meat	0.02	96, 94, 94	94–96	95	1.2
	0.2	80, 84, 85	80–85	83	3.2
Eggs	0.02	77, 95, 86	77–95	86	10.5
	0.2	89, 89, 89	89–89	89	0.0
Fat	0.02	80, 95, 95, 91, 91	80–95	90	6.8

Matrix	Fortification level (mg/kg)	Individual recoveries (%)	Range of recoveries (%)	Mean Recovery (%)	RSD (%)
Liver	0.02	88, 102, 102, 93, 84	84–102	94	8.7
Kidney	0.02	76, 96, 77, 80, 93	76–96	84	11.1

Applicability of multi-residue methods

Methods have been reported in the scientific literature for the analysis of acibenzolar-S-methyl in food, including multi-residue screens (Banerjee *et al*. 2012, Christia *et al*. 2015, Eitzer *et al*. 2014, Fillâtre *et al*. 2010, González-Curbelo *et al*. 2014, Ishibashi *et al*. 2015, Kwon *et al*. 2011, Kwon *et al*. 2012, López *et al*. 2014, Okihashi *et al*. 2007, Pang *et al*. 2009, Pérez-Ortega *et al*. 2016, Pihlström *et al*. 2007). As these methods do not include a hydrolysis step, the analyte measured is parent compound.

The published DFG S19 multi-residue method was found to be not acceptable for the determination of acibenzolar-S-methyl residues in crops as the parent compound did not chromatograph under the described gas chromatography conditions.

Moreover, methods for the determination of acibenzolar-S-methyl analysing per some residue definitions established by regulators must include acibenzolar acid (CGA210007), and therefore a hydrolysis step is required. Common multi-residue methods listed above do not allow the inclusion of a hydrolysis step and therefore are not applicable to the determination of acibenzolar-S-methyl in products of plant or animal origin.

Stability of residues in stored analytical samples

The freezer storage stability of acibenzolar-S-methyl in homogenised plant, animal tissues, milk and eggs samples fortified with acibenzolar-S-methyl and acibenzolar acid (CGA210007) was studied.

Stability of residues in analytical sample extracts

Gemrot (2011b 10043) studied the stability of acibenzolar acid (CGA210007) (expressed as acibenzolar-S-methyl equivalents) in final extracts stored between 0 and 9 °C for a range of crop matrices (dry tobacco leaves, tomatoes, apples, bananas (peel and pulp), hazelnuts, spinach and peaches) fortified at a nominal concentration of 0.01 mg/kg. After the initial analysis, samples were reanalysed after periods in storage (16 days for dry tobacco leaves, 8 days for peaches, 10 days for tomatoes, 14 days for hazelnuts and 9 days for apples, bananas (peel and pulp) and spinach). Samples were analysed per the validated analytical method GRM051.01A. Recovery results determined were generally like those obtained during the initial analyses. It is concluded that acibenzolar acid (CGA210007) is stable in sample extracts for periods of 8 to 16 days when stored at 0–9 °C.

Storage residues in stored plant products

Formica (1996b 0248) studied the freezer storage stability of acibenzolar-S-methyl and acibenzolar acid (CGA210007) in wheat commodities. Samples of wheat grain were fortified separately with acibenzolar-S-methyl and acibenzolar acid (CGA210007) at a concentration of 0.5 mg/kg and stored frozen at –18 °C. Samples were taken for analysis at intervals up to 24 months. Samples were analysed per the validated analytical method REM172.02.

No decrease in residues of acibenzolar-S-methyl or acibenzolar acid (CGA210007) were observed in wheat grain stored frozen for up to 24 months (2 years). The recoveries of acibenzolar-S-methyl and acibenzolar acid (CGA210007) after frozen storage at –18 °C are summarized in Tables 47 and 48.

Table 47 Stability of acibenzolar-S-methyl residues in wheat grain during frozen storage

Storage period (months)	Procedural recoveries (%)	Residues (mg/kg)	Mean % remaining [a]
0		0.45, 0.45, 0.43, 0.49	100

1	87, 88	0.43, 0.44	96
2	89, 95	0.44, 0.39	91
4	88, 91	0.47, 0.43	99
8	84, 83	0.44, 0.43	96
12	84, 87	0.41, 0.40	89
18	80, 80	0.36, 0.41	85
24	86, 88	0.38, 0.44	90

[a] % remaining = 100× (Residue at storage period *x*)/(Residue at storage period 0)

Table 48 Stability of acibenzolar acid (CGA210007) residues in wheat grain during frozen storage

Storage period (months)	Procedural recoveries (%)	Residues (mg/kg)	Mean % remaining [a]
0		0.47, 0.46, 0.48, 0.45	100
1	87, 88	0.46, 0.48	101
2	89, 95	0.46, 0.46	99
4	88, 91	0.44, 0.49	100
8	84, 83	0.44, 0.49	100
12	84, 87	0.44, 0.46	97
18	80, 80	0.43, 0.42	91
24	86, 88	0.48, 0.43	98

N.B. Control and procedural recovery values are the same as those reported in Table 47.

[a] % remaining = 100× (Residue at storage period *x*)/(Residue at storage period 0)

Walser (1999c 0569) studied the stability of acibenzolar-S-methyl and acibenzolar acid (CGA210007) in homogenised banana samples stored frozen at –18 °C. Samples of whole banana fruits were fortified with acibenzolar-S-methyl at a concentration of 1.0 mg/kg and stored frozen at –18 °C. Samples were taken for analysis at intervals up to 24 months. Samples were analysed per the validated analytical method REM172.11.

No decrease in acibenzolar-S-methyl residues were observed in whole banana fruits stored frozen for up to 24 months (2 years). The recoveries of acibenzolar-S-methyl residues after frozen storage at –18 °C are summarized in Table 49.

Table 49 Stability of acibenzolar-S-methyl residues in banana during frozen storage

Storage period (months)	Procedural recoveries (%)	Residue (mg/kg)	Mean % remaining [a]
0		0.90, 0.87, 0.90, 0.86	100
1	90, 90	0.87, 0.82	96
2	88, 89	0.86, 0.90	99
4	90, 89	0.83, 0.90	98
8	93, 104	1.02, 0.90	109
24	106, 105	0.97, 0.90	106

[a] % remaining = 100× (Residue at storage period *x*)/(Residue at storage period 0)

In a freezer storage stability study, Hayworth (1999a 0716) spiked samples of tobacco, lettuce, tomato, wheat, cabbage, squash and turnip as well as the processed commodity tomato paste. Samples were fortified separately with acibenzolar-S-methyl and acibenzolar acid (CGA210007) at a concentration of 1.0 mg/kg and stored frozen at –18 °C. Samples were taken for analysis at regular intervals throughout the duration of frozen storage (ca. 20–23 months). Samples were analysed per the validated analytical method AG-671.

There was no observable decrease in residues of acibenzolar-S-methyl or acibenzolar acid (CGA210007) in all commodities stored frozen for at least 20 months. The recoveries of acibenzolar-S-methyl and acibenzolar acid (CGA210007) residues after frozen storage at –18 °C are summarized in Tables 50 and 51.

Table 50 Stability of acibenzolar-S-methyl residues in various commodities during frozen storage acibenzolar-S-methyl

Storage period (months)	Procedural recoveries (%)	Residue (mg/kg)	Mean % remaining [a]
Burley (green) tobacco			
0	72, 80	0.783, 0.801	100
2.5	65, 77	0.698, 0.672	86
8.6	82, 82	NR, 0.831	105
13.1	81, 82	0.841, 0.834	106
22.9	92, 93	0.915, 0.897	114
Flue-cured tobacco			
0	77, 64	0.797, 0.800	100
3.5	79, 73	0.741, 0.739	93
8.4	71, 72	0.785, 0.713	94
14.2	77, 81	0.753, 0.734	93
22.8	88, 88	0.747, 0.741	99
Leaf lettuce			
0	86, 90	0.859, 0.854, 0.865	100
2.5	88, 81	0.852, 0.892	91
8.6	81, 85	0.750, 0.814	78
13.8	89, 90	0.645, 0.689	112
21.8	89, 90	0.827, 1.104	92
Tomato fruit			
0	84, 85, 86	0.847, 0.899, 0.913	100
2.2	91, 89	0.925, 0.895	103
8.3	91, 88	0.941, 0.909	104
13.3	87, 87	0.794, 0.908	96
22.3	NR, 97	0.909, 0.918	103
Tomato paste			
0	93, 86, 88	0.946, 0.972, 0.975	100
2.7	84, 93	0.869, 0.899	92
8.2	95, 85	0.706, 0.871	82
12.9	80, 82	0.808, 0.903	89
22.3	93, 107	0.918, 0.877	93
Wheat grain			
0	80, 79, 88	0.923, 0.765, NR	100
2.7	64, 67	0.700, 0.575	76
7.8	63, 58	0.604, 0.669	75
13.4	59, 86	0.795, 0.785	94
21.9	69, 86	0.620, NR	73
Wheat straw			
0	78, 71, 84	0.856, 0.880, 0.733	100
2.7	77, 80	0.827, 0.736	95
7.9	90, 80	0.813, 0.790	97
13.4	79, 88	0.789, 0.633	86
21.5	77, 81	0.814, 0.842	101
Cabbage			
0	82, 81, 89	0.877, 0.880, 0.837	100
1.9	101, 96	0.970, 1.115	121
7.2	92, 91	0.879, 0.939	105
13.2	77, 70	0.726, 0.735	84
21.1	93, 95	0.874, 0.916	104
Squash			
0	80, 87, 87	0.862, 0.808, 0.797	100
2.0	101, 105	0.951, 1.025	120
6.3	77, 70	0.800, 0.726	93
13.4	68, 74	0.729, 0.585	80
20.3	95, 92	0.832, 0.844	102
Turnip roots			
0	84, 87, 90	0.886, 0.829, 0.823	100
2.0	102, 100	0.958, 0.953	113
7.1	92, 93	0.844, 0.932	105

Storage period (months)	Procedural recoveries (%)	Residue (mg/kg)	Mean % remaining [a]
12.6	68, 78	0.711, 0.689	83
20.4	93, 94	0.879, 0.860	103

NR = data not reported due to problems during sample purification

[a] % remaining = 100× (Residue at storage period *x*)/(Residue at storage period 0)

Table 51 Stability of acibenzolar acid (CGA210007) residues in various commodities during frozen storage

Storage period (months)	Procedural recoveries (%)	Residue (mg/kg)	Mean % remaining [a]
Burley (green) tobacco			
0	73, 71	0.654, 0.726	100
2.5	68, 61	0.696, 0.736	104
8.6	75, NR	0.691, 0.587	93
13.1	62, 85	0.782, 0.584	99
22.9	78, 88	0.927, 0.892	132
Flue-cured tobacco			
0	68, 77	0.732, 0.749	100
3.7	83, 85	0.836, 0.816	112
9.0	70, 74	0.754, 0.839	108
14.3	67, 81	0.866, 0.540	95
22.8	81, 76	0.866, 0.764	110
Leaf lettuce			
0	83, 85, 84	0.887, 0.896, 0.925	100
2.5	82, 87	0.865, 0.569	79
8.6	83, 82	0.799, 0.851	91
13.7	63, 69	0.613, 0.725	74
21.8	81, 87	0.845, 0.759	89
Tomato fruit			
0	82, 90, 93	0.881, 0.954, 0.875	100
2.2	92, 87	0.714, 0.872	88
8.3	84, 75	0.894, 0.879	98
12.9	70, 75	0.477, 0.570	58
22.3	83, 90	0.883, NR	98
Tomato paste			
0	78, 87, 86	0.850, 0.884, 0.875	100
2.5	97, 103	0.959, 0.955	110
8.2	91, 81	0.937, 0.881	105
12.9	69, 69	0.759, 0.705	84
22.3	89, 90	0.875, 0.901	102
Wheat grain			
0	64, 81, NR	0.739, 0.839, 0.718	100
2.7	67, 69	0.760, 0.707	96
7.8	72, 71	0.771, 0.751	99
13.2	78, 68	0.869, 0.792	109
22.2	NR 75	0.649, 0.730	90
Wheat straw			
0	94, 101, 113	0.982, 1.219, 0.990	100
2.7	74, 80	0.778, 0.960	82
7.9	80, 78	0.816, 0.721	72
13.3	82, 77	0.863, 0.868	81
21.5	86, 71	0.808, 0.798	75
Cabbage			
0	78, 89, 88	0.844, 0.894, 0.886	100
1.9	97, 99	0.960, 0.878	105
7.2	84, 88	0.901, 0.909	103
12.9	79, 82	0.845, 0.712	89
21.1	88, 86	0.855, 0.855	98
Squash			
0	76, 82, 86	0.651, 0.861, 0.881	100
2.0	102, 108	1.087, 1.054	134

Storage period (months)	Procedural recoveries (%)	Residue (mg/kg)	Mean % remaining [a]
6.3	69, 67	0.737, 0.718	91
13.3	63, 65	0.753, 0.648	88
20.4	85, 87	0.911, 0.931	115
Turnip roots			
0	77, 80, 82	0.856, 0.823, 0.876	100
2.0	90, 102	0.995, 0.940	114
7.1	86, 96	0.932, 0.972	112
12.6	83, 76	0.692, 0.699	82
20.4	88, 86	0.911, 0.898	106

NR = data not reported due to problems during sample purification

[a] % remaining = 100× (Residue at storage period *x*)/(Residue at storage period 0)

Stability of residues in stored animal products

In a freezer storage stability study (Walser 1998d 0465), samples of bovine muscle, liver, kidney, fat, milk and blood were fortified with acibenzolar-S-methyl at a concentration of 0.2 mg/kg in tissues, 0.05 mg/kg in milk and 0.5 mg/kg in blood (Table 52). Samples were stored at –20 °C for a period of 4.5 months, which covered the sample storage time in the transfer study. Samples were analysed using several analytical methods: REM172.11 for milk, REM172.12 for tissues (meat, fat, liver and kidney) and REM172.13 for blood samples. The LOQ of the methods was 0.005 mg/kg for milk; 0.02 mg/kg for tissues and fat and 0.05 mg/L for blood.

Table 52 Stability of acibenzolar-S-methyl residues in various animal commodities during frozen storage

Substrate	Fortification level (mg/kg)	Storage period (days)	Residue (mg/kg)	Average % remaining [a]
Muscle	0.20	87	0.131, 0.151, 0.160, 0.137, 0.158	74
Liver	0.20	124	0.102, 0.104, 0.102, 0.0803, 0.122	51
Kidney	0.20	131	0.178, 0.133, 0.168, 0.141, 0.107	73
Fat	0.20	129	0.174, 0.179, 0.176, 0.171, 0.170	87
Milk	0.05	115	0.0395, 0.0481, 0.04, 0.0355, 0.0415	82
Blood	0.50	136	0.434, 0.372, 0.434, 0.471, 0.508	89

[a] % remaining = 100× (Residue/(Fortification level))

Introduction to use patterns

Acibenzolar-S-methyl is used for the control of *Erysiphe graminis* (powdery mildew of cereals) and *Mycosphaerella musci* (black Sigatoka of banana) in a variety of crops and in partial control of *Septoria* spp. (side effect only) and *Puccinia* spp. (side effect only) in cereals.

Table 53 Selected registered uses of acibenzolar-S-methyl

Crop	Country	Method	GS	Rate (g ai/ha)	Water (L/ha)	No.	Interval (days)	PHI (days)
Apple	Italy	F		75–100 [7.5 g ai/hL]		6	5–14	7
Banana	France	F		40		6	40	2
Banana	Guatemala (Belize, Honduras, Costa Rica, Panama)	F		40	15–30 air	–	35–40	no restriction
Beans	Brazil	F	Start beginning vegetative developmen	12.5	200–300 grd 20–40 air	3	14	14

Crop	Country	Method	GS	Rate (g ai/ha)	Water (L/ha)	No.	Interval (days)	PHI (days)
			t					
Brassica (Cole) crops Group 5	USA	F	Begin 7–10 days after thinning	17.5–35, max 140 g ai/ha/yr	≥ 187 grd ≥ 93.5 air		7	7
Cabbage	Indonesia	F	1st application 7–14 days after planting	[2.5 g ai/hL]			7	n/s
Cacao	Brazil	F		[150 g ai/hL]	30–50 mL/plant	3/year	60	–
Chili	Indonesia	F	1st application at flowering	[1.25 g ai/hL]			3–4	n/s
Citrus	USA	S		17.5–112, max 112 g ai/ha/spray, max 448 g ai/year		12	30	0
Citrus	USA	Chem		17.5–112, max 112 g ai/ha/spray, max 448 g ai/year		12	30	0
Citrus seedlings	Brazil	F	Start as seedlings	[40 g ai/hL]	30–50 mL/plant	4/year	45	–
Cotton	Brazil	F	Start beginning vegetative development	7.5–12.5	200–300 grd 20–40 air	8	7	21
Cucurbits crop Group 9	USA	F		17.5–70, max 280 g ai/ha/yr	≥ 187 grd ≥ 93.5 air		7	0
Hazelnut	Italy	F	From budding and during vegetative development	25 [2.5 g ai/hL]		4	21–28	28
Herbs	France	F		12.5		3	–	10
Kiwifruit	Italy (emergency)	F		50–100 In case of application at 14 day interval use 50–75 g ai/ha [5–10 g ai/hL]		6	14–21	90
Kiwifruit	NZ	F	Apply to actively growing plants	100 [10 g ai/hL]		4	21	DO NOT apply between flowering and harvest
Kiwifruit	NZ	S	Apply to actively growing plants	100		4	21	14 (if applied after flowering, avoid drift onto crop canopy and fruit)

Crop	Country	Method	GS	Rate (g ai/ha)	Water (L/ha)	No.	Interval (days)	PHI (days)
Lettuce	USA	F		26.2–35 max 332 g ai/ha/yr	≥ 187 grd ≥ 93.5 air	4	7–10	7
Lettuce, broad-leaved endives, curly endive, rocket (all field)	France	F	After BBCH18 (8 leaves)	17.5		2		10
Low growing berry subgroup 13-07G including strawberry	USA	F	Do not apply within 5 days of transplanting	17.5–26.2 max 210 g ai/ha/yr			7–14	0
Melon	Brazil	F	Start beginning vegetative development	12.5	300–500	4	7	1
Onion	Brazil	F	Start as seedlings	12.5	500	5	7–14	14
Onion crop Group 3-07A	USA	F	Begin after thinning or after 3–4 leaf stage	26.2–35 max 140 g ai/ha/yr	≥ 187 grd ≥ 46.75 air		7–10	7
Peach	Italy	F		50–75 [5 g ai/hL]		5	7–14	7
Pear	Italy	F	From pre-flowering	75–100 [7.5 g ai/hL]		6	5–7 pre-flowering 14–28 fruiting	14
Peppers (non-Bell/chili/hot)	USA	F	Apply 26.2 g ai/ha within 1 week of transplanting or emergence, apply > 11.5 g ai/ha early season rising to 26.2 g ai/ha end of season.	11.5–26.2 max 210 g ai/ha/yr	≥ 187 grd ≥ 93.5 air	8	7	14
Peppers and other fruiting vegetables except tomato	USA	F	Apply 26.2 g ai/ha within 1 week of transplanting, apply 17.5 g ai/ha 7 days after 1st spray. Apply 11.5 g ai/ha until plans begin to bloom. Do not apply once plants begin to bloom	11.5–26.2 max 210 g ai/ha/yr	≥ 187 grd ≥ 93.5 air	8	7	14

Crop	Country	Method	GS	Rate (g ai/ha)	Water (L/ha)	No.	Interval (days)	PHI (days)
Pome	USA	F	Do not apply prior to thinning or within 5 days transplanting	17.5–112, max 112 g ai/ha/spray, max 448 g ai/year			7	60
Pome	USA	S		0.0168–0.49 g ai/tree, max 112 g ai/ha				0
Pome	USA	P2C		12.7 g ai/L				60
Potato	Brazil	F	Start beginning vegetative development	12.5	300–500	6	7	14
Potato	Indonesia	F	1st application 21 days after planting	[1.25–2.5 g ai/hL]			7	n/s
Spinach	France	F		12.5		3	–	10
Spinach	USA	F	Begin at 1st to 2nd true leaf or after thinning. Do not apply to young seedlings	17.5–26.2 max 332 g ai/ha/yr		3 consecutive	7–10	7
Tomato	Brazil	F	Staked tomatoes, when reach 1st wire. Ground tomatoes when 30 days old	[2.5 g ai/hL]	500–1000	10	5–7	5
Tomato	Indonesia	F	1st application 7–14 days after planting	[2.5 g ai/hL]			3–4	n/s
Tomato	Italy	F		12.5–25 [1.25–2.5 g ai/hL]		4	7–10	3
Tomato	USA	F	Begin 1 week after transplanting or emergence, apply > 11.5 g ai/ha early season rising to 26.2 g ai/ha end of season.	11.6–26.2	≥ 187 grd ≥ 93.5 air	8	7	14
Tomato, field	France	F		25		6	7	3
Tomato/peppers transplant	USA	DS		0.00625–0.025 g ai/cell	≥ 187 grd ≥ 93.5 air	8	7	14
Watermelon	Brazil	F	Start beginning vegetative	12.5	500	4	7–14	1

Crop	Country	Method	GS	Rate (g ai/ha)	Water (L/ha)	No.	Interval (days)	PHI (days)
			development					
Wheat	Brazil	F	Start beginning vegetative development	12.5	150 grd 20–40 air	3	14	21

USA PBI: for fruiting vegetables, Brassica (cole) crops, cucurbits, lettuce, spinach, onion (dry bulb group) and shallot—0 days. All other crops for food or feed—30 days.

F = foliar, S = soil, Chem = chemigation, DS = drip system, P2C = paint to cuts

US Onion group 3-07A = Daylily, bulb; fritillaria, bulb; garlic, bulb; garlic, great-headed, bulb; garlic, serpent, bulb; lily, bulb; onion, bulb; onion, Chinese, bulb; onion, pearl; onion, potato, bulb; shallot, bulb; cultivars, varieties, and/or hybrids of these.

US Brassica (cole) crops Group 5 = Broccoli (Brassica oleracea var. botrytis), Broccoli, Chinese (gai lon) (Brassica alboglabra), Broccoli raab (rapini) (Brassica campestris), Brussels sprouts (Brassica oleracea var. gemmifera), Cabbage (Brassica oleracea), Cabbage, Chinese (bok choy) (Brassica chinensis), Cabbage, Chinese (napa) (Brassica pekinensis), Cabbage, Chinese mustard (gai choy) (Brassica campestris), Cauliflower (Brassica oleracea var. botrytis), Cavalo broccolo (Brassica oleracea var. botrytis), Collards (Brassica oleracea var. acephala), Kale (Brassica oleracea var. acephala), Kohlrabi (Brassica oleracea var. gongylodes), Mizuna (Brassica rapa Japonica Group), Mustard greens (Brassica juncea), Mustard spinach (Brassica rapa Perviridis Group), Rape greens (Brassica napus)

US Low growing berry subgroup 13-07G = Bearberry; bilberry; blueberry, lowbush; cloudberry; cranberry; lingonberry; muntries; partridgeberry; strawberry; cultivars, varieties, and/or hybrids of these.

RESULTS OF SUPERVISED RESIDUE TRIALS ON CROPS

The Meeting received information on supervised field trials for acibenzolar-S-methyl on the following crops or crop groups:

Crop	Table No.
Citrus fruit (orange, grapefruit, lemon)	Table 54
Pome fruit (apple, pear)	Table 55
Stone fruit (peach, apricot)	Table 56
Strawberries	Table 57
Banana	Table 58
Kiwi fruit	Table 59
Onion	Table 60
Brassica vegetables (cabbage, broccoli)	Table 61–62
Fruiting vegetables, cucurbits (cucumber, melon, squash)	Table 63–65
Tomato	Table 66
Leafy vegetables (lettuce, spinach, mustard greens)	Table 67–69
Potato	Table 70
Wheat	Table 71

Trials were generally well documented with laboratory and field reports. Laboratory reports included method validation with procedural recoveries from spiking at residue levels like those occurring in samples from the supervised trials. Dates of analyses or duration of residue sample storage were also provided. Although trials included control plots, no control data are recorded in the tables except where residues in control samples exceeded the LOQ. Control samples are indicated in the summary tables with a "c". Unless stated otherwise, residue data are recorded unadjusted for recovery.

Residues and application rates have generally been rounded to two significant figures or, for residues near the LOQ, to one significant figure. Residue values from the trials conducted per maximum GAP have been used for the estimation of maximum residue levels. Those results included in the evaluation are underlined.

Conditions of the supervised residue trials were generally well reported in detailed field reports. Trial designs used non-replicated plots. Field reports provided data on the sprayers used, plot size, field sample size and sampling date.

Where duplicate field samples from an un-replicated plot were taken at each sampling time and were analysed separately, the mean of the two analytical results was taken as the best estimate of the residues in the plot and only the means are recorded in the tables. Similarly, where samples were collected from replicate plots the mean result is reported (see general consideration JMPR 2010).

Citrus fruit

Willard (2012a 50026) conducted 23 trials in the USA on citrus (12 orange; 6 grapefruit; 5 lemon) during 2010/11. Acibenzolar-S-methyl was applied four times as a WG formulation. Four applications were made as a soil-drenching application under the canopy and within the dripline of the canopy in the range of 89–118 g ai/ha and a PHI of zero days. In two of the orange trials a second plot was established with elevated application rate (ca. 560 g ai/ha) to obtain fruit samples for processing. Samples of whole fruit were analysed for residues of acibenzolar-S-methyl according to the validated method GRM051.05A. Procedural recoveries were acceptable: orange: mean = 98.7%, RSD = 3.7% (n = 6 in 0.01–1.0 mg/kg spiking range); grapefruit: mean = 97.3%, (n = 2 in 0.01–1 mg/kg spiking range); lemon: mean = 104%, RSD = 8.2% (n = 4 in 0.01–1 mg/kg spiking range). Samples from the trials were stored for a maximum of 339 days (11.1 months).

Table 54 Residues in citrus following soil-drench application using a WG formulation, Willard (2012a 50026) (replicate samples from treated plots).

Location, year, variety	No.	Rate	Spray	GS	DALA	Sample	Residues (mg/kg) [a]
CITRUS		(kg ai/ha)	volume (L/ha)	(BBCH)			
ORANGE							
Groveland Florida USA (2010) Navel [b] Last 17 Dec	4 (29 33 31)	113 114 114 112	1149 1229 1180 1108	75 77 81 89	0	fruit	< 0.01 < 0.01 (< 0.01)
Groveland Florida USA (2010) Hamlin [b] Last 17 Dec	4 (29 33 31)	112 113 112 112	1132 1218 1160 1107	75 79 81 89	0	fruit	< 0.01 < 0.01 (< 0.01)
Winter Garden Florida USA (2010) Hamlin	4 (28 32 30)	112 111 112 113	1893 1919 1878 1879	81 81 85 88	0	fruit	< 0.01 < 0.01 (< 0.01)
Chuluota, FL, USA (2010) Hamlin	4 (28 27 27)	114 112 114 112	2623 2569 2611 2573	75 78 79 83	0	fruit	< 0.01 < 0.01 (< 0.01)
Oviedo, FL, USA (2010) Navel	4 (28 27 27)	114 112 113 114	4107 4055 4078 4111	75 78 79 83	0 3 7 9 14	Fruit	< 0.01 < 0.01 (< 0.01) < 0.01 < 0.01 0.01 < 0.01
Clermont FL, USA (2010) Mid-sweet [b] Last 16 Nov	4 (27 28 27)	113 114 112 111	3303 3312 3258 3222	75 78 79 83	0	Fruit	< 0.01 < 0.01 (< 0.01)

Location, year, variety	No.	Rate	Spray	GS	DALA	Sample	Residues (mg/kg) [a]
CITRUS		(kg ai/ha)	volume (L/ha)	(BBCH)			
Clermont FL, USA (2010) Hamlin [b] Last 16 Nov	4 (27 28 27)	116 113 114 112	2663 2601 2615 2580	75 78 79 83	0	Fruit	< 0.01 < 0.01 (< 0.01)
Clermont FL, USA (2010) Mid-sweet Last 16 Dec	4 (28 32 30)	115 113 112 113	1916 1882 1870 1900	74 79 83 87	0	Fruit	< 0.01 < 0.01 (< 0.01)
Raymondville, TX, USA (2010) N-33	4 (29 31 32)	113 114 115 114	972 969 974 952	78 79 83 89	0	Fruit	< 0.01 < 0.01 (< 0.01)
Fresno, CA, USA (2010) Navel	4 (29 31 30)	112 112 112 113	1123 1118 1122 1172	72 73 85 89	0	Fruit	< 0.01 < 0.01 (< 0.01)
Orland, CA, USA (2010) Satsuma	4 (30 30 28)	112 112 112 112	1872 1882 1888 1877	90d 60d 30d Ready harv	0 3 7 10 14	Fruit	< 0.01 < 0.01 (< 0.01) < 0.01 < 0.01 < 0.01 < 0.01
Porterville, CA, USA (2010) Atwood	4 (30 30 27)	112 112 113 112	1828 1968 2037 1975	75 75 85 89	0	Fruit	< 0.01 < 0.01 (< 0.01)
LEMON							
Clermont FL, USA (2010) Bearss	4 (28 33 30)	115 115 114 115	3635 3628 3602 3637	72 79 83 85	0	Fruit	< 0.01 < 0.01 (< 0.01)
Porterville, CA, USA (2010) Pryor	4 (30 30 27)	112 112 112 112	1831 1963 1966 1977	75 75 85 89	0	Fruit	< 0.01 < 0.01 (< 0.01)
Madera, CA, USA (2010) Limco 8A Lisbon	4 (30 31 30)	112 113 114 113	1027 1035 1045 1040	73 75 81 89	0	Fruit	< 0.01 < 0.01 (< 0.01)
Lindsay, CA, USA (2010) Lisbon	4 (30 30 30)	112 112 112 114	2012 2230 2178 2330	75 75 87 89	0	Fruit	< 0.01 < 0.01 (< 0.01)
Arroyo Grande, CA, USA (2011) Eureka	4 (30 32 29)	112 112 112 113	1169 1174 1207 1179	77 79 81 85	0 3 7 10 14	Fruit	< 0.01 < 0.01 (< 0.01) < 0.01 < 0.01 < 0.01 < 0.01
GRAPEFRUIT							
Clermont, FL, USA (2010) Ray Last 16 Nov	4 (31 30 29)	113 111 112 111	2194 2156 2168 2156	75 79 81 83	0 3 7 9 14	Fruit	< 0.01 < 0.01 (< 0.01) < 0.01 < 0.01 < 0.01 < 0.01
Clermont, FL, USA (2010) White Last 17 Dec	4 (29 33 31)	113 88.7 112 113	991 759 989 993	73 75 81 89	0	Fruit	< 0.01 < 0.01 (< 0.01)
Alturas, FL, US (2010) White	4 (28 33 30)	114 114	1226 1228	75 77	0	Fruit	< 0.01 < 0.01 (< 0.01)

Location, year, variety	No.	Rate	Spray	GS	DALA	Sample	Residues (mg/kg) [a]
CITRUS		(kg ai/ha)	volume (L/ha)	(BBCH)			
		112 112	1158 1054	81 89			
Raymondville, TX, USA (2010) Rio Red	4 (29 31 32)	113 114 114 118	979 970 966 985	78 79 83 89	0	Fruit	< 0.01 < 0.01 (< 0.01)
Porterville, CA, USA (2010) Mellogold	4 (28 31 28)	116 112 112 112	1994 2010 1948 2158	75 77 81 89	0	Fruit	< 0.01 < 0.01 (< 0.01)
Sanger, CA, US (2010) Marsh White	4 (32 28 29)	112 113 112 112	1354 1389 1358 1355	76 78 81 89	0	Fruit	< 0.01 0.01 (< 0.01)

[a] Figures in brackets are means of results for replicate samples

[b] Trials not considered independent, application dates are the same

Pome fruits

Apples

Sixteen trials were conducted in Europe on apples with six applications of a WG formulation in the range of 90–123 g ai/ha and a PHI of seven days. Four of the trials in 2002 were conducted with five applications of a WG formulation, two of which included samples taken at a PHI of 7 days. Fruit samples were taken immediately after last application (0 DALA) and at intervals up to 35 DALA. Samples of whole fruit were analysed for residues derived from acibenzolar-S-methyl per methods AGRI 061 (a modification of method REM 172.11) or GRM051.01A. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 12 months (378 days).

Pears

Pear trees were treated with acibenzolar-S-methyl in eight residue trials with a WG formulation across Europe. The trials were conducted over two growing seasons and were carried out in two countries (Italy, Greece) with climates typical of Europe.

Table 55 Residues in pome fruit following foliar application of acibenzolar-S-methyl as a WG formulation (Apple: Valli & Baravelli 2003a 10070, Amic 2010ab 10020, 10019, 2011ab 10040 10039; Pear: Sack 2000ab 0725 0726, Kuhne 2001abcdef 0782 0781 0780 0779 0777 0775)

Location, year, variety	No	Rate	Water	GS	DALA	Sample	Residues (mg/kg)
POME FRUIT	(int)	(kg ai/ha)	(L/ha)	(BBCH)			
APPLE							
Solarolo, Ravenna, Italy (2002) Pink Lady	5 (16 12 14 14)	98 100 99 101 101	1177 1208 1184 1211 1209	75 76 78 79 81	–0 0 7 14 21	fruit	< 0.01 0.76 0.16 0.08 < 0.01
Isola Della Scala, Verona, Italy (2002) Pink Lady	5 (13 14 15 15)	97 99 101 97 99	1166 1188 1210 1164 1188	77 78–80 82 83 84–85	–0 0 7 14 21	fruit	0.08 1.4 0.17 0.09 0.02
Belfióre Zervio, Verona, Italy (2002) Granny Smith	5 (15 13 14 14)	99 101 100 99	1188 1210 1203 1190	77 78 81 83–84	0 15	fruit	0.59 0.09

Location, year, variety	No	Rate	Water	GS	DALA	Sample	Residues (mg/kg)
POME FRUIT	(int)	(kg ai/ha)	(L/ha)	(BBCH)			
		96	1151	84–86			
Baricella, Bologna, Italy (2002) Pink Lady	5 (14 14 14 14)	97	1159	73	0	fruit	0.77
		98	1173	75–77	14		0.06
		100	1200	79–80			
		100	1205	80			
		99	1189	86			
Obernai, Alsace, France (2009) Pink Lady	6 (4 6 5 6 4)	91	996	81–85	0	Fruit	0.08
		105	1153	81–85	7		< 0.01
		97	1057	81–85	14		< 0.01
		101	1105	83–85	21		< 0.01
		104	1138	85	35		< 0.01
		95	1038	85			< 0.01
Corzé, Maine et Loire, Pays de Loire, France (2009) Braeburn	6 (5 5 5 5 5)	105	1150	76–78	0	Fruit	0.05
		98	1074	76–78	7		0.01
		101	1112	76–78	14		< 0.01
		97	1067	77–79	21		< 0.01
		101	1115	77–78	35		< 0.01
		104	1139	79–81			
Fuilla, Pyrénées-Orientales, Languedoc-Roussillon, France (2009) Golden	6 (5 5 5 5 5)	103	1134	77–78	0	Fruit	0.08
		102	1123	79	7		0.01
		100	1102	79	14		0.01
		99	1093	79	21		0.01
		99	1085	81	35		< 0.01
		105	1150	84			
Malalbergo, Emilia Romagna, Bologna, Italy (2009) Imperatore	6 (5 4 6 5 5)	94	944	72–74	0	Fruit	0.05
		99	994	74–75	7		0.01
		98	979	79	14		< 0.01
		98	977	80	21		< 0.01
		102	1018	82	35		< 0.01
		100	998	82–84			
Blumber, Brandenburg, Germany (2010) Elstar	6 (4 6 4 6 5)	90	904	74	0	Fruit	0.06
		95	954	74	7		< 0.01
		95	954	74–75	14		< 0.01
		101	1014	74–75	21		< 0.01
		102	1018	75	35		< 0.01
		97	966	75–79			
Ensingen, Baden-Württemberg, Germany (2010) Gloster	6 (5 5 5 5 6)	98	1081	75	0	Fruit	0.06
		103	1133	75	7		< 0.01
		104	1139	76–77	14		< 0.01
		105	1150	77–78	21		< 0.01
		103	1136	78–79	35		< 0.01
		93	1022	79–86			
Innenheim, Alsace, France (2010) Braeburn	6 (4 6 4 4 6)	100	1294	77	0	Fruit	0.03
		109	1405	77	7		< 0.01
		103	1339	81	14		< 0.01
		103	1331	81	21		< 0.01
		105	1364	85	35		< 0.01
		106	1372	83–85			
St Hilaire St Mesmin, Loiret France (2010) Golden	6 (5 5 5 5 5)	104	1036	75	0	Fruit	0.03
		99	994	75	7		< 0.01
		97	967	75	14		< 0.01
		98	982	76	21		< 0.01
		99	985	77	35		< 0.01
		105	1054	78			
Les Rosiers sur Loire, Pays de la Loire, France (2010) Tentation Duplicate analyses	6 (5 6 4 5 5)	101	1108	72–73	0	Fruit	0.16 0.17 (0.17)
		96	1051	72–73	7		0.03 0.02 (0.03)
		103	1136	72–73	14		< 0.01
		104	1139	74–75	21		< 0.01
		99	1086	75–76	35		< 0.01
		103	1136	75–76			
Hollern, Niedersachsen, Germany (2010) Golden	6 (5 5 5 6 4)	109	1307	77	0	Fruit	0.04
		103	1232	78	7		< 0.01

Location, year, variety	No	Rate	Water	GS	DALA	Sample	Residues (mg/kg)
POME FRUIT	(int)	(kg ai/ha)	(L/ha)	(BBCH)			
Delicious		98 94 96 123	1173 1133 1147 1480	78–79 81–83 83 83–85	14 21 35		< 0.01 < 0.01 < 0.01
Bages, Languedoc-Roussillon, France (2010) Fuji	6 (5 5 5 5 5)	101 99 101 100 100 101	1010 988 1010 998 999 1014	71 71–72 73 73–74 75 85	0 7 14 21 35	Fruit	0.10 < 0.01 < 0.01 < 0.01 < 0.01
San Pietro in Casale, Emilia Romagna, Italy (2010) Stayman	6 (6 4 5 5 5)	101 101 102 98 102 100	1308 1313 1324 1270 1329 1304	72–73 74–75 75–76 76–81 79–82 82–85	0 7 14 21 34	Fruit	0.03 < 0.01 < 0.01 < 0.01 < 0.01
Malalbergo, Emilia Romagna, Italy (2010) Imperatore	6 (6 4 5 5 5)	96 102 106 100 102 103	964 1019 1275 1201 1223 1236	71–72 72–75 73–77 77–79 79–81 83	0 7 14 21 35	Fruit	0.02 < 0.01 < 0.01 < 0.01 < 0.01
Calatorao, Zaragoza, Spain (2010) Fuji clone 08	6 (5 6 4 5 5)	97 97 98 99 102 98	1161 1161 1179 1188 1227 1177	74–75 75 76–77 76–77 76–78 79–81	0 7 14 21 35	Fruit	0.06 < 0.01 < 0.01 < 0.01 < 0.01
Salillas de Jalon, Zaragoza, Spain (2010) Fuji clone 12	6 (5 6 4 5 5)	98 98 98 95 108 103	1176 1170 1181 1134 1292 1233	74–75 75 75–76 76–77 76–78 81–83	0 7 14 21 35	Fruit	0.04 < 0.01 < 0.01 < 0.01 < 0.01
Meauzac, Midi Pyrénées, France (2010) Pink Lady	6 (5 5 5 5 6)	101 107 102 101 95 102	1108 1181 1122 1106 1047 1122	75–79 75–79 75–81 75–83 75–85 85	0 7 14 21 35	Fruit	0.06 0.01 < 0.01 < 0.01 < 0.01
PEAR							
Monestirolo (FE) Italy (1999) Abate Fetel	4 (14 14 14)	100 100 100 100	1500 1500 1500 1500	75 76 77 77–78	0 7 14 21 21 28	Fruit	0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02
Stretti di Erachlea (VE) Italy (1999) S. Maria [a] Last spray 15 Jul 1999 Replicate samples	4 (12 11 13)	100 100 100 100	1000 1000 1000 1000	74 74–75 75 77–79	0 7 14 21 28	Fruit	0.05 < 0.02 < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02
Stretti di Erachlea (VE) Italy (1999) S. Maria [a] Last spray 15 Jul 1999 Replicate samples	8 (6 6 6 37 12 11 13)	100 100 100 100 100 100 100 100	1000 1000 1000 1000 1000 1000 1000 1000	65 69 69–71 71 74 74–75 75 77–79	0 7 14 21 28	Fruit	0.05 < 0.02 < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02
Tyrnavos, larisa, Greece	8 (5 7	97	1158	61	0	Fruit	0.06

Location, year, variety	No	Rate	Water	GS	DALA	Sample	Residues (mg/kg)
POME FRUIT	(int)	(kg ai/ha)	(L/ha)	(BBCH)			
(2000) Kristali [a]	7 83	97	1160	63	7		< 0.02
Last spray 28 Aug 2000	13 14	96	1157	64	14		< 0.02
Replicate samples	14)	97	1159	65	21		< 0.02 < 0.02
		97	1359	72–73	28		(< 0.02)
		97	1358	74			< 0.02
		97	1356	77			
		97	1359	79			
Tyrnavos, larisa, Greece	8 (5 7	97	1158	61	0	Fruit	0.03
(2000) Kristali [a]	7 83	97	1160	63	7		< 0.02
Last spray 28 Aug 2000	13 14	97	1157	64	14		< 0.02
Replicate samples	14)	97	1159	65	21		< 0.02 < 0.02
		97	1256	72–73	28		(< 0.02)
		97	1359	74			< 0.02
		97	1357	77			
		97	1358	79			
Monestirolo (FE) Italy	8 (5 6	100	1500	59–61	21	Fruit	< 0.02 < 0.02
(2000) Abate Fetel	5 76	100	1500	62–64			(< 0.02)
Last spray 10 Aug 2000	12 12	100	1500	65–67			
Replicate samples	10)	100	1500	69			
		100	1500	76			
		100	1500	76–77			
		100	1500	77			
		100	1500	79–81			
Monestirolo (FE) Italy	8 (5 6	100	1500	61	0	Fruit	0.08
(2000) Conference	5 76	100	1500	61–63	7		< 0.02
Last spray 3 Aug 2000	12 12	100	1500	65	14		< 0.02
Replicate samples	10)	100	1500	67	21		< 0.02 < 0.02
		100	1500	75–77	28		(< 0.02)
		100	1500	76–77			< 0.02
		100	1500	77			
		100	1500	77–79			
Stretti di Erachlea (VE)	8 (6 6	100	1000	65	21	Fruit	< 0.02 < 0.02
Italy (2000) Abate Fetel	6 72	100	1000	67			(< 0.02)
Last spray 16 Aug 2000	11 14	100	1000	69–71			
Replicate samples	9)	100	1200	71			
		100	1200	75–77			
		100	1300	77–79			
		100	1300	81			
		100	1300	81			
Stretti di Erachlea (VE)	8 (6 6	100	1200	65	21	Fruit	< 0.01 < 0.01
Italy (2000) Conference	6 44	100	1200	67			(< 0.01)
last spray 18 Jul 2000	11 11	100	1200	69–71			
Replicate samples	11)	100	1200	71			
		100	1200	72–74			
		100	1200	72–75			
		100	1200	75–77			
		100	1200	77–79			

[a] Trials not considered independent, variety and application dates are the same

Stone fruit

Acibenzolar-S-methyl was applied five times as a WG formulation. All trials were conducted with five applications at nominal 75 g ai/ha and a PHI of seven days. In the decline trials, peach fruit samples were taken immediately before and after the last application (0 DALA) and at nominal intervals of 3, 7 and 14 days after last application. In the harvest trials, fruit samples were taken 7 days after last application. Apricot samples were taken immediately after the last application (0 DALA) and at 3, 7, 10 and 14 DALA. Peach samples were analysed for residues derived from acibenzolar-S-methyl per the validated method AGRI 066, identical to method REM172.02. Apricot samples were analysed for residues derived from acibenzolar-S-methyl per the validated method

GRM051.01B. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 11 months (347 days).

Table 56 Residues in stone fruit following foliar application of a WG formulation of acibenzolar-S-methyl (Valli & Baravelli 2003b 0849, Baravelli 20034 0848)

Location, year, variety STONE FRUIT	N (int)	Rate kg ai/ha	Volume L/ha	GS (BBCH)	DALA	Sample	Residues (mg/kg)
PEACH							
Barbiano di Cotignola, Ravenna, Italy (2002) Fayette	5 (10 10 10 11)	74	1190	72	–0	Fruit	0.06
		74	1184	73	0		1.05
		74	1181	74	4		0.26
		73	1167	78	7		0.05
		75	1198	83	14		0.03
Minerbio, Bologna, Italy (2002) Padana	5 (11 11 10 12)	76	1218	73	–0	fruit	< 0.01
		74	1176	74	0		0.77
		73	1172	75	2		0.14
		79	1270	79	7		0.09
		75	1197	81	14		< 0.01
S Giorgio di Cesena, Forli-Cesena, Italy (2002) Rich Lady	5 (11 10 11 11)	73	1174	73	7	fruit	0.05
		72	1151	75			
		73	1174	77			
		72	1154	79–81			
		74	1190	85			
Isola della Scala, Verona, Italy (2002) Franca	5 (12 12 11 11)	74	1189	72	7	fruit	0.05
		74	1192	74			
		77	1228	76			
		76	1218	78			
		71	1133	81–83			
Barbiano di Cotignola, Ravenna, Italy (2003) Rich Lady	5 (11 11 11 12)	78	1253	72	–0	fruit	< 0.01
		74	1186	72	0		0.86
		75	1206	73–74	3		0.16
		76	1210	75	7		0.02
		79	1259	81	14		< 0.01
Minerbio, Bologna, Italy (2003) Red Moon	5 (12 10 11 10)	75	1197	72	–0	fruit	0.02
		76	1210	73	0		0.74
		72	1147	74	3		0.12
		74	1180	75–76	7		0.02
		76	1207	84	14		< 0.01
Maria Codifiume, Ferra, Italy (2003) Glohaven	5 (12 10 11 12)	78	1242	72	7	fruit	0.02
		70	1124	74			
		76	1217	75			
		70	1118	75–76			
		76	1209	84			
Sarna di Faenza, Ravenna, Italy (2003) Simphony	5 (11 12 10 11)	74	1479	73	7	fruit	0.02
		74	1479	74			
		69	1377	76			
		74	1475	78			
		77	1541	79–81			
APRICOT							
Valencia, Comunidad Valenciana, Spain (2013) Mitger de Castelló Last 3 June -652309 38.924457	5 (7 7 7 6)	76	1059	75	0	Fruit	0.27
		78	1088	76	3		0.06 0.06 0.05 (0.06)
		75	1045	77	7		0.13
		73	1015	79	10		0.11
		77	1075	81	14		0.06
Valencia, Comunidad Valenciana Spain (2013) Tadeo Last 17 June -0.39460181 39.9401585	5 (7 6 8 6)	75	1048	75	0	Fruit	0.11
		80	1122	76	3		0.14
		80	1121	77	7		0.08
		69	961	78	10		0.06
		74	1036	81	14		0.06

Location, year, variety STONE FRUIT	N (int)	Rate kg ai/ha	Volume L/ha	GS (BBCH)	DALA	Sample	Residues (mg/kg)
Ferrara, Emilia Romagna, Italy (2013) Portici Last 20 June	5 (7 7 7 7)	79	1051	75–77	0	Fruit	0.09
		77	1031	75–77	3		0.07
		73	979	77	7		0.04
		103	1376	78–79	11		0.05
		77	1020	79–81	14		0.04
Bologna, Emilia Romagna, Italy (2013) Precoce D'Imola Last 11 Jun	5 (7 7 7 7)	72	961	73–75	0	Fruit	0.13
		75	1000	74–77	3		0.08
		76	1013	75–77	7		0.06
		75	994	77–81	10		0.07
		76	1007	81–83	14		0.05

Strawberry

Ten trial were conducted in the USA on strawberries where acibenzolar-S-methyl was applied eight to nine times as a WG formulation at 24.8–29.2 g ai/ha. Samples of strawberry fruit were analysed for residues of acibenzolar-S-methyl per the validated method AG-671A. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 314 days (10.3 months).

Table 57 Residues in strawberries following foliar application of acibenzolar-S-methyl as a WG formulation (replicate analytical samples) (Thompson 2010 50155)

Location, year, variety	No	Rate	Spray volume	Growth stage at	DALA	Sample	Residues (mg/kg)
STRAWBERRIES	(int)	kg ai/ha	L/ha	application			
Marion NY, USA (2008) Allstar	8 (8 6 7 7 7 7 8)	26	285	1st leaves	0	fruit	0.02 0.03 (0.03)
		26	286	3rd trifoliate			
		26	283	4–5 trifolate			
		26	284	1st bud			
		25	280	3–5 buds/plant			
		25	279	Fruit 12.5 mm dia			
		25	280	Fruit 25 mm dia			
		26	282	Mature berries			
Arlington WI, USA (2008) Kent	8 (6 6 7 6 7 7 6)	26	248	Vegetative	0	fruit	0.04 0.02 (0.03)
		26	267	Vegetative-bud			
		26	280	Early bloom			
		28	269	Bloom			
		27	279	Bloom			
		26	272	Fruiting			
		27	285	Fruiting			
		27	280	Fruiting			
Clinton NC, USA (2008) Chandler	8 (7 6 6 7 7 6 7)	26	321	Bloom	0	fruit	0.05 0.04 (0.05)
		25	313	Early fruit			
		25	332	Fruiting			
		26	322	Fruiting			
		26	325	Fruiting			
		26	324	Fruiting			
		26	326	Fruiting			
		26	327	Fruiting			
Wimauma FL, USA (2008) Festival	8 (7 8 7 7 7 6 8)	27	383	Fruiting	0	Fruit	0.07 0.06 (0.07)
		26	367	Fruiting			
		27	380	Fruiting			
		27	389	Fruiting			
		27	384	Fruiting			
		28	390	Fruiting			
		27	385	Fruiting			
		27	381	Fruiting			
Parlier CA, USA (2008) Seascape	8 (7 7 8	27	291	Vegetative	0	Fruit	0.06 0.06 (0.06)
		26	286	Vegetative	3		0.04 0.04 0.04 0.04
		27	289	Bloom-Fruiting	7		(0.04)

Location, year, variety	No	Rate	Spray volume	Growth stage at	DALA	Sample	Residues (mg/kg)
STRAWBERRIES	(int)	kg ai/ha	L/ha	application			
	6 9 6 6)	27 27 28 27 27	273 288 281 281 295	Bloom-Fruiting Bloom-Fruiting Fruiting Fruiting Fruiting	10 14		0.02 0.02 (0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 < 0.02 (< 0.02)
Aurora OR, USA (2008) Totem	9 (7 7 6 7 6 6 9 7)	26 26 28 27 27 27 26 27 27	276 275 443 433 437 436 425 427 430	Late dormant 5% bloom 30% bloom 50% bloom Grn fruit 6% bloom left Grn fruit Green-pink fruit Fruiting 5% ripe Fruiting	0	Fruit	0.04 0.04 0.04 0.04 (<u>0.04</u>)
Salinas CA, USA (2008) Albion last spray 19 Nov 2008	8 (7 7 7 7 8 8 6)	27 27 26 26 26 26 26 26	255 282 295 362 459 448 512 554	Flowering-fruiting Flowering-fruiting Flowering-fruiting Flowering-fruiting Flowering-fruiting Flowering-fruiting Flowering-fruiting Fruit colouring	0 [a]	Fruit	0.08 0.05 (<u>0.06</u>)
Salinas CA, USA (2008) Albion last spray 24 Sept 2008	8 (6 6 7 8 8 6 6)	25 27 26 26 27 27 27 26	287 389 517 478 459 473 468 531	Flowering-fruiting Flowering-fruiting Flowering-fruiting Flowering-fruiting Flowering-fruiting Fruiting Flowering-fruiting Fruit ripening	0 [b]	Fruit	0.08 0.09 (<u>0.08</u>)
L'Acadie QB, Canada (2008) Seascape	8 (7 7 7 6 7 7 7)	27 26 27 26 25 26 26 27	360 351 356 349 348 341 3532 364	65 65 65 65 65 65 85 85	0	Fruit	0.02 0.02 (<u>0.02</u>)
Harrow ON, Canada (2008) Honeoye	8 (7 7 7 8 6 6 7)	27 28 28 27 26 28 26 29	313 324 316 312 294 316 297 333	New leaves New leaves-some flowers Some flowers Flowering Flowering-fruit set Maturing fruit Maturing fruit Maturing fruit	0	Fruit	0.02 0.02 (<u>0.02</u>)

[a] In this Salinas, CA trial, red fruit was noticeably smaller in the treated plots than in the untreated plot.

[b] In this Salinas CA trial, fruit was small and stunted in the treated plots compared to the untreated plot. In this trial, medium to large green fruit was absent from the treated plots, and the berries were stunted and red but were about one-eighth to one-quarter the size of commercially acceptable fruit.

Tank adjuvants: Marion NY, LI700; Arlington WI Herbimax; Clinton NC none; Wimauma FL none; Parlier CA Induce for last three sprays; Aurora OR Prime oil last 7 sprays; Salinas CA R-11; L'Acadie QC Agral 90; Harrow ON Agro 90.

Banana

Trials in <u>bananas</u> were conducted in South America and Malaysia.

Supervised trials in Central America

Acibenzolar-S-methyl was applied four to eight times to bagged and unbagged banana bunches, either as a WG formulation, in the 1995 trials, or as a SC formulation in the 1998 and 2000/2001 trials. Fourteen trials were conducted per the Guatemala GAP, with the numbers of applications only restricted by the vegetation period and an application interval of 35 days, at a nominal rate of 40 g ai/ha. In two of the 1998 trials a second plot was established with eight applications at an elevated rate of nominal 80 g ai/ha. Fruit samples were taken immediately after last application, when the spray deposit has dried and at up to 10 DALA. Samples of whole fruit were separated into peel and pulp, which were analysed separately for residues per method REM 172.11 (modified). Recoveries were acceptable.

Supervised trials in Malaysia

In trials conducted in Malaysia, acibenzolar-S-methyl was applied six times to bagged and unbagged banana bunches, as a WG formulation. Fruit samples were taken immediately after last application, when the spray deposit has dried and at two and seven DALA. Samples of whole fruit were separated into peel and pulp, which were analysed separately for residues per method REM 172.11 (modified).

Table 58 Residues in banana following foliar application of acibenzolar-S-methyl (Formica 1996c 0301, Anon 1996 0340, Formica 1996d 0315, Walser 1999defghijklmn 0588 0589 0590 0591 0592 0593 0594 0595 0596 0597 0598, Kuhne 2001gh 0769 0770)

Location, year, variety	N	Rate		GS	DALA	Sample	Residues (mg/kg)
BANANA		kg ai/ha	L/ha	application			
Finca Ombu, Pococi, Costa Rica (1995) Gran Naine	7 (21 21 21 21 21 21)	50 49 51 52 47 52 52	22 22 22 23 21 23 23	Before flowering until harvest	0 2 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02)
					0 2 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	0.02 0.04 0.02 (0.03) < 0.02 < 0.02 < 0.02 (< 0.02) 0.03 0.06 0.02 (0.04) 0.02 0.03 0.02 (0.02) < 0.02 < 0.02 < 0.02 (< 0.02) 0.03 0.05 0.02 (0.03) 0.03 0.03 0.02 (0.03) < 0.02 < 0.02 < 0.02 (< 0.02) 0.04 0.04 0.02 (0.03)
San Rafael, Pococi, Costa Rica (1998) Cavendish last spray 24 Nov 1998 10 18 N 83 48 W SC formulation	8 (28 28 28 29 27 28 28)	34 37 38 43 45 48 43 41	19 20 24 23 24 25 25 22	Mature fresh green	0 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02

Location, year, variety	N	Rate		GS	DALA	Sample	Residues (mg/kg)
BANANA		kg ai/ha	L/ha	application			
							(< 0.02) < 0.02 < 0.02 (< 0.02)
					0 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
		111 73 72 80 83 78 88 88	30 20 22 21 22 22 26 23	Mature fresh green	0	Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0	Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) 0.02 0.04 (0.03)
Cariari, Pococi, Costa Rica (1998) Grand Naine last spray 24 Nov 1998 10 21 N 83 42 W SC formulation	8 (28 28 28 29 27 28 28)	47 45 40 44 42 47 42 41	26 25 25 23 22 25 25 21	Mature fresh green	0 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
Roxana, Pococi, Costa Rica (1998) Valery Last spray 24 Nov 98 10 18 N 83 40 W SC formulation	8 (28 28 28 29 27 28 28)	34 50 34 43 42 40 45 39	18 28 21 23 22 22 26 21	Mature fresh green	0 1 3 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02

Location, year, variety	N	Rate		GS	DALA	Sample	Residues (mg/kg)
BANANA		kg ai/ha	L/ha	application			
					 10	Peel Fruit (bgd) Pulp Peel	(< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0 1 3 7 10	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) 0.02 0.02 (0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
Achiote, El Triunfo, Ecuador (1998) Cavendish Last spray 2 Dec 98 2 18 S 79 18 W SC formulation	8 (28 29 26 28 28 29 28)	41 45 53 52 39 38 38 41	23 25 30 29 22 22 22 23	Mature fresh green	0 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0 7	Fruit (unbgd) Pulp Peel Fruit (unbgd)	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)

Location, year, variety	N	Rate		GS	DALA	Sample	Residues (mg/kg)
BANANA		kg ai/ha	L/ha	application			
						Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
		74 82 93 70 81 84 83 78	21 23 26 20 23 24 24 22	Mature fresh green	0	Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0	Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
Lorenzo de Garaycoa, Simon Bolivar, Ecuador (1998) Cavendish Last spray 2 Dec 98 2 3 S 80 20 N SC formulation	8 (28 29 26 28 28 29 28)	50 42 43 46 38 41 45 41	28 24 24 26 22 24 26 23	Mature fresh green	0 1 3 7 10	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0 1 3 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)

Location, year, variety	N	Rate		GS	DALA	Sample	Residues (mg/kg)
BANANA		kg ai/ha	L/ha	application			
					10	Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
Lorenzo de Garaycoa, Simon Bolivar, Ecuador (1998) Cavendish Last spray 2 Dec 98 2 3 S 80 19 N SC formulation	8 (28 29 26 28 28 29 28)	39 48 46 44 41 42 43 38	22 27 26 25 24 24 24 22	Mature fresh green	0 1 3 7 10	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0 1 3 7 10	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)

Location, year, variety	N	Rate		GS	DALA	Sample	Residues (mg/kg)
BANANA		kg ai/ha	L/ha	application			
							< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
El Reposo, Cienága, Colombia (1998) Valery last spray 4 Nov 10 16 S 74 10 W SC formulation	8 (28 28 28 28 29 28 27)	35 42 44 37 43 43 44 43	17 22 23 19 22 23 23 22	Mature fresh green	0 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
Riofrio, Cienága, Colombia (1998) Grand Naine last spray 4 Nov 10 07 S 74 18 W SC formulation	8 (28 28 28 28 29 28 27)	36 44 37 43 40 42 41 44	19 23 19 23 21 22 22 23	Mature fresh green	0 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
Cuidad Hidalgo, Suchiate, Mexico	8 (29	39 42	24 27	Mature fresh green	0	Fruit (bgd) Pulp	< 0.02 < 0.02 (< 0.02)

Location, year, variety	N	Rate		GS	DALA	Sample	Residues (mg/kg)
BANANA		kg ai/ha	L/ha	application			
(1998) Grand Naine SC formulation	29 26 26 28 28 28)	43 38 38 43 43 36	30 24 24 25 28 26		 7	Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
Tiquisate, Tiquisate, Guatemala (1998) Grand Naine Last spray 8 Jan 99 91 25 W 14 10 N SC formulation	8 (28 29 27 28 28 28 30)	35 37 39 34 41 37 39 37	22 23 24 21 25 23 24 23	Mature fresh green	0 1 3 7 10	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0 1 3 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)

Location, year, variety	N	Rate		GS	DALA	Sample	Residues (mg/kg)
BANANA		kg ai/ha	L/ha	application			
					10	Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
	8 (28 29 27 28 28 28 30)	35 34 39 39 42 46 41 36	22 21 24 24 25 27 25 22	Mature fresh green	0 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
					0 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 (< 0.02)
Rivière Lézarde, Central Region, Martinique (2000) Grand Nain last spray 15 Jan 2001 SC formulation	4 (36 35 37)	40 40 40 40	28 25 25 25	Pre-flower Pre-harvest Pre-harvest Mature	0 3 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02
					0 3 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02
Le Francois, South Region, Martinique (2000) Grand Nain last spray 15 Jan 2001	4 (36 35 37)	40 40 40 40	30 25 25 25	Pre-flower Pre-harvest Pre-harvest Mature	0 3	Fruit (bgd) Pulp Peel Fruit (bgd)	< 0.02 < 0.02 < 0.02 < 0.02

Location, year, variety	N	Rate		GS	DALA	Sample	Residues (mg/kg)
BANANA		kg ai/ha	L/ha	application			
SC formulation					 7	Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 < 0.02 < 0.02 < 0.02
					0 3 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	< 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02
Rembaur, Negeri Sembilan, Malaysia (1995) Cavendish last spray 19 Dec	6 (21 21 21 21 21)	50 50 50 50 50 50	23 23 23 23 23 23	39–61 39–61 51–71 51–71 71–75 97–98	0 2 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 0.02 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 < 0.02 (< 0.02)
					0 2 7	Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel Fruit (unbgd) Pulp Peel	0.07 0.04 0.03 (0.05) < 0.02 < 0.02 < 0.02 (< 0.02) 0.13 0.06 0.05 (0.08) 0.07 0.02 0.04 (0.05) 0.05 0.04 < 0.02 < 0.02 (0.03) 0.11 0.03 0.06 (0.07) 0.04 0.03 0.03 (0.03) 0.02 0.02 < 0.02 < 0.02 (0.02) 0.07 0.04 0.04 (0.05)
Johor Baharu, Rembau, Malaysia (1995) Cavendish last spray 23 Dec	6 (28 19 23 20 23)	50 50 50 50 50 50	23 23 23 23 23 23	51–59 60–69 70–71 73–79 79–81 83–87	0 2 7	Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel Fruit (bgd) Pulp Peel	< 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02) < 0.02 < 0.02 < 0.02 (< 0.02)
					0	Fruit (unbgd) Pulp	0.05 0.02 0.04 (0.04) 0.02 < 0.02 0.02

Location, year, variety	N	Rate		GS	DALA	Sample	Residues (mg/kg)
BANANA		kg ai/ha	L/ha	application			
						Peel	(0.02)
					2	Fruit (unbgd)	0.09 0.03 0.06 (0.06)
						Pulp	0.04 0.02 0.03 (0.03)
						Peel	0.02 < 0.02 0.02
					7	Fruit (unbgd)	(0.02)
						Pulp	0.07 0.03 0.04 (0.05)
						Peel	0.04 0.02 0.02 (0.03)
							0.02 < 0.02 < 0.02
							(0.02)
							0.07 0.03 0.03 (0.04)

Residues in whole fruit were calculated based on weight of pulp and peel and residues in pulp and peel.

Adjuvants: Tiquisate, Guatemala, Orchex spray oil; Suchiate Mexico Pemex Citrolina spray oil; Simon Bolivar Ecuador Aceite Agricola spray oil; El Triunfo Ecuador Aceite Agricola spray oil; Pococi Costa Rica Mobil Prorex spray oil; Cienaga Columbia Banole (Total) spray oil; Martinique Texaco Emulsified spray oil; Malaysia mineral oil or none

Kiwi fruit

Trials on kiwifruit conducted in Europe and New Zealand were made available.

Supervised trials in France, Italy, Portugal and Spain (Europe)

Eight trials were conducted in Europe during 2013 using a WG formulation. Eight applications in the range of 91–111 g ai/ha were made, with spray intervals of 10–18 days and a PHI at harvest of 59–64 days. In all trials, a second plot was established where acibenzolar-S-methyl was applied eight times by spray drench application or fertigation to soil at a nominal rate of 100 g ai/ha and a PHI of zero days. Samples of kiwifruits were taken immediately after the last application and at nominally 10, 20, 40 and 60 DALA. Samples of whole fruit were separated into peel and pulp, which were analysed separately for residues per method GRM051.01B. Procedural recoveries were acceptable. Samples from the trials were stored for less than 11 months from sampling to analysis.

Supervised trials in New Zealand

In New Zealand trials acibenzolar-S-methyl was applied four times by spray application as a WG formulation. All trials were conducted by foliar application at the critical use rate of 100 g ai/ha and at an elevated rate of 200 g ai/ha in a second plot. In a third and fourth plot of each trial, acibenzolar-S-methyl was applied four times by soil application at rates of 100 and 200 g ai/ha. In the eight trials performed in 2011, all applications were done post-blossom, whereas in the six trials performed in 2012, all applications were done pre-blossom until up to 15% blossom opening. Samples of whole kiwifruits were taken at four to five sampling intervals in the range of 13 to 191 DALA with the latest sampling at normal commercial harvest. Samples of whole fruit were analysed for residues per method KB item 30880 that is based on method GRM051.01A. Procedural recoveries were acceptable. Samples from the trials were stored for up to 7.7 months (233 days) from sampling to analysis.

Table 59 Residues of acibenzolar-S-methyl in kiwifruit following foliar or soil applications (Amic 2014 10563, Haller 2012 10300, Munro 2013 10544)

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
Picassent, Valencia, Spain (2013) Green Lay	8 (13 13 14 14 14 13 14)	109 Foliar	1634	59	0	Peel	1.13
		100 Foliar	1497	67	0	Pulp	0.03
		101 Foliar	1519	71	0	Whole fruit	0.20
		93 Foliar	1394	72	10	Peel	0.85
		101 Foliar	1511	73	10	Pulp	0.02
		105 Foliar	1579	74	10	Whole fruit	0.10
		93 Foliar	1397	75	21	Peel	0.47
		101 Foliar	1508	78	21	Pulp	0.05
					21	Whole fruit	0.09
					42	Peel	0.26
					42	Pulp	0.02
					42	Whole fruit	0.05
					64	Peel	0.16
					64	Pulp	0.01
					64	Whole fruit	0.03
	8 (8 22 15 14 14 14 12)	100 soil	10/vine	74	0	Peel	0.02
		100 soil	10/vine	75		Pulp	< 0.01
		100 soil	10/vine	77		Whole fruit	0.01
		100 soil	10/vine	79			
		100 soil	10/vine	81			
		100 soil	10/vine	85			
		100 soil	10/vine	88			
		100 soil	10/vine	89			
Carlet, Valencia, Spain (2013) Hayward	8 (13 13 14 14 14 13 14)	93 foliar	1389	61	0	Peel	0.99
		97 foliar	1450	69	0	Pulp	0.02
		100 foliar	1503	71	0	Whole fruit	0.12
		96 foliar	1446	72	10	Peel	0.57
		96 foliar	1441	73	10	Pulp	0.02
		106 foliar	1585	74	10	Whole fruit	0.07
		99 foliar	1491	75	21	Peel	0.18
		100 foliar	1500	78	21	Pulp	0.02
					21	Whole fruit	0.04
					42	Peel	0.27
					42	Pulp	0.02
					42	Whole fruit	0.04
					64	Peel	0.21
					64	Pulp	0.02
					64	Whole fruit	0.03
	8 (15 15 15 14 14 14 12)	100 soil	10/vine	74	0	Peel	< 0.01
		100 soil	10/vine	75		Pulp	< 0.01
		100 soil	10/vine	77		Whole fruit	< 0.01
		100 soil	10/vine	79			
		100 soil	10/vine	81			
		100 soil	10/vine	85			
		100 soil	10/vine	88			
		100 soil	10/vine	89			
Valença do Minho, Ganfei, Portugal (2013) Hayward	8 (14 14 14 15 13 13 15)	99 foliar	1482	70–72	0	Peel	2.10
		96 foliar	1437	73–74	0	Pulp	0.07
		99 foliar	1489	75–76	0	Whole fruit	0.34
		94 foliar	1411	76–77	9	Peel	1.33
		91 foliar	1366	78–79	9	Pulp	0.06
		95 foliar	1431	79–80	9	Whole fruit	0.23
		97 foliar	1451	80–81	21	Peel	1.18
		96 foliar	1438	82–83	21	Pulp	0.08
					21	Whole fruit	0.24
					43	Peel	0.84
					43	Pulp	0.04
					43	Whole fruit	0.14
					61	Peel	0.73
					61	Pulp	0.04
					61	Whole fruit	0.12

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
	8 (15 14 14 14 15 14 13)	100 soil	10/vine	77–80	0	Peel	< 0.01
		100 soil	10/vine	81–82		Pulp	< 0.01
		100 soil	10/vine	83–84		Whole fruit	< 0.01
		100 soil	10/vine	85–86			
		100 soil	10/vine	85–86			
		100 soil	10/vine	86–87			
		100 soil	10/vine	87–88			
		100 soil	10/vine	88–89			
Amares, Enre Douro E Minho, Portugal (2013) Hayward	8 (14 14 14 15 13 13 15)	99 foliar	1485	70–71	0	Peel	1.54
		101 foliar	1522	71–72	0	Pulp	0.06
		96 foliar	1436	72–73	0	Whole fruit	0.30
		100 foliar	1500	73–74	9	Peel	1.56
		95 foliar	1420	75–76	9	Pulp	0.10
		100 foliar	1511	79–80	9	Whole fruit	0.31
		101 foliar	1501	78–79	21	Peel	1.39
		92 foliar	1382	80–81	21	Pulp	0.07
					21	Whole fruit	0.27
					43	Peel	0.76
					43	Pulp	0.05
					43	Whole fruit	0.15
					61	Peel	0.76
					61	Pulp	0.08
					61	Whole fruit	0.17
	8 (15 14 14 14 15 14 13)	100 soil	10/vine	77–78	0	Peel	< 0.01
		100 soil	10/vine	80–81		Pulp	< 0.01
		100 soil	10/vine	82–83		Whole fruit	< 0.01
		100 soil	10/vine	84–85			
		100 soil	10/vine	85–86			
		100 soil	10/vine	86–87			
		100 soil	10/vine	87–88			
		100 soil	10/vine	88–89			
Veneto, Verona, Italy (2013) Summer 3373	8 (14 14 14 14 14 14 14)	101 foliar	1514	63–65	0	Peel	0.44
		106 foliar	1589	65–66	0	Pulp	0.02
		100 foliar	1493	65–69	0	Whole fruit	0.07
		102 foliar	1529	67–69	10	Peel	0.19
		98 foliar	1475	71–72	10	Pulp	0.02
		111 foliar	1668	74	10	Whole fruit	0.04
		103 foliar	1546	75–77	21	Peel	0.12
		92 foliar	1375	77–78	21	Pulp	0.01
					21	Whole fruit	0.03
					42	Peel	0.14
					42	Pulp	0.01
					42	Whole fruit	0.03
					59	Peel	0.10
					59	Pulp	< 0.01
					59	Whole fruit	0.02
	8 (14 14 14 14 13 15 13)	100 soil	10/vine	72	0	Peel	0.01
		100 soil	10/vine	76		Pulp	< 0.01
		100 soil	10/vine	77		Whole fruit	0.01
		100 soil	10/vine	77–78			
		100 soil	10/vine	77–78			
		100 soil	10/vine	81–82			
		100 soil	10/vine	85–86			
		100 soil	10/vine	89			

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
Emilia Romagna, Bologna, Italy (2013) Hayward	8 (14 14 16 12 14 14 15)	102 foliar	1532	69	0	Peel	0.26
		105 foliar	1568	72–73	0	Pulp	0.01
		100 foliar	1504	74–75	0	Whole fruit	0.02
		101 foliar	1518	76–77	10	Peel	0.16
		100 foliar	1504	77–78	10	Pulp	0.01
		103 foliar	1550	79–81	10	Whole fruit	0.04
		109 foliar	1636	81–83	21	Peel	0.17
		97 foliar	1454	82–84	21	Pulp	0.02
					21	Whole fruit	0.04
					42	Peel	0.10
					42	Pulp	0.01
					42	Whole fruit	0.03
					59	Peel	0.05
					59	Pulp	< 0.01
					59	Whole fruit	0.01
	8 (13 15 14 14 14 13 15)	100 soil	10/vine	77–78	0	Peel	< 0.01
		100 soil	10/vine	79–81		Pulp	< 0.01
		100 soil	10/vine	81–83		Whole fruit	< 0.01
		100 soil	10/vine	82–84			
		100 soil	10/vine	84–85			
		100 soil	10/vine	85–86			
		100 soil	10/vine	86–87			
		100 soil	10/vine	89			
Tarn et Garonne, Midi–Pyrénées, France (2013) Howard	8 (18 10 11 14 14 15 14)	95 foliar	1431	65–69	0	Peel	1.45
		101 foliar	1511	71	0	Pulp	0.05
		98 foliar	1470	73–74	0	Whole fruit	0.25
		101 foliar	1521	74	10	Peel	0.50
		101 foliar	1515	75–77	10	Pulp	0.02
		99 foliar	1489	77	10	Whole fruit	0.09
		101 foliar	1508	78	20	Peel	0.95
		92 foliar	1375	79	20	Pulp	0.03
					20	Whole fruit	0.13
					41	Peel	0.49
					41	Pulp	0.02
					41	Whole fruit	0.07
					59	Peel	0.48
					59	Pulp	0.02
					59	Whole fruit	0.07
	8 (14 14 14 14 14 14 14)	100 soil	10/vine	75	0	Peel	< 0.01
		100 soil	10/vine	77–78		Pulp	< 0.01
		100 soil	10/vine	78		Whole fruit	< 0.01
		100 soil	10/vine	79			
		100 soil	10/vine	79–80			
		100 soil	10/vine	79			
		100 soil	10/vine	79–81			
		100 soil	10/vine	87–89			
Latina, Lazio, Italy (2013) Hayward	8 (14 15 13 14 13 15 14)	106 foliar	1859	71–72	0	Peel	1.66
		100 foliar	1505	72–73	0	Pulp	0.07
		99 foliar	1481	72–73	0	Whole fruit	0.41
		102 foliar	1526	73–74	11	Peel	2.12
		95 foliar	1425	74–75	11	Pulp	0.09
		100 foliar	1496	77	11	Whole fruit	0.37
		104 foliar	1558	78	21	Peel	2.51
		101 foliar	1514	79	21	Pulp	0.11
					21	Whole fruit	0.40
					42	Peel	< 0.01
					42	Pulp	< 0.01
					42	Whole fruit	< 0.01
					60	Peel	0.65
					60	Pulp	0.05
					60	Whole fruit	0.16

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
	8 (14 14 14 14 14 14 14)	100 soil 100 soil 100 soil 100 soil 100 soil 100 soil 100 soil 100 soil	10/vine 10/vine 10/vine 10/vine 10/vine 10/vine 10/vine 10/vine	75 78 78 79 79 81 83 89	0	Peel Pulp Whole fruit	< 0.01 < 0.01 < 0.01
Whangarata Rd, Tuakau, South Auckland NZ (2011) Hort 16A Last foliar 14 Feb 12 -37.26714 174.96310	4 (21 19 20)	99 foliar 100 foliar 99 foliar 100 foliar	745 1004 996 1003	42 days post fruit set Fruit 60 mm Fruit 65 mm Fruit 70 mm	13 71 100 120 133	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.64 0.24, 0.26 (0.25) 0.13 0.13 0.10
	4 (21 19 20)	201 foliar 199 foliar 199 foliar 199 foliar	755 1001 1001 1000	42 days post fruit set Fruit 60 mm Fruit 65 mm Fruit 70 mm	13 71 100 120 133	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	1.41 0.38 0.33, 0.33 (0.33) 0.27 0.20
	4 (21 19 20)	99 soil 100 soil 100 soil 100 Soil	215 232 236 222	42 days post fruit set Fruit 60 mm Fruit 65 mm Fruit 70 mm	13 71 100 120 133	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.02 < 0.01 < 0.01 < 0.01 < 0.01
	4 (21 19 20)	199 soil 199 soil 200 soil 199 Soil	215 232 236 222	Fruit 60 mm Fruit 65 mm Fruit 70 mm 42 days post fruit set	13 71 100 120 133	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.02 0.02 < 0.01 < 0.01 < 0.01
Whangarata Rd, Tuakau, South Auckland NZ (2011) Hayward Last spray 14 Feb 12 -37.26540 174.96217	4 (21 19 20)	99 foliar 99 foliar 100 foliar 99 foliar	743 995 1003 1003	10 days post fruit set Fruit 60 mm Fruit 60 mm Fruit 60 mm	13 71 100 120 133	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.51 0.19 0.15 0.13 0.15
	4 (21 19 20)	199 foliar 200 foliar 198 foliar 200 foliar	746 1003 995 1000	10 days post fruit set Fruit 60 mm Fruit 60 mm Fruit 60 mm	13 71 100 120 133	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	1.81 0.64 0.59, 0.55 (0.57) 0.58 0.36

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
	4 (21 19 20)	99 soil 100 soil 100 soil 100 soil	215 232 236 222	10 days post fruit set Fruit 60 mm Fruit 60 mm Fruit 60 mm	13 71 100 120 133	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.01 < 0.01 < 0.01 < 0.01 0.01
	4 (21 19 20)	199 soil 199 soil 200 soil 199 soil	215 232 236 222	Fruit 60 mm Fruit 60 mm Fruit 60 mm 10 days post fruit set	13 71 100 120 133	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.02, 0.02 (0.02) < 0.01 < 0.01 < 0.01 < 0.01
Bruntwood Dr, Tauranga, Bay of Plenty NZ (2011) Gold 3 Last foliar 29 Feb 12 -37.66900 176.03279	4 (21 21 20)	100 foliar 99 foliar 99 foliar 99 foliar	991 994 997 989	Fruit 50mm Fruit 60 mm Fruit 60 mm Fruit 70 mm	14 70 100 121	Whole fruit Whole fruit Whole fruit Whole fruit	0.18 0.23 0.09 0.04
	4 (21 21 20)	197 foliar 196 foliar 198 foliar 198 foliar	988 986 992 992	Fruit 50mm Fruit 60 mm Fruit 60 mm Fruit 70 mm	14 70 100 121	Whole fruit Whole fruit Whole fruit Whole fruit	0.53 0.41, 0.38 (0.40) 0.19 0.14
	4 (21 21 20)	99 soil 99 soil 99 soil 99 Soil	216 217 220 223	Fruit 50mm Fruit 60 mm Fruit 60 mm Fruit 70 mm	14 70 100 121	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01
	4 (21 21 20)	198 soil 199 soil 200 soil 199 Soil	216 217 220 223	Fruit 50mm Fruit 60 mm Fruit 60 mm Fruit 70 mm	14 70 100 121	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01
Knyvett Rd, Kati Kati, Bay of Plenty NZ (2011) Green 14 Last foliar 29 Feb 12 -37.64170 175.94740	4 (21 21 20)	100 foliar 99 foliar 98 foliar 99 foliar	1003 991 988 997	Fruit 50mm Fruit 60 mm Fruit 60 mm Fruit 70 mm	14 70 100 121	Whole fruit Whole fruit Whole fruit Whole fruit	0.22 0.13 0.08 0.06
	4 (21 21 20)	200 foliar 199 foliar 197 foliar 199 foliar	1005 998 991 1000	Fruit 50mm Fruit 60 mm Fruit 60 mm Fruit 70 mm	14 70 100 121	Whole fruit Whole fruit Whole fruit Whole fruit	0.39 0.26, 0.18 (0.22) 0.17 0.10

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
	4 (21 21 20)	99 soil 99 soil 99 soil 99 soil	216 217 220 223	Fruit 50mm Fruit 60 mm Fruit 60 mm Fruit 70 mm	14 70 100 121	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01
	4 (21 21 20)	198 soil 199 soil 200 soil 199 soil	216 217 220 223	Fruit 50mm Fruit 60 mm Fruit 60 mm Fruit 70 mm	14 70 100 121	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01
Belk Rd, Tauranga, Bay of Plenty NZ (2011) Hort 16A Gold Last foliar 15 Feb 12 -37.785267 176.094250	4 (18 19 20)	99 foliar 99 foliar 100 foliar 99 foliar	1482 1491 1500 1482	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	0.07, 0.08 (0.08) 0.03 0.02 0.02
	4 (18 19 20)	198 foliar 198 foliar 198 foliar 196 foliar	1491 1491 1491 1473	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	0.12 0.04, 0.05 (0.05) 0.04 0.03
	4 (18 19 20)	99 soil 99 soil 99 soil 99 soil	299 299 299 299	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	0.02 < 0.01 0.01 < 0.01
	4 (18 19 20)	199 soil 199 soil 199 soil 199 soil	299 299 299 299	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01
Griffin Rd, Tauranga, Bay of Plenty NZ (2011) Hort 16A Gold Last foliar 15 Feb 12 -37.775433 176.081900	4 (18 19 21)	99 foliar 100 foliar 98 foliar 98 foliar	1491 1500 1473 1473	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	0.07 0.04 < 0.01 < 0.01
	4 (18 19 21)	198 foliar 199 foliar 195 foliar 196 foliar	1491 1500 1464 1473	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	0.09 0.07, 0.06 (0.07) 0.03 0.01
	4 (18 19 21)	99 soil 99 soil 99 soil 99 Soil	299 299 299 299	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01
	4 (18 19 21)	199 soil 199 soil 199 soil 199 Soil	299 299 299 299	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01
Melville Rd, Tauranga, Bay of Plenty NZ (2011) Hayward Last foliar 15 Feb 12 -37.778433 176.093833	4 (18 19 21)	98 foliar 98 foliar 97 foliar 97 foliar	1473 1473 1464 1464	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	0.12 0.10 0.04 0.05
	4 (18 19 21)	195 foliar 196 foliar 196 foliar 195 foliar	1464 1473 1473 1464	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	0.36 0.21, 0.19 (0.20) 0.04 0.08
	4 (18 19 21)	99 soil 99 soil 99 soil 99 soil	299 299 299 299	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
	4 (18 19 21)	199 soil 199 soil 199 soil 199 soil	299 299 299 299	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01
Belk Rd, Tauranga, Bay of Plenty NZ (2011) Hayward Last foliar 15 Feb 12 -37.784533 176.093283	4 (18 19 21)	99 foliar 99 foliar 98 foliar 100 foliar	1491 1491 1473 1500	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	0.15 0.14 0.05 0.04
	4 (18 19 21)	199 foliar 198 foliar 196 foliar 197 foliar	1500 1491 1473 1482	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	0.42 0.12, 0.11 (0.12) 0.05 0.04
	4 (18 19 21)	99 soil 99 soil 99 soil 99 soil	299 299 299 299	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01
	4 (18 19 21)	199 soil 199 soil 199 soil 199 soil	299 299 299 299	74 75 76 77	16 68 99 127	Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01
Whangarata Rd, Tuakau NZ (2012) Hort 16A last foliar 14 Oct 12	4 (9 10 9)	100 (foliar) 100 (foliar) 100 (foliar) 100 (foliar)	1000 1000 1000 1000	10% at 10 cm extn 5–7 mm flower buds 10 mm flower buds 15% open bloom	29 43 58 100 191	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.14 0.05 0.02 < 0.01 < 0.01
	4 (9 10 9	200 (foliar) 200 (foliar) 200 (foliar) 200 (foliar)	1000 1000 1000 1000	10% at 10 cm extn 5–7 mm flower buds 10 mm flower buds 15% open bloom	29 43 58 100 191	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.34 0.12 0.03 0.02 0.01
	4 (9 10 9	100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300	10% at 10 cm extn 5–7 mm flower buds 10 mm flower buds 15% open bloom	29 43 58 100 191	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01
	4 (9 10 9	200 (soil) 200 (soil) 200 (soil) 200 (soil)	300 300 300 300	10% at 10 cm extn 5–7 mm flower buds 10 mm flower buds 15% open bloom	29 43 58 100 191	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01, < 0.01 (< 0.01) < 0.01 < 0.01, < 0.01 (< 0.01) < 0.01 < 0.01

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
	8 (21 21 21 21 21 21 22)	100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300 300 300 300 300	15 mm fruit size Fruit 40 mm Fruit 60 mm Fruit 60 mm Fruit 60 mm Fruit 60 mm mature fruit mature fruit	Prior 1st application 14	Whole fruit Whole fruit	< 0.01 < 0.01
Whangarata Rd, Tuakau NZ (2012) Hayward Last foliar 12 Nov 12	4 (10 12 26)	100 (foliar) 100 (foliar) 100 (foliar) 100 (foliar)	1000 1000 1000 1000	5–10 cm extn 20 cm extn 10 mm flower buds 1% open bloom	29 42 56 100 183	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.10 0.07 0.03 0.01 < 0.01, < 0.01 (< 0.01)
		200 (foliar) 200 (foliar) 200 (foliar) 200 (foliar)	1000 1000 1000 1000	5–10 cm extn 20 cm extn 10 mm flower buds 1% open bloom	29 42 56 100 183	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.41 0.05 0.03 0.01 < 0.01
	4 (10 12 26)	100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300	5–10 cm extn 20 cm extn 10 mm flower buds 1% open bloom	29 42 56 100 183	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.11 0.02 < 0.01 < 0.01 < 0.01
		200 (soil) 200 (soil) 200 (soil) 200 (soil)	300 300 300 300	5–10 cm extn 20 cm extn 10 mm flower buds 1% open bloom	29 42 56 100 183	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.33 0.02 0.01, 0.01 (0.01) < 0.01 < 0.01
	6 (26 21 21 21 21)	100 (foliar) 100 (foliar) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	1000 1000 300 300 300 300	10 mm flower buds 1% open bloom fruit set Fruit 40 mm Fruit 50 mm Fruit 60 mm	–0 (pre-1st soil) 28 42 57 99	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.86 < 0.01 < 0.01 < 0.01 < 0.01

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
	8 (21 21 21 21 21 22 20)	100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300 300 300 300 300	fruit set Fruit 40 mm Fruit 50 mm Fruit 60 mm Fruit 60 mm Full size fruit mature fruit mature fruit	–0 (pre-1st soil) 15	Whole fruit Whole fruit	0.43 < 0.01
Bruntwood Rd, Omokoroa NZ (2012) G3 Last foliar 26 Oct 13	4 (12 9 9)	100 (foliar) 100 (foliar) 100 (foliar) 100 (foliar)	1000 1000 1000 1000	10–12 cm extn 15 cm extn 20 cm extn 30 cm extn	29 42 56 100 188	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.37 0.04 0.02 0.02 < 0.01
	4 (12 9 9)	200 (foliar) 200 (foliar) 200 (foliar) 200 (foliar)	1000 1000 1000 1000	10–12 cm extn 15 cm extn 20 cm extn 30 cm extn	29 42 56 100 188	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.82 0.13 0.12, 0.11 (0.12) 0.03 < 0.01
	4 (12 9 9)	100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300	10–12 cm extn 15 cm extn 20 cm extn 30 cm extn	29 42 56 100 188	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.11 0.02 < 0.01 < 0.01 < 0.01
	4 (12 9 9)	200 (soil) 200 (soil) 200 (soil) 200 (soil)	300 300 300 300	10–12 cm extn 15 cm extn 20 cm extn 30 cm extn	29 42 56 100 188	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.10 0.02 < 0.01 < 0.01, < 0.01 (< 0.01) < 0.01
	5 (37 21 21 21)	100 (foliar) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	1000 300 300 300 300	20 cm extn fruit set Fruit 40 mm Fruit 50 mm Fruit 60 mm	–0 (pre-1st soil) 28 42 55 97	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.13 < 0.01 < 0.01 < 0.01 < 0.01
	8 (21 21 21 21 21 20 23)	100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300 300 300 300 300	fruit set Fruit 40 mm Fruit 50 mm Fruit 60 mm Fruit 70 mm Full size fruit mature fruit mature fruit	–0 (pre-1st soil) 12	Whole fruit Whole fruit	0.05 < 0.01
Knyvett Rd, Kati Kati NZ (2012) G14 Last foliar 7 Nov 12	4 (12 9 21)	100 (foliar) 100 (foliar) 100 (foliar) 100 (foliar)	1000 1000 1000 1000	5–10 cm extn 15 cm extn 20 cm extn 3% flowering	29 42 56 100 176	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.07 0.01 < 0.01 < 0.01 < 0.01

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
	4 (12 9 21)	200 (foliar) 200 (foliar) 200 (foliar) 200 (foliar)	1000 1000 1000 1000	5–10 cm extn 15 cm extn 20 cm extn 3% flowering	29 42 56 100 176	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.29 0.06, 0.07 (0.07) 0.02 0.03 0.03
	4 (12 9 21)	100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300	5–10 cm extn 15 cm extn 20 cm extn 3% flowering	29 42 56 100 176	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01
	4 (12 9 21)	200 (soil) 200 (soil) 200 (soil) 200 (soil)	300 300 300 300	5–10 cm extn 15 cm extn 20 cm extn 3% flowering	29 42 56 100 176	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01
	6 (21 16 21 21)	100 (foliar) 100 (foliar) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	1000 1000 300 300 300 300	20 cm extn 3% flowering fruit set Fruit 25 mm Fruit 50 mm Fruit 60 mm	–0 (pre-1st soil) 28 42 55 98	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.44 < 0.01 < 0.01 < 0.01 < 0.01
	8 (21 21 21 21 20 23)	100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300 300 300 300 300	fruit set Fruit 25 mm Fruit 50 mm Fruit 60 mm Fruit 60 mm Fruit 60 mm Fruit 60 mm mature fruit	–0 (pre-1st soil) 12	Whole fruit Whole fruit	< 0.01 < 0.01, < 0.01 (< 0.01)
Melville Rd, Tauriko, Tauranga NZ (2012) Hort 16A Last foliar 18 Oct 12	3 (19 16)	100 (foliar) 100 (foliar) 100 (foliar)	1500 1500 1500	Shoots Shoots pre-flower	28 42 56 100 172	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.11 0.02 < 0.01 < 0.01 < 0.01
	3 (19 16)	200 (foliar) 200 (foliar) 200 (foliar)	1500 1500 1500	Shoots Shoots pre-flower	28 42 56 100 172	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.12 0.04 0.01 < 0.01 < 0.01
	3 (19 16)	100 (soil) 100 (soil) 100 (soil)	300 300 300	Shoots Shoots pre-flower	28 42 56 100 172	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01

Crop (Variety) KIWIFRUIT	N	Application Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Matrix	Residue (mg/kg) [a]
	3 (19 16)	200 (soil) 200 (soil) 200 (soil)	300 300 300	Shoots Shoots pre-flower	28 42 56 100 172	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01
	5 (33 13 16 26)	100 (foliar) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	1500 300 300 300 300	pre-flower fruit fruit fruit fruit	28 42 56 100	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01, < 0.01 (< 0.01) < 0.01 < 0.01 < 0.01 < 0.01
	8 (13 16 26 17 12 25 19)	100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300 300 300 300 300	Fruit Fruit Fruit Fruit Fruit Fruit Fruit fruit	–0 (pre-1st soil) 14	Whole fruit Whole fruit	< 0.01 < 0.01, < 0.01 (< 0.01)
Melville Rd, Tauriko, Tauranga, NZ (2012) Hayward Last foliar 15 Nov 12	4 (16 13 15)	100 (foliar) 100 (foliar) 100 (foliar) 100 (foliar)	1500 1500 1500 1500	Shoots Shoots Flower Bud pre-flower	28 42 56 100 148	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.15 0.02 0.02 < 0.01 < 0.01
	4 (16 13 15)	200 (foliar) 200 (foliar) 200 (foliar) 200 (foliar)	1500 1500 1500 1500	Shoots Shoots Flower Bud pre-flower	28 42 56 100 148	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	0.64, 0.70 (0.67) 0.05 0.02 0.02 < 0.01
	4 (16 13 15)	100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300	Shoots Shoots Flower Bud pre-flower	28 42 56 100 148	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01
	4 (16 13 15)	200 (soil) 200 (soil) 200 (soil) 200 (soil)	300 300 300 300	Shoots Shoots Flower Bud pre-flower	28 42 56 100 148	Whole fruit Whole fruit Whole fruit Whole fruit Whole fruit	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01
	5 (34 19 17 12)	100 (foliar) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	1500 300 300 300 300	pre-flower fruit fruit fruit fruit	28 42 56 100	Whole fruit Whole fruit Whole fruit Whole fruit	0.15 < 0.01 < 0.01 < 0.01 < 0.01
	8 (19 17 12 22 19 14 22)	100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil) 100 (soil)	300 300 300 300 300 300 300 300	Fruit Fruit Fruit Fruit Fruit Fruit Fruit fruit	–0 (pre-1st soil) 14	Whole fruit Whole fruit	< 0.01 < 0.01, < 0.01 (< 0.01)

[a] Residue in whole fruit calculated as follows: (Total amount (mg) Kiwi peel + Kiwi pulp)/(total weight (kg) fruit)

In the calculation of residues in whole fruit, individual residues of < 0.01 mg/kg have been assigned a value of 0.01 mg/kg, except where peel and pulp contained residues < 0.01 mg/kg, in which case the overall residue is also < 0.01 mg/kg.

Onions

Eleven supervised trials on onions were carried out in the USA. Acibenzolar-S-methyl was applied as four foliar applications at 33.6–38.1 g ai/ha as a WG formulation. Samples of onion bulb were collected from the treated plots 7 ± 1 DALA. For decline trials/plots additional samples were collected at 3, 10 and 14 DALA. No adjuvants were used in the 2005 trials. Duplicate samples were collected from plots for the 2005 and 2010 trials. Samples of onion bulbs were analysed for residues per the validated methods AG-671A for the 2005/06 trials and GRM051.05A for the 2010/11 trials. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 463 days (15.2 months).

Table 60 Residues of acibenzolar-S-methyl following foliar application to onions (Thompson 2008 467403, Willard 2012b 50017)

Crop (Variety) ONION	N (int)	Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage	DALA	Crop Part	Residue (mg/kg)
Holtville, CA USA (2005) Ebano	4 (6 8 7)	36 36 36 35	325 323 321 315	small bulbs Bulb Bulb Bulb	7 (0)	Dry Bulb	< 0.05, < 0.05 (< 0.05)
Five Points CA USA (2005)–	4 (7 7 7)	34 36 35 38	372 391 381 410	Bulb 7.5–10 cm dia Bulbs present Bulbs present Bulbs present—drying down	3 (0) 7 (0) 10 (0) 14 (0)	Dry Bulb Dry Bulb Dry Bulb Dry Bulb	< 0.05, < 0.05 (< 0.05) < 0.05, < 0.05 (< 0.05) < 0.05, < 0.05 (< 0.05) < 0.05, < 0.05 (< 0.05)
Fort Collins CO USA (2005) Vantage	4 (7 7 7)	36 35 37 35	240 240 246 236	Bulbing Bulbing Bulbing—Plant tipped over Bulb	7 (+3)	Dry Bulb	< 0.05, < 0.05 (< 0.05)
Parma ID USA (2005) Vaquero	4 (7 6 7)	35 35 36 36	458 467 471 473	Maturing Maturing Maturing maturing	8 (0)	Dry Bulb	< 0.05, < 0.05 (< 0.05)
Laingsburg MI USA (2005) Millennium	4 (7 8 7)	35 36 35 34	366 385 377 358	Vegetative Vegetative Vegetative vegetative—drying down	7 (+14)	Dry Bulb	0.06, 0.05 (0.06)
Freeville NY USA (2005) Millennium	4 (7 7 7)	35 37 36 35	189 199 194 187	8 true leaves–9 true leaves 9 true leaves 9 true leaves 9 true leaves	7 (+4)	Dry Bulb	< 0.05, < 0.05 (< 0.05)
Aurora OR USA (2005) Gunnison	4 (7 7 7)	36 36 36 37	275 278 276 283	vegetative—Bulb 5 cm dia vegetative—Bulb 7.5 cm dia vegetative—Bulb 7.5 cm dia vegetative—Bulb 7.5 cm dia	6 (+4)	Dry Bulb	< 0.05, < 0.05 (< 0.05)
Weslaco TX USA (2005) Cirrus	4 (8 7 6)	36 35 35 36	189 305 304 309	Bulb 5 cm dia Bulb 5 cm dia 5–7.5 cm roots 7.5–10 cm dia roots	6 (0)	Dry Bulb	< 0.05, < 0.05 (< 0.05)
Kerman CA USA (2011) Stockton Early Yellow	4 (7 7 7)	35 foliar 35 foliar 35 foliar 35 foliar	184 184 186 186	48 49 49 49	7 10 14	Dry Bulb Dry Bulb Dry Bulb	< 0.01, < 0.01 (< 0.01) < 0.01 < 0.01

Crop (Variety) ONION	N (int)	Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage	DALA	Crop Part	Residue (mg/kg)
	4 (7 7 7)	35 foliar 35 foliar 35 foliar 35 foliar (+ Agridex at 0.5–0.625%)	185 186 187 187	48 49 49 49	7	Dry Bulb	< 0.01, < 0.01 (< 0.01)
	4 (7 7 7)	35 soil 35 soil 35 soil 35 soil	1403 1402 1396 1408	48 49 49 49	7 10 14	Dry Bulb Dry Bulb Dry Bulb	< 0.01, < 0.01 (< 0.01) < 0.01 < 0.01
King City, CA USA (2010) Varsity last 9 Feb 10	4 (7 7 7)	36 foliar 36 foliar 36 foliar 36 foliar	339 334 337 330	47 47 47 49	7 10 14	Dry Bulb Dry Bulb Dry Bulb	0.01, 0.01 (0.01) 0.02 < 0.01
	4 (7 7 7)	35 foliar 35 foliar 36 foliar 34 foliar (+ Dyne-Amic at 0.375%)	329 327 337 321	47 47 47 49	7	Dry Bulb	0.02, 0.02 (0.02)
	4 (7 7 7)	37 soil 36 soil 36 soil 35 soil	1463 1433 1423 1411	47 47 47 49	7 10 14	Dry Bulb Dry Bulb Dry Bulb	< 0.01, < 0.01 (< 0.01) < 0.01 < 0.01
King City CA USA (2010) Redwing Last 10 Jan 10	4 (7 7 7)	35 foliar 35 foliar 34 foliar 35 foliar	306 331 331 340	85 47 49 49	7 10 14	Dry Bulb Dry Bulb Dry Bulb	< 0.01, < 0.01 (< 0.01) < 0.01 < 0.01
	4 (7 7 7)	36 foliar 36 foliar 35 foliar 36 foliar (+ Pro 90 at 0.50%)	308 331 334 342	85 47 49 49	7	Dry Bulb	< 0.01, 0.01 (0.01)
	4 (7 7 7)	36 soil 35 soil 35 soil 35 soil	1917 1914 1951 1906	85 47 49 49	7 10 14	Dry Bulb Dry Bulb Dry Bulb	< 0.01, < 0.01 (< 0.01) < 0.01 0.018
Ephrata, WA USA (2011) Colorado #6	4 (7 7 7)	36 foliar 36 foliar 35 foliar 35 foliar	191 189 187 186	45 46 47 48	7 10 14	Dry Bulb Dry Bulb Dry Bulb	< 0.01, < 0.01 (< 0.01) < 0.01 < 0.01
	4 (7 7 7)	35 foliar 35 foliar 35 foliar 35 foliar (+ Slither at 0.03%)	186 186 186 189	45 46 47 48	7	Dry Bulb	< 0.01, < 0.01 (< 0.01)
	4 (7 7 7)	35 soil 35 soil 35 soil 35 soil	1880 1866 1885 1889	45 46 47 48	7 10 14	Dry Bulb Dry Bulb Dry Bulb	< 0.01, < 0.01 (< 0.01) < 0.01 < 0.01

Values in parentheses = mean of results

In four of the trials the samples were harvested and allowed to dry for 3 to 14 days before collecting samples. Drying is the local practice in these areas.

Brassica vegetables

Supervised trials were conducted in the USA on Brassica vegetables (cabbage and broccoli). Acibenzolar-S-methyl was applied as four foliar applications of a WG formulation at about 53 g ai/ha. In the three cabbage trials performed in 2010, a second plot was conducted with addition of an additive to the tank-mix (Helfire, Preference, Agridex) and a third plot where the four applications were as soil drenches. At all trial sites samples of cabbage and broccoli were collected from the treated plots at day seven after application, and immediately after application for trials performed in 1997/98. For decline trials/plots additional samples were collected at nominally 1, 3, 5, 10 and 14 DALA. Samples were analysed for residues per methods AG-671A for the 1997/98 trials and GRM051.05A for the 2010 trials. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 636 days (21 months).

Table 61 Residues of acibenzolar-S-methyl following foliar application to cabbage (Eudy 1999 0660)

Location CABBAGE	N (int)	Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Sample	Residue (mg/kg)
Fresno CA USA (1997) Copenhagen	4 (7 7 7)	53 53 53 53	187 187 187 187	vegetative—heading heading heading—Immature head heading	0 7 7 7	Head Head w/o wrapper Wrapper leaves Head	0.59, 0.58 (0.59) 0.09, 0.10 (0.10) 0.45, 0.64 (0.55) 0.45, 0.57 (0.51)
Indian River FL USA (1997) Bravo	4 (7 9 5)	53 53 53 53	570 570 570 570	25–28 cm HT 25–28 cm HT 28–30 cm HT 28–30 cm HT	0 7 7 7	Head Head w/o wrapper Wrapper leaves Head	0.36, 0.65 (0.51) 0.20, 0.31 (0.26) 0.29, 0.24 (0.27) 0.19, 0.22 (0.21)
San Patricio TX USA (1997) Gensis	4 (7 7 7)	53 53 53 53	234 234 234 234	Small heads Small heads Small heads Medium heads	0 7 7 7	Head Head w/o wrapper Wrapper leaves Head	0.34, 0.28 (0.31) 0.20, 0.21 (0.21) 0.23, 0.14 (0.19) 0.28, 0.36 (0.32)
Sampson NC USA (1997) Early Jersey Wakefield	4 (8 7 7)	53 53 53 53	187 187 187 187	8–12 leaf stage 14–15 leaf stage 14–15 leaf stage 15–20 leaf stage	0 7 7 7	Head Head w/o wrapper Wrapper leaves Head	0.46, 0.53 (0.50) 0.12, 0.11 (0.12) 0.15, 0.13 (0.14) 0.14, 0.12 (0.13)
Walworth WI USA (1997) Vantage Point	4 (7 7 8))	53 53 53 53	234 243 243 243	15 cm. head 18 cm head 20 cm head 23 cm head	0 7 7 7	Head Head w/o wrapper Wrapper leaves Head	0.29, 0.35 (0.32) 0.11, 0.12 (0.12) 0.13, 0.13 (0.13) 0.06, 0.09 (0.08)
Wayne NY USA (1997) Heads Up	4 (7 7 7)	53 53 53 53	187 187 187 187	10–13 cm head 10–15 cm. head 15–20 cm head 15–20 cm. head	0 1 3 5 7 7 7	Head Head Head Head Head w/o wrapper Wrapper leaves Head	0.36, 0.29 (0.33) 0.25, 0.32 (0.29) 0.39, 0.37 (0.38) 0.21, 0.21 (0.21) 0.16, 0.20 (0.18) 0.40, 0.40 (0.40) 0.27, 0.35 (0.31)
North Rose NY USA (2010) Constellation	4 (7 7 7)	54 foliar 54 foliar 55 foliar 54 foliar	283 282 289 282	BBCH 43 BBCH 45 BBCH 47 BBCH 49	7 10 14	Head Head Head	0.38, 0.26 (0.32) 0.36 0.29

Location CABBAGE	N (int)	Rate (g ai/ha)	Spray volume (L/ha)	GS	DALA	Sample	Residue (mg/kg)
	4 (7 7 7)	54 foliar 55 foliar 54 foliar 54 foliar (+ Agridex at 1.0%)	286 283 288 283	BBCH 45 BBCH 47 BBCH 49 BBCH 43	7	Head	0.60, 0.56 (0.58)
	4 (7 7 7)	53 soil 52 soil 54 soil 51 soil	985 961 989 981	BBCH 43 BBCH 45 BBCH 47 BBCH 49	7 10 14	Head Head Head	0.27, 0.26 (0.27) 0.21 0.24
Bagley IA USA (2010) Copenhagen	4 (7 7 7)	54 foliar 58 foliar 56 foliar 53 foliar	25 144 94 132	BBCH 12 BBCH 19 BBCH 19 BBCH 85	6 9 12	Head Head Head	0.14, 0.17 (0.16) 0.14 0.17
	4 (7 7 7)	53 foliar 55 foliar 58 foliar 51 foliar (+ Helfire at 0.25%)	213 137 97 128	BBCH 12 BBCH 19 BBCH 19 BBCH 85	6	Head	0.19, 0.18 (0.19)
	4 (7 7 7)	52 soil 51 soil 50 soil 51 soil	1820 1798 1757 1786	BBCH 12 BBCH 19 BBCH 19 BBCH 85	6 9 12	Head Head Head	< 0.01, < 0.01 (< 0.01) < 0.01 < 0.01
Fitchburg WI USA (2010) Stonehead	4 (7 7 7)	54 foliar 54 foliar 52 foliar 53 foliar	238 237 232 225	BBCH 47–51 BBCH 51 7.5–13 cm heads 11–14 cm dia head	7 10 14	Head Head Head	0.32, 0.30 (0.31) 0.29 0.30
	4 (7 7 7)	55 foliar 55 foliar 53 foliar 54 foliar (+ Preference at 0.25%)	241 241 234 231	BBCH 47–51 BBCH 51 7.5–13 cm heads 11–14 cm dia head	7	Head	0.38, 0.39 (0.39)
	4 (7 7 7)	56 soil 53 soil 52 soil 51 soil	987 1012 1080 1105	BBCH 47–51 BBCH 51 7.5–13 cm heads 11–14 cm dia head	7 10 14	Head Head Head	0.15, 0.13 (0.14) 0.14 0.20

Table 62 Residues of acibenzolar-S-methyl following foliar application to broccoli (Eudy 1999 0660) (replicate samples)

Crop (Variety) BROCCOLI	N (int)	Application Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Fresno CA USA (1997) Decicco	4 (7 7 7)	53 53 53 53	47 47 47 47	Vegetative Vegetative/heading Heading Heading/immature	0 7	Inflorescence Inflorescence	0.53 0.64 (0.58) 0.34 0.28 (0.31)
San Patricio TX USA (1997) Southern Comet Vegetables	4 (7 7 5)	53 53 53 53	234 234 234 234	Small heads large heads large heads large heads	0 7	Inflorescence Inflorescence	0.49, 0.60 (0.55) 0.60, 0.32 (0.46)

Crop (Variety) BROCCOLI	N (int)	Application Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
San Luis Obispo CA USA (1997) Marathon	4 (7 6 7)	53 53 53 53	234 234 234 234	Pre-Head Pre-Head Early head Early head	0 1 3 5 7 9	Inflorescence Inflorescence Inflorescence Inflorescence Inflorescence Inflorescence	0.47, 0.41 (0.44) 0.51, 0.60 (0.56) 0.95, 0.88 (0.92) 1.08, 1.41 (1.25) 0.63, 0.60 (0.62) 0.39, 0.53 (0.46)
Santa Barbara CA USA (1997) Arcadia	4 (6 8 7)	53 53 53 53	234 234 234 234	Pre-Head Heading Heading Head sizing	0 7	Inflorescence Inflorescence	0.43, 0.50 (0.47) 0.52, 0.42 (0.47)
Yuma AZ USA (1998) HMX 7144	4 (7 5 9)	53 53 53 53	281 281 281 281	Head development Head development maturing–mature mature–immature	0 7	Inflorescence Inflorescence	0.47, 0.42 (0.45) 0.58, 0.51 (0.55)
Linn OR USA (1997) Gem Hybrid	4 (7 6 8)	53 53 53 53	271 271 262 281	Pre-Heading heading–2.5 cm diam 1.2–7.5 cm. diam 5.1–15.2 cm diam	0 7	Inflorescence Inflorescence	0.25, 0.25 (0.25) 0.17, 0.22 (0.20)

Fruiting vegetables, Cucurbits

Supervised trials on cucurbits were conducted in the USA.

In trials on cucumber, acibenzolar-S-methyl was applied as eight foliar applications at 68.1–72.3 g ai/ha as a WG formulation. In the three trials performed in 2010 a second plot was conducted with addition of an additive to the tank-mix (Agridex, Scanner, Induce) and a third plot applications were made as a soil drench. At all trial sites samples of cucumber were collected from the treated plots immediately after application, when the spray deposit has dried. For decline trials/plots additional samples were collected at nominal 3, 7 and 10 days after the last application (DALA). Samples of cucumbers were analysed for residues of acibenzolar-S-methyl per the validated methods AG-671A for the 1998 trials and GRM051.05A for the 2010 trials. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 353 days (11.6 months).

Table 63 Residues of acibenzolar-S-methyl following foliar application to cucumber (Timothy 1999 4782, Willard 2012b 50017)

Crop (Variety) CUCUMBER	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Madera CA USA (1998) Dasher II	8 (7 7 7 7 7 7 7)	70 70 70 70 70 70 70 70	94 94 94 94 94 94 94 94	2 leaves–4 leaves 2 leaves–5 leaves 8 leaves–12 leaves Early bloom–small fruit Early bloom–small fruit Blooming–5 cm fruit Blooming–15 cm fruit Blooming–mature fruit	0	Fruit	0.07, 0.07 (0.07)
Indian River FL USA (1998) Straight Eight	8 (7 7 7 7 7 7 7)	70 70 70 70 70 70 70 70	19 19 19 19 19 19 19 19	Seedling 2 leaves–6 leaves Pre-bloom Blooming fruit set fruit set fruit set fruit set	0	Fruit	0.12, 0.18 (0.15)

Crop (Variety) CUCUMBER	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Hidalgo TX USA (1998) Poinsett	8 (7 7 8 7 7 7 7)	70	168	Vegetative	0	Fruit	0.12, 0.14 (0.13)
		70	159	Vegetative			
		70	168	Vining			
		70	168	fruiting–Vining			
		70	168	fruiting–Vining			
		70	168	fruiting			
		70	168	Early senescencing			
		70	159	Senescencing			
Sampson NC USA (1998) Poinsett 76	8 (7 7 7 7 7 7 7)	70	94	Seedling	0	Fruit	0.13, 0.11 (0.12)
		70	94	2 leaves–3 leaves			
		70	94	4 leaves–5 leaves			
		70	94	Early Running			
		70	94	Running			
		70	94	5.1–10 cm fruit			
		70	94	Fruiting			
		70	94	Fruiting			
Barnwell SC USA (1998) Poinsett 76	8 (8 7 8 7 7 8 8)	70	187	Vegetative	0	Fruit	0.15, 0.13 (0.14)
		70	187	Vegetative			
		70	187	Vegetative			
		70	187	Early bloom			
		70	187	Blooming			
		70	187	Blooming–5.1 cm fruit			
		70	187	Blooming–12.7 cm fruit			
		70	187	mature fruit			
Ottawa MI USA (1998) Marketmore	8 (7 7 7 7 7 7)	70	206	1 leaf–3 leaves	0	Fruit	0.33, 0.43 (0.38)
		70	196	4 leaves–7 leaves	1	Fruit	0.33, 0.32 (0.33)
		70	196	First Bloom	3	Fruit	0.53, 0.43 (0.48)
		70	224	mid bloom–fruiting	7	Fruit	0.44, 0.46 (0.45)
		70	224	mid bloom–fruiting	10	Fruit	0.14, 0.14 (0.14)
		70	224	Blooming–fruiting			
		70	215	Blooming–fruiting			
		70	224	mature fruit			
Walworth WI USA (1998) Marketmore 86	8 (7 7 8 5 7 5 7)	70	159	Vegetative	0	Fruit	0.16, 0.19 (0.18)
		70	159	Starting to vine			
		70	159	Blooming			
		70	159	Blooming			
		70	159	Blooming			
		70	159	Blooming–fruiting			
		70	150	Blooming–fruiting			
		70	159	Blooming–large fruit			
Tulare CA USA (1998) Dasher II	8 (7 7 7 7 7 7 7)	70	140	2 leaves–4 leaves	0	Fruit	0.14, 0.19 (0.17)
		70	131	6 leaves–8 leaves			
		70	131	10 leaves–12 leaves			
		70	131	20 leaves			
		70	131	Blooming			
		70	131	Blooming			
		70	131	15–20 cm fruit			
		70	140	mature fruit			
Seven Springs NC USA (2010) Lancer 152	8 (7 7 7 7 7 7 7)	70 foliar	138	BBCH 13	0	Fruit	0.52, 0.60 (0.56)
		70 foliar	148	BBCH 14	3	Fruit	0.45
		71 foliar	148	BBCH 18	6	Fruit	0.53
		68 foliar	143	BBCH 23	9	Fruit	0.52
		71 foliar	156	BBCH 53			
		71 foliar	161	BBCH 74			
		69 foliar	167	BBCH 81			
		69 foliar	164	BBCH 89			

Crop (Variety) CUCUMBER	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
	8 (7 7 7 7 7 7 7)	71 foliar 70 foliar 72 foliar 71 foliar 72 foliar 70 foliar 70 foliar 69 foliar (+Agridex at 0.29%)	140 148 151 148 157 159 168 164	BBCH 13 BBCH 14 BBCH 18 BBCH 23 BBCH 53 BBCH 74 BBCH 81 BBCH 89	0	Fruit	0.77, 0.76 (0.77)
	8 (7 7 7 7 7 7 7)	70 soil 71 soil 70 soil 70 soil 71 soil 69 soil 72 soil 71 soil	1100 1069 1080 1101 1091 1044 1058 1057	BBCH 13 BBCH 14 BBCH18 BBCH 23 BBCH 53 BBCH 74 BBCH 81 BBCH 89	0 3 6 9	Fruit Fruit Fruit Fruit	0.46, 0.56 (0.51) 0.31 0.40 0.47
Elko SC USA (2010) Marketmore 76	8 (7 7 7 7 7 7 7)	70 foliar 70 foliar 70 foliar 70 foliar 70 soil 71 foliar 70 foliar 70 foliar	208 209 214 211 210 212 218 214	BBCH 14 BBCH 17 Vegetative Bloom to Fruit 2.5 cm Bloom to Fruit 5 cm Bloom to Fruit 10 cm Bloom to Mature Fruit Bloom to Mature Fruit	0 3 7 9	Fruit Fruit Fruit Fruit	0.21, 0.21 (0.21) 0.23 0.095 0.13
	8 (7 7 7 7 7 7 7)	70 foliar 71 foliar 70 foliar 70 foliar 70 foliar 71 foliar 70 foliar 70 foliar (+ Scanner at 0.24–0.26%)	207 211 213 211 210 212 218 213	BBCH 14 BBCH 17 Vegetative Bloom to Fruit 2.5 cm Bloom to Fruit 5 cm Bloom to Fruit 10 cm Bloom to Mature Fruit Bloom to Mature Fruit	0	Fruit	0.23, 0.32 (0.28)
	8 (7 7 7 7 7 7 7)	70 soil 70 soil 70 soil 70 soil 71 soil 70 soil 70 soil 70 soil	989 981 965 988 1013 998 978 970	BBCH 14 BBCH 17 Vegetative Bloom to Fruit 2.5 cm Bloom to Fruit 5 cm Bloom to Fruit 10 cm Bloom to Mature Fruit Bloom to Mature Fruit	0 3 7 9	Fruit Fruit Fruit Fruit	0.30, 0.19 (0.25) 0.29 0.20 0.12
Athens GA USA (2010) Poinsett	8 (7 7 7 7 7 7 7)	70 foliar 69 foliar 70 foliar 70 foliar 69 foliar 70 foliar 70 foliar 69 foliar	337 326 275 264 262 269 272 290	BBCH 11 BBCH 12–13 BBCH 15–17 BBCH 22 BBCH 68 BBCH 71–72 BBCH 78–79 BBCH 79	0 3 7 10	Fruit Fruit Fruit Fruit	0.32, 0.26 (0.29) 0.42 0.46 0.42

Crop (Variety) CUCUMBER	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
	8 (7 7 7 7 7 7 7)	70 foliar 69 foliar 70 foliar 69 foliar 69 foliar 70 foliar 70 foliar 70 foliar (+ Induce at 0.13%)	336 327 276 262 261 271 274 294	BBCH 11 BBCH 12–13 BBCH 15–17 BBCH 22 BBCH 68 BBCH 71–72 BBCH 78–79 BBCH 79	0	Fruit	0.21, 0.27 (0.24)
	8 (7 7 7 7 7 7 7)	70 soil 69 soil 69 soil 69 soil 69 soil 69 soil 70 soil 70 soil	1075 1084 1127 1073 1085 1086 1107 1091	BBCH 11 BBCH 12–13 BBCH 15–17 BBCH 22 BBCH 68 BBCH 71–72 BBCH 78–79 BBCH 79	0 3 7 10	Fruit Fruit Fruit Fruit	0.19, 0.20 (0.20) 0.22 0.27 0.21

Adjuvant added, crop oil: Madera CA Kinetic; Indian River FL Kinetic; Hidalgo TX LI-700; Sampson NC EXCEL 2000; Barnwell SC Freeway; Ottawa MI Silwet L-77; Walworth WI Kinetic; Tulare CA Kinetic

Trials on melons were conducted in the USA where eight foliar applications of acibenzolar-S-methyl were applied at 61.8–73.4 g ai/ha as WG formulation. All trials included a plot on cantaloupe melon and in four trials, three performed in 2010 and one performed in 2011, a second plot was conducted on a different variety (Honeydew melon) either in parallel or with a temporary shift. Samples of melon were collected from the treated plots immediately after application, when the spray deposit has dried. For decline trials/plots additional samples were collected at nominal 1, 3, 5, 7 and 10 DALA. Samples of melons were analysed for residues per methods AG-671A for the 1998/99 trials and GRM051.05A for the 2010/11 trials. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 470 days (15.5 months).

Table 64 Residues of acibenzolar-S-methyl following foliar application to melon (Timothy 1999 4782, Willard 2012b 50017, Willard 2012c 50020)

Crop (Variety) MELON	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Imperial CA USA (1999) Cantaloupe—Im-Pac	8 (8 5 7 8 7 7 8)	70 70 70 70 70 70 70 70	187 187 187 187 187 187 187 187	Blooming Blooming–fruiting Bedtop canopy full bloom full bloom Full net on fruit Fruit to colour Full slip	0 1 3 7 10	Fruit Fruit Fruit Fruit Fruit	0.15, 0.15 (0.15) 0.11, 0.17 (0.14) 0.13, 0.13 (0.13) 0.13, 0.12 (0.13) 0.13, 0.12 (0.13)
Tift GA USA (1999) Cantaloupe—Edisto 47	8 (6 7 7 7 7 7 7)	70 70 70 70 70 70 70 70	159 168 140 168 159 168 168 178	Early vegetative Early bloom Blooming Blooming–Fruit developing Blooming–10 cm fruit Blooming–14 cm fruit Fruit to full size Fruit to full size	0	Fruit	0.71, 0.95 (0.83)

Crop (Variety) MELON	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Hamilton IN USA (1998) Cantaloupe—Burpee Hybrid	8 (7 7 6 8 6 5 6)	70	28	Vegetative	0	Fruit	0.27, 0.22 (0.25)
		70	28	Vining			
		70	28	Very Early Flower			
		70	28	Flower			
		70	28	fruit set			
		70	28	fruit set			
		70	28	Full fruit			
		70	28	mature fruit			
Hidalgo TX USA (1998) Cantaloupe—Mission Hybrid	8 (7 7 7 7 7 7 7)	70	150	Early vegetative	0	Fruit	0.33, 0.28 (0.31)
		70	150	flowering–Vining			
		70	159	Vining–Early Fruit			
		70	150	Vining–fruiting			
		70	150	Vining–fruiting			
		70	150	Vining–fruiting			
		70	141	Vining–fruiting			
		62	159	Vining–fruiting			
Yuma AZ USA (1998) Cantaloupe—Mission	8 (7 8 8 6 9 5 7)	70	187	25 cm vines	0	Fruit	0.33, 0.33 (0.33)
		70	187	36 cm vines			
		70	187	47 cm vines			
		70	187	56 cm vines			
		70	187	76 cm vines			
		70	187	76 cm vines			
		70	187	135 cm vines			
		70	187	127 cm vines			
Tulare CA USA (1998) Cantaloupe—Hale's Best Jumbo	8 (7 7 7 7 7 7 7)	70	131	5 cm fruit	0	Fruit	0.35, 0.35 (0.35)
		70	131	7.5 cm fruit			
		70	131	10 cm fruit			
		70	131	10–13 cm fruit			
		70	131	15–18 cm fruit			
		70	140	15–18 cm fruit			
		70	131	15–18 cm fruit			
		70	131	mature fruit			
Raymondville TX USA (2010) Cantaloupe—Earli-Star[a] Last 29 Jul	8 (7 7 7 7 7 7 7)	72	190	BBCH 65	0	Fruit	0.54, 0.48 (0.51)
		72	192	BBCH 66	1	Fruit	0.46, 0.53 (0.50)
		71	188	BBCH 69	3	Fruit	0.50, 0.45 (0.48)
		72	190	BBCH 71	5	Fruit	0.49, 0.49 (0.49)
		72	190	BBCH 82	7	Fruit	0.47, 0.58 (0.53)
		72	190	BBCH 85	9	Fruit	0.40, 0.43 (0.42)
		73	194	BBCH 88			
Raymondville TX USA (2010) Honeydew melon—Earli-Dew[a] Last 29 Jul	8 (7 7 7 7 7 7 7)	72	190	BBCH 65	0	Fruit	0.78, 0.90 (0.84)
		72	191	BBCH 66	1	Fruit	0.74, 0.81 (0.78)
		72	191	BBCH 69	3	Fruit	0.60, 0.62 (0.61)
		72	190	BBCH 71	5	Fruit	0.66, 0.59 (0.63)
		72	191	BBCH 82	7	Fruit	0.65, 0.74 (0.70)
		73	193	BBCH 85	9	Fruit	0.95, 1.00 (0.98)
		73	194	BBCH 88			
Hughson CA USA (2010) Cantaloupe—Hale's Best Jumbo[a] Last 23 Sept	8 (7 7 7 7 7 7 7)	70	281	BBCH 65	0	Fruit	0.38, 0.48 (0.43)
		70	281	BBCH 69	1	Fruit	0.45, 0.37 (0.41)
		70	279	BBCH 71	3	Fruit	0.57, 0.42 (0.50)
		70	280	BBCH 73	5	Fruit	0.50, 0.47 (0.49)
		70	281	BBCH 77	8	Fruit	0.59, 0.45 (0.52)
		70	280	BBCH 79	10	Fruit	0.59, 0.60 (0.60)
		70	280	BBCH 85			
		68	273	BBCH 89			

Crop (Variety) MELON	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Hughson CA USA (2010) Honeydew melon—Green Flesh [a] Last 23 Sept	8 (7 7 7 7 7 7 7)	70 69 70 70 70 70 70 69	282 276 278 279 278 280 280 278	BBCH 73 BBCH 73 BBCH 75 BBCH 77 BBCH 79 BBCH 81 BBCH 85 BBCH 89	0 1 3 5 8 10	Fruit Fruit Fruit Fruit Fruit Fruit	0.21, 0.25 (0.23) 0.16, 0.23 (0.20) 0.21, 0.26 (0.24) 0.096, 0.12 (0.11) 0.16, 0.17 (0.17) 0.14, 0.19 (0.17)
Kerman CA USA (2010) Cantaloupe—Durango [a] Last 15 Oct	8 (7 7 7 7 7 7 7)	70 70 70 70 70 70 70 70	282 281 281 282 281 281 280 279	BBCH 71 BBCH 71 BBCH 73 BBCH 79 BBCH 79 BBCH 79 BBCH 87 BBCH 87	0 1 3 5 7 10	Fruit Fruit Fruit Fruit Fruit Fruit	0.50, 0.54 (0.52) 0.45, 0.50 (0.48) 0.57, 0.63 (0.60) 0.45, 0.65 (0.55) 0.61, 0.64 (0.63) 0.58, 0.46 (0.52)
Kerman CA USA (2010) Honeydew melon—Green Flesh [a] Last 15 Oct	8 (7 7 7 7 7 7 7)	70 70 70 70 70 70 70 70	280 281 280 282 281 283 279 280	BBCH 81 BBCH 81 BBCH 81 BBCH 79 BBCH 79 BBCH 79 BBCH 87 BBCH 87	0 1 3 5 7 10	Fruit Fruit Fruit Fruit Fruit Fruit	0.25, 0.37 (0.31) 0.23, 0.38 (0.31) 0.35, 0.38 (0.37) 0.36, 0.41 (0.39) 0.36, 0.33 (0.35) 0.47, 0.49 (0.48)
Elko SC USA (2011) Cantaloupe—Ariel	8 (7 7 7 7 7 7 7)	70 70 70 70 70 70 70 70	227 236 228 229 227 228 230 225	BBCH 61 BBCH 62 BBCH 66 BBCH 69 BBCH 71 BBCH 71 BBCH 72 BBCH 83	0 1 3 5 6 9	Fruit Fruit Fruit Fruit Fruit Fruit	0.31, 0.16 (0.24) 0.19, 0.33 (0.26) 0.20, 0.18 (0.19) 0.18, 0.23 (0.21) 0.15, 0.26 (0.21) 0.16, 0.16 (0.16)
Fitchburg WI USA (2011) Cantaloupe—Aphrodite	8 (7 7 7 7 7 7 7)	71 70 70 70 71 70 72 69	274 273 273 267 271 269 290 281	BBCH 61 BBCH 63 BBCH 65 BBCH 65–77 BBCH 75 BBCH 75 BBCH 80 BBCH 81–88	0 1 3 5 7 11	Fruit Fruit Fruit Fruit Fruit Fruit	0.73, 0.53 (0.63) 0.64, 0.57 (0.61) 0.87 0.64 0.74 0.84
Raymondville TX USA (2011) Cantaloupe—Earli-Star [A] Last 13 Jun	8 (7 7 7 7 7 7 7)	73 71 72 71 71 71 72 71	194 189 192 189 188 188 192 189	BBCH 68 BBCH 69 BBCH 69 BBCH 71 BBCH 71 BBCH 72 BBCH 73 BBCH 86	0 1 3 5 7 9	Fruit Fruit Fruit Fruit Fruit Fruit	0.14, 0.22 (0.18) 0.21, 0.16 (0.19) 0.11, 0.17 (0.14) 0.14, 0.15 (0.15) 0.14, 0.15 (0.15) 0.13, 0.12 (0.13)
Raymondville TX USA (2011) Honeydew melon—Earli-Dew [a] Last 13 Jun	8 (7 7 7 7 7 7 7)	71 73 72 72 71 71 72 71	194 190 192 189 188 188 191 189	BBCH 71 BBCH 68 BBCH 69 BBCH 69 BBCH 71 BBCH 72 BBCH 73 BBCH 86	0 1 3 5 7 9	Fruit Fruit Fruit Fruit Fruit Fruit	0.28, 0.30 (0.29) 0.29, 0.32 (0.31) 0.30, 0.28 (0.29) 0.27, 0.22 (0.25) 0.27, 0.26 (0.27) 0.24, 0.20 (0.22)

Adjuvant added, crop oil: Tifton GA Kinetic; Hidalgo TX LI-700; Yuma AZ Kinetic; Tulare CA Kinetic; Imperial CA Kinetic;

[a] Trials not considered independent, different variety but same spray dates and harvest times

Trials on summer squash were conducted in the USA where eight foliar applications of acibenzolar-S-methyl were applied at 70 g ai/ha as WG formulation. Samples of squash were collected from the treated plots immediately after application, when the spray deposit has dried. For decline trial/plot additional samples were collected at nominal 1, 3, 5, 7 and 10 DALA. Samples of squash were analysed for residues per method AG-671A. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 470 days (15.5 months).

Table 65 Residues of acibenzolar-S-methyl following foliar application to summer squash (Timothy 1999 4782)

Crop (Variety) SQUASH	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Tifton GA USA (1998) Dixie	8 (6 7 7 7 7 7 7)	70	187	Early vegetative	0	Fruit	0.14 0.12 (0.13)
		70	178	Early vegetative			
		70	187	Vegetative			
		70	187	Fruiting			
		70	178	Fruiting			
		70	196	Fruiting			
		70	187	fruiting			
		70	187	Late fruiting			
Indian River FL USA (1998) Black Beauty	8 (7 7 7 7 7 7 7)	70	19	Seedling	0	Fruit	0.03 0.05 (0.04)
		70	19	2 leaves–6 leaves			
		70	19	Pre-bloom			
		70	19	Blooming			
		70	19	fruit set			
		70	19	fruit set			
		70	19	fruit set			
		70	19	fruit set			
Ottawa MI USA (1998) Yellow Straight Neck	8 (7 7 7 7 7 7 7)	70	206	1 true leaf	0	Fruit	0.18
		70	215	2–3 leaves			
		70	206	4–7 leaves			
		70	196	7–11 leaves			
		70	215	Early bloom/fruit			
		70	224	Mature fruit			
		70	224	Mature fruit			
		70	224	Mature fruit			
Tulare CA USA (1998) Ambasador	8 (7 7 7 7 7 7 7)	70	140	4 leaf stage	0	Fruit	0.07 0.06 (0.06)
		70	131	8 leaf stage	1		0.13 0.11 (0.12)
		70	131	14 leaf stage	3		0.15 0.13 (0.14)
		70	131	18 leaf stage	7		0.06 0.05 (0.06)
		70	131	Bloom	10		0.02 0.03 (0.02)
		70	131	Developing fruit			
		70	131	Developing fruit			
		70	140	Mature fruit			

Adjuvant added, crop oil: Tifton GA Kinetic; Indian River FL Kinetic; Ottawa MI Silwet L-77; Tulare CA Kinetic;

Fruiting vegetables, other than Cucurbits

Trials on tomatoes were conducted in Europe where acibenzolar-S-methyl was applied as five to six foliar applications at 25 g ai/ha as a WG formulation. For decline trials, fruit samples were taken immediately after the last application and at 3, 7, 14 and 21 DALA. In some trials fruits were also sampled immediately before the last application (–0 days). Samples of whole fruit were analysed for residues per method REM 172.11. For studies performed in 1997 or later, the method was modified by omission of the silica gel clean-up. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 12 months (378 days).

Table 66 Residues of acibenzolar-S-methyl following foliar application to tomato (replicate analyses) (Formica 1995fg 0222 0223, Walser 1998defghijklm 0508 0509 0534 0536 0535 0521 0524 0525 0523 0522 Walser 1999o 0634, Sack 1999abcd 0644 0640 0643 0642)

Crop (Variety) TOMATO	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Les Barges, Vouvry, Switzerland (1995) Selhardy F1	6 (11 10 11 10 11)	25	1500	BBCH 50	0	Fruit	0.07
		25	1500	BBCH 62	2	Fruit	0.06
		25	1500	BBCH 65	4	Fruit	0.05
		25	1500	BBCH 66	7	Fruit	0.04
		25	1500	BBCH 68–80	14	Fruit	0.04
		25	1500	BBCH 81			
Fully, Switzerland (1995) Paola	6 (13 11 11 10 10)	25	1500	BBCH 61	0	Fruit	0.05
		25	1500	BBCH 62	2	Fruit	0.05
		25	1500	BBCH 65	4	Fruit	0.04
		25	1500	BBCH 67	7	Fruit	0.06
		25	1500	BBCH 68	14	Fruit	0.05
		25	1500	BBCH 81–82			
Nocera Inferiore, Italy (1997) San Marzano	6 (7 7 7 7 7)	25	800	BBCH 70	–0	Fruit	0.05
		25	800	BBCH 70–72	0	Fruit	0.05
		25	1000	BBCH 70–76	3	Fruit	0.05
		25	1100	BBCH 70–78	8	Fruit	0.09
		25	1200	BBCH 72–80	15	Fruit	0.07
		25	1200	BBCH 76–82	22	Fruit	0.07
Giorgi Piacentino, Piacenza, Italy (1997) 98063	6 (7 7 7 7 7)	25	600	BBCH 61	–0	Fruit	0.04, 0.04 (0.04)
		25	600	BBCH 64–66	0	Fruit	0.06, 0.06 (0.06)
		25	600	BBCH 71	3	Fruit	0.06, 0.05 (0.06)
		25	600	BBCH 72	7	Fruit	0.05, 0.05 (0.05)
		25	600	BBCH 72–73	14	Fruit	0.08, 0.09 (0.09)
		25	600	BBCH 81–82	21	Fruit	0.11, 0.09 (0.10)
Chemin des Landes, Tierce France (1997) Promo [a]	6 (7 7 7 7)	25	400	BBCH 71–72	–0	Fruit	0.03
		25	400	BBCH 72	0	Fruit	0.03
		25	400	BBCH 73	3	Fruit	0.03
		25	400	BBCH 74	7	Fruit	0.03
		25	400	BBCH 75–807	14	Fruit	0.03
					21	Fruit	0.05
Chemin des Landes, Tierce, France (1997) Promo [a] processing	5 (7 6 8 7)	25	400	BBCH 71	3	Fruit	0.03
		25	400	BBCH 72			
		25	400	BBCH 72			
		25	400	BBCH 74			
		25	400	BBCH 75–807			
Chatillon le Roi, France (1997) Promo [a] Last 2 Sept	6 (6 7 7 7 8)	25	400	BBCH 71	–0	Fruit	0.04
		25	400	BBCH 72	0	Fruit	0.06
		25	400	BBCH 73	3	Fruit	0.06
		25	400	BBCH 74–75	7	Fruit	0.06
		25	400	BBCH 76	14	Fruit	0.07
		25	400	BBCH 76–77	21	Fruit	0.06
Chatillon le Roi, France (1997) Promo [a] processing	6 (7 7 7 7 7)	25	400	BBCH 72	3	Fruit	0.06, 0.07 (0.07)
		25	400	BBCH 72–73			
		25	400	BBCH 74–75			
		25	400	BBCH 76			
		25	400	BBCH 76–77			
		25	400	BBCH 77			
Le Terras, Mus, France (1997) Promo [a]	6 (8 7 6 7 7)	25	400	BBCH 605	–0	Fruit	0.08
		25	400	BBCH 607	0	Fruit	0.12
		25	400	BBCH 701	3	Fruit	0.11
		25	400	BBCH 801	7	Fruit	0.13
		25	400	BBCH 801	14	Fruit	0.13
		25	400	BBCH 805	21	Fruit	0.12

Crop (Variety) TOMATO	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Le Terras, Mus, France (1997) Promo Processing [a]	6 (6 7 7 7 7)	25 25 25 25 25 25	400 400 400 400 400 400	BBCH 701 BBCH 801 BBCH 801 BBCH 805 BBCH 807 BBCH 809	3	Fruit	0.08, 0.08 (0.08)
Figairasse, Marsillargues, France (1997) Promo [a]	6 (6 7 7 8 6)	25 25 25 25 25 25	400 400 400 400 400 400	BBCH 69 BBCH 71 BBCH 73 BBCH 74 BBCH 81 BBCH 84	–0 0 3 7 14 21	Fruit Fruit Fruit Fruit Fruit Fruit	0.04 0.04 0.05 0.04 0.06 0.05
Figairasse, Marsillargues, France (1997) Promo [a] processing	6 (7 8 6 7 7)	25 25 25 25 25 25	400 400 400 400 400 400	BBCH 73 BBCH 74 BBCH 81 BBCH 84 BBCH 84 BBCH 86	3	Fruit	0.05
Nocera Inferiore SA Italy (1998) S. Marzano	6 (7 7 7 7 7) + Cu	25 25 25 25 25 25	800 800 800 850 900 900	BBCH 62 BBCH 65 BBCH 67 BBCH 70 BBCH 72–76 BBCH 82	0 3 7 14 21	Fruit Fruit Fruit Fruit Fruit	0.08 0.07 0.08 0.15 0.09
	6 (7 7 7 7 7)	25 25 25 25 25 25	800 800 800 850 900 900	BBCH 62 BBCH 65 BBCH 67 BBCH 70 BBCH 72–76 BBCH 82	3	Fruit	0.07, 0.07 (0.07)
Lombardelli Giancarlo, Italy (1998) 98063	6 (7 7 7 7 7) +Cu	25 25 25 25 25 25	1000 1000 1000 1000 1000 1000	BBCH 63 BBCH 64 BBCH 66 BBCH 71 BBCH 81 BBCH 82	0 3 7 14 21	Fruit Fruit Fruit Fruit Fruit	0.09 0.10 0.10 0.14 0.11
	6 (7 7 7 7 7)	25 25 25 25 25 25	1000 1000 1000 1000 1000 1000	BBCH 63 BBCH 64 BBCH 66 BBCH 71 BBCH 81 BBCH 82	3	Fruit	0.09, 0.08 (0.09)
Gartenacker, Vouvry Switzerland (1998) Selhardy Replicate plot	6 (7 7 7 7 8)	25 25 25 25 25 25	2000 2000 2000 2000 2000 2000	BBCH 63 BBCH 64 BBCH 65 BBCH 66 BBCH 71 BBCH 80	0 3 7 14 21	Fruit Fruit Fruit Fruit Fruit	0.05 0.04 (0.04) 0.05 0.04 (0.04) 0.05 0.03 (0.04) 0.03 0.03 (0.03) 0.03 0.03 (0.03)
Polidendri, Domokos, Greece (1998) Heinz 1999 Diff farm same times	6 (7 7 7 7 7)	25 25 25 25 25 25	993 990 990 987 983 987	BBCH 63 BBCH 63 BBCH 64 BBCH 64 BBCH 73 BBCH 83	0 3 7 14 21	Fruit Fruit Fruit Fruit Fruit	0.12 0.10 0.10 0.12 0.12

Crop (Variety) TOMATO	N (int)	Application rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Polidendri, Domokos Greece (1998) Oval Red Diff farm same times	6 (7 7 7 7 7)	25 25 25 25 25 25	990 987 990 990 990 987	BBCH 63 BBCH 63 BBCH 64 BBCH 64 BBCH 73 BBCH 83	0 3 7 14 21	Fruit Fruit Fruit Fruit Fruit	0.07 0.07 0.09 0.07 0.10

[a] Trials not considered independent, same variety, spray dates and harvest times

Leafy vegetables

Trials on leafy vegetables were conducted in the USA.

Four foliar applications of acibenzolar-S-methyl at 35 g ai/ha as a WG formulation. In two decline trials, samples were taken immediately after the last application and at 1, 3, 7 and 14 DALA. In the ten harvest trials samples were taken 5 or 7 DALA. Samples of leaves and heads with or without wrapper leaves were analysed for residues per method AG 671. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 14.6 months (447 days).

Table 67 Residues of acibenzolar-S-methyl following foliar application to lettuce (Hayworth 1999 0672) (replicate analyses)

Crop (Variety) LETTUCE	N (int)	Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Head lettuce							
Fresno, California USA (1996) Great Lakes 659	4 (7 7 7)	35 35 35 35	234 234 234 234	Immature Plants Immature Plants Immature Plants Small heads	0 1 3 7 7 7 14	Head + Wrapper Leaves Head + Wrapper Leaves Head + Wrapper Leaves Head Head + Wrapper Leaves Wrapper Leaves Head + Wrapper Leaves	0.36, 0.44 (0.40) 0.15, 0.11, 0.10, 0.11 (0.12) 0.18, 0.19, 0.10, 0.07 (0.14) 0.02, 0.02 (0.02) 0.08, 0.08 (0.08) 0.27, 0.28, 0.32 (0.29) 0.03, 0.05 (0.04)
Lake, Florida (1996) Great Lakes	4 (7 7 7)	35 35 35 35	357 367 386 376	Head initiation Heading Heading mature heads	5 5 5	Head Head + Wrapper Leaves Wrapper Leaves	0.07, 0.06 (0.06) 0.10, 0.09 (0.10) 0.56, 0.39 (0.47)
Monterey California USA (1996) Detroit Last 6/6	4 (7 7 7)	35 35 35 35	280 281 278 283	Head formation Head formation Head formation mature	7 7 7	Head Head + Wrapper Leaves Wrapper Leaves	0.02, 0.02 (0.02) 0.05, 0.07 (0.06) 0.16, 0.18, 0.19 (0.18)
Monterey California USA (1996) Patriot Last 13/6	4 (7 7 7)	35 35 35 35	281 282 282 280	Maturation Head formation Head formation mature	7 7 7	Head Head + Wrapper Leaves Wrapper Leaves	0.03, 0.02 (0.02) 0.03, 0.04 (0.04) 0.16, 0.14 (0.15)
Yuma Arizona USA (1996) Winter Haven	4 (7 7 7)	35 35 35 35	281 187 187 187	Heading Heading Head mature	7 7 7	Head Head + Wrapper Leaves Wrapper Leaves	0.05, 0.04 (0.04) 0.04, 0.04 (0.04) 0.15, 0.30 (0.22)

Crop (Variety) LETTUCE	N (int)	Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Columbia, New York USA (1996) Iceberg (Green) Crispino MTO	4 (7 7 7)	35 35 35 35	327 327 327 327	Heads forming Heads forming Heads forming mature	7 7 7	Head Head + Wrapper Leaves Wrapper Leaves	0.02, 0.02, 0.02, 0.01 (0.02) 0.04, 0.06 (0.05) 0.22, 0.22 (0.22)
Leaf lettuce							
Fresno, California USA (1996) Waldmanns green	4 (7 7 7)	35 35 35 35	234 234 234 234	Immature Plants Immature Plants Immature Plants mature plants	0 1 3 7 14	Leaves Leaves Leaves Leaves Leaves	0.52 0.27, 0.26 (0.26) 0.15, 0.16 (0.16) 0.10, 0.10 (0.10) 0.02, 0.02 (0.02)
Indian River, Florida USA (1996) Black Seeded Simpson	4 (7 7 7)	35 35 35 35	567 567 567 567	303 303 303 307	7	Leaves	0.15, 0.14 (0.14)
Monterey California USA (1996) Waldmanns green	4 (7 7 7)	35 35 35 35	280 281 278 282	Rosette Stage Rosette Stage Mature mature	7	Leaves	0.07, 0.04 (0.06)
San Luis Obispo California USA (1996) Esmerelda	4 (7 7 7)	35 35 35 35	315 307 308 306	Immature leaves Immature leaves Immature leaves mature leaves	7	Leaves	0.11, 0.18 (0.14)
Yuma Arizona USA (1996) Romain Green Towers	4 (7 7 7)	35 35 35 35	374 374 374 374	Immature leaves 8–10 leaves Immature leaves mature leaves	7	Leaves	0.18, 0.18 (0.18)
Columbia, New York USA (1996) Black Seeded Simpson	4 (7 7 7)	35 35 35 35	327 327 327 327	Leaf extension Leaf extension Leaf extension Marketable	7	Leaves	0.04, 0.04 (0.04)

Spinach

Trials on spinach were conducted in Europe and the USA.

Supervised trials in Europe were conducted in France, Italy and Switzerland. Three foliar applications of a WG formulation containing 40 g acibenzolar-S-methyl/kg of product in combination with 400 g metalaxyl M/kg. Five trials were conducted with three applications at 10 g ai/ha and a nominal PHI of ten days. Three additional trials were conducted with three applications at 14 g ai/ha. In four decline trials, samples were taken immediately after the last application and at 3, 7, 10 and 14 DALA. In the four harvest trials samples were taken 10–13 days after last application. Spinach leaves samples were analysed for residues per method REM 172.11. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 12.7 months (385 days).

Supervised trials in the USA

Acibenzolar-S-methyl was applied four times as a WG formulation. Nine trials were conducted with four applications at 35 g ai/ha and a nominal PHI of seven days. In the three trials performed in 2010/11 a second plot was conducted with addition of an additive to the tank-mix (Herbimax, Induce) and a third plot was conducted by four soil drench applications. In the one decline trial, samples were taken immediately after the last application and at 1, 3, 7 and 14 DALA. In the five harvest trials samples were taken 7 days after the last application. Spinach leaves samples were analysed for residues per method AG 671 for the 1996 trials and GRM051.05A for the 2010/11 trials. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 14 months (430 days).

Table 68 Residues of acibenzolar-S-methyl following foliar application to spinach in Europe (Sack 2000cdefgh 0740 0719 0741 0723 0742 0724 Sack 2001ab 0753 0752) (replicate samples) and the USA (replicate analyses) (Willard 2012b 50017, Hayworth 1998c 0487)

Crop (Variety) SPINACH	N	Application rate (g ai/ha)	Spray volume	Growth Stage at Application	DALA	Residue (mg/kg)
	(int)		(L/ha)			
Serravalle a Po (MN) Italy (1999) Lorelay	3 (9 11)	14 14 14	100 1000 1000	BBCH 16–18 BBCH 41–43 BBCH 44–45	0 3 7 10 14	0.27 0.18 0.18 0.09, 0.12 (0.11) 0.07
Camisano Vic.-Vi. Italy (1999) San Verdo (SG6153)	3 (10 10)	14 14 14	1000 1000 1000	BBCH 12–14 BBCH 13–19 BBCH 19–46	0 3 7 10 14	0.31 0.24 0.13 0.13, 0.11 (0.12) 0.08
Sannicandro di Bari, Italy (1999) Riccio America	3 (11 10)	14 14 14	1000 1000 1000	BBCH 19 BBCH 41 BBCH 45–49	10	0.19, 0.13 (0.16)
Les Barges, Vouvry, Switzerland (1999) Monnopa [a] Last 7 Jun	3 (10 10)	10 10 10	800 800 800	BBCH 14 BBCH 15 BBCH 10	0 3 7 10 14	0.19 0.10 0.06 0.04, 0.04 (0.04) 0.03
	3 (10 10)	14 14 14	800 800 800	BBCH 14 BBCH 15 BBCH 45	0 3 7 10 14	0.34 0.15 0.08 0.05, 0.07 (0.06) 0.04
Les Barges, Vouvry, Switzerland (1999) Chica F1 Last 27 Aug	3 (11 10)	10 10 10	800 800 800	BBCH 12 BBCH 15 BBCH 41	0 3 7 10 14	0.34 0.12 0.05 0.04, 0.03 (0.04) 0.02
	3 (11 10)	14 14 14	800 800 800	BBCH 12 BBCH 15 BBCH 41	0 3 7 10 14	0.45 0.20 0.07 0.05, 0.03 (0.04) 0.03
Les Barges, Vouvry, Switzerland (1999) Monnopa [a] Last 7 Jun	3 (10 10)	10 10 10	800 800 800	BBCH 13 BBCH 14 BBCH 44	10	0.03, 0.04 (0.04)
	3 (10 10)	14 14 14	800 800 800	BBCH 13 BBCH 14 BBCH 44	10	0.06, 0.05 (0.06)

Crop (Variety) SPINACH	N	Application rate (g ai/ha)	Spray volume	Growth Stage at Application	DALA	Residue (mg/kg)
	(int)		(L/ha)			
Jalons, France (1999) Santana	3 (10 10)	10 9 10	393 374 381	BBCH 10 BBCH 14 BBCH 22	10	0.06, 0.05 (0.06)
	3 (10 10)	14 14 14	399 387 387	BBCH 10 BBCH 14 BBCH 22	10	0.08, < 0.01, 0.06, < 0.01 (0.04)
Mauguio, France (1999) Nordane	3 (14 11)	11 11 10	427 431 420	BBCH 12–14 BBCH 14–15 BBCH 18–19	13	0.14, 0.13 (0.14)
	3 (14 11)	15 15 15	418 424 431	BBCH 12–14 BBCH 14–15 BBCH 18–19	13	0.18, 0.17 (0.18)
Fresno, California USA (1996) Polka	4 (7 7 7)	35 35 35 35	234 234 234 234	Immature Plants Immature Plants Immature Plants mature plants	0 1 3 7 14	1.25, 1.21 (1.23) 0.72, 0.72 (0.72) 0.48, 0.52 (0.50) 0.26, 0.27 (0.26) 0.12, 0.17 (0.14)
Boulder, Colorado, USA (1996) Melody	4 (7 7 7)	35 35 35 35	188 188 187 187	Vegetative Vegetative Vegetative Vegetative–Fully Extended	7	0.11, 0.14 (0.12)
Montgomery, Virginia USA (1996) Bloomsdale Long Standing	4 (7 7 7)	35 35 35 35	210 210 210 210	6 cm diameter 10–13 cm diameter 13–15 cm diameter 15–20 cm diameter	7	0.23, 0.26 (0.24)
Hunterdon, New Jersey, USA (1996) Tyee (F1)	4 (7 7 7)	35 35 35 35	205 208 206 206	Young Medium Mature Mature	7	0.23, 0.19 (0.21)
San Luis Obispo, California USA (1996) SC[b] Space F1	4 (7 7 7)	35 35 35 35	315 307 308 306	Immature leaves Immature leaves Immature leaves Mature leaves	7	0.42, 0.54 (0.48)
San Patricio, Texas, USA (1997) Cascade	4 (7 7 7)	35 35 35 35	234 234 234 234	Vegetative Vegetative Vegetative Vegetative	7	0.33, 0.25 (0.29)
Elko, South Carolina, USA (2011) Bloomsdale Long Standing)	4 (7 7 7)	35 foliar 35 foliar 35 foliar 35 foliar	204 201 203 200	BBCH 14 BBCH 14 BBCH 15 BBCH 19	7 10 14	0.15, 0.17 (0.16) 0.08 0.06
	4 (7 7 7)	35 foliar 35 foliar 35 foliar 35 foliar (+ Herbimax at 1.0%)	204 201 204 202	BBCH 14 BBCH 14 BBCH 15 BBCH 19	7	0.18, 0.18 (0.18)
	4 (7 7 7)	35 soil 35 soil 35 soil 35 soil	950 948 960 946	BBCH 14 BBCH 14 BBCH 15 BBCH 19	7 10 14	0.04, 0.04 (0.04) 0.03 0.02

Crop (Variety) SPINACH	N	Application rate (g ai/ha)	Spray volume	Growth Stage at Application	DALA	Residue (mg/kg)
	(int)		(L/ha)			
Athens, Georgia USA (2011) Bloomsdale Long Standing	4 (7 7 7)	35 foliar 35 foliar 34 foliar 35 foliar	270 270 272 268	BBCH 41–42 BBCH 42–43 BBCH 43–44 BBCH 45–46	7 10 14	0.26, 0.26 (0.26) 0.13 0.06
	4 (7 7 7)	35 foliar 35 foliar 35 foliar 35 foliar (+ Induce at 0.13%)	270 267 272 268	BBCH 41–42 BBCH 42–43 BBCH 43–44 BBCH 45–46	7	0.27, 0.30 (0.29)
	4 (7 7 7)	35 soil 35 soil 34 soil 35 soil	1008 1019 1039 1006	BBCH 41–42 BBCH 42–43 BBCH 43–44 BBCH 45–46	7 10 14	0.30, 0.35 (0.33) 0.24 0.14
Chula, Georgia, USA (2010) Bloomsdale Long Standing	4 (7 7 7)	35 foliar 35 foliar 35 foliar 35 foliar	244 231 235 222	BBCH 15 BBCH 16 BBCH 18 BBCH 19	7 10 14	0.27, 0.27 (0.27) 0.26 0.25
	4 (7 7 7)	35 foliar 35 foliar 35 foliar 35 foliar (+ Induce at 0.125%)	241 230 236 222	BBCH 15 BBCH 16 BBCH 18 BBCH 19	7	0.34, 0.32 (0.33)
	4 (7 7 7)	35 soil 34 soil 35 soil 35 soil	1205 1047 1073 1062	BBCH 15 BBCH 16 BBCH 18 BBCH 19	7 10 14	0.06, 0.06 (0.06) 0.07 0.04

Mustard greens

Supervised trials were conducted in the USA on mustard greens. Acibenzolar-S-methyl was applied as four foliar applications of a WG formulation at about 53 g ai/ha. At all trial sites samples of mustard green were collected from the treated plots at day seven after application, and immediately after application for trials performed in 1997/98. For decline trials/plots additional samples were collected at nominally 1, 3, 5, 10 and 14 DALA. Samples were analysed for residues per methods AG-671A for the 1997/98 trials and GRM051.05A for the 2010 trials. Procedural recoveries were acceptable. Samples from the trials were stored for a maximum of 636 days (21 months).

Table 69 Residues of acibenzolar-S-methyl following foliar application to mustard greens (Eudy 1999 0660)

Crop (Variety) MUSTARD GREENS	N (int)	Application Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Fresno CA USA (1998) Florida Broadleaf	4 (7 7 7)	53 53 53 53	94 94 94 94	Seedling Vegetative growth vegetative–bolting bolting	0 7	Leaves Leaves	2.24, 3.08 (2.66) 0.59, 0.74 (0.67)
San Patricio TX USA (1997) Florida Broadleaf	4 (6 7 9)	53 53 53 53	187 187 187 187	Vegetative Vegetative Vegetative vegetative	0 1 3 5 7 9	Leaves Leaves Leaves Leaves Leaves Leaves	1.80, 1.85 (1.83) 1.03, 0.83 (0.93) 0.13, 0.32 (0.23) 0.27, 0.19 (0.23) 0.14, 0.18 (0.16) 0.08, 0.13 (0.11)

Crop (Variety) MUSTARD GREENS	N (int)	Application Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Tift GA USA (1997) Southern Giant Curled	4 (6 6 7)	53 53 53 53	271 271 252 299	Rapid Growth Vegetative Rapid Growth Rapid Growth	0 7	Leaves Leaves	3.41, 2.59 (3.00) 0.53, 0.64 (0.59)
St Landry Parish LA USA (1997) Florida Broadleaf	4 (7 7 7)	53 53 53 53	196 206 215 271	Vegetative Vegetative Vegetative vegetative	0 7	Leaves Leaves	2.55, 1.96 (2.26) 0.26, 0.32 (0.29)
Clinton IL USA (1997) Florida Broadleaf	4 (7 7 6)	53 53 53 53	196 196 215 215	Vegetative Vegetative Vegetative vegetative	0 7	Leaves Leaves	3.02, 1.82 (2.42) 0.71, 0.80 (0.76)

Potatoes

Trials were conducted on potatoes in Brazil, Germany and Indonesia.

Supervised trials in Brazil

Eight foliar applications of acibenzolar-S-methyl as a WG formulation were made to potatoes at 18.8 g ai/ha or 37.5 g ai/ha. Samples of potato tubers were collected from the treated plots immediately after the last application, when the spray deposit had dried, and at 3, 7, 14 and 21 DALA. Samples were analysed for residues per the analytical method POPIT MET.003 with an LOQ of 0.1 mg/kg.

Supervised trials in Germany (Europe)

Acibenzolar-S-methyl was applied as eight foliar applications as a WG formulation. Samples of potato tubers were collected from the treated plots immediately after the last application, when the spray deposit had dried, and at 3, 7 ± 1 and 13 DALA. Samples were analysed for residues using analytical method REM 172.11 (modified) with an LOQ of 0.02 mg/kg.

Supervised trial in Indonesia

One trial was conducted in Indonesia in which acibenzolar-S-methyl was applied either at a rate of 12.5 g ai/ha or at a rate of 25 g ai/ha. Each rate was applied in two different plots each, either eight or sixteen times as a WP formulation. Samples of potato tubers were collected 14 DALA. Potato tuber samples were analysed for residues per the validated analytical method REM 172.11 (modified) with an LOQ of 0.02 mg/kg.

Storage of samples

Samples from the trials were stored for a maximum of 410 days (13.5 months).

Table 70 Residues of acibenzolar-S-methyl following foliar application to potatoes in Brazil, Germany and Indonesia (replicate analyses) (Smith 1999ab 0626 0627, Salvi 2001 0768, Roncato *et al*. 2003 10014)

Crop (Variety) POTATO	N (int)	Application Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop part	Residue (mg/kg)

Crop (Variety) POTATO	N (int)	Application Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop part	Residue (mg/kg)
Engenheiro Coelho–SP, Brazil (2000) Asterix	8 (7 7 7 7 7 7 7)	19	400	BBCH 17	0	Tuber	< 0.10
		19	400	BBCH 19	3	Tuber	< 0.10
		19	400	BBCH 51	7	Tuber	< 0.10
		19	400	BBCH 68	14	Tuber	< 0.10
		19	400	BBCH 75	21	Tuber	< 0.10
		19	400	BBCH 80			
		19	400	BBCH 85			
		19	400	BBCH 92			
	8 (7 7 7 7 7 7 7)	38	400	BBCH 17	0	Tuber	< 0.10
		38	400	BBCH 19	3	Tuber	< 0.10
		38	400	BBCH 51	7	Tuber	< 0.10
		38	400	BBCH 68	14	Tuber	< 0.10
		38	400	BBCH 75	21	Tuber	< 0.10
		38	400	BBCH 80			
		38	400	BBCH 85			
		38	400	BBCH 92			
Palmeira PR, Brazil (2001) Monalisa	8 (7 7 7 7 7 7 6)	19	400	BBCH 33–34	0	Tuber	< 0.10
		19	400	BBCH 33–36	3	Tuber	< 0.10
		19	400	BBCH 51–53	8	Tuber	< 0.10
		19	400	BBCH 54–55	14	Tuber	< 0.10
		19	400	BBCH 62–65	21	Tuber	< 0.10
		19	400	BBCH 66–68			
		19	400	BBCH 75–78			
		19	400	BBCH 78–81			
	8 (7 7 7 7 7 7 6)	38	400	BBCH 33–34	0	Tuber	< 0.10
		38	400	BBCH 33–36	3	Tuber	< 0.10
		38	400	BBCH 51–53	8	Tuber	< 0.10
		38	400	BBCH 54–55	14	Tuber	< 0.10
		38	400	BBCH 62–65	21	Tuber	< 0.10
		38	400	BBCH 66–68			
		38	400	BBCH 75–78			
		38	400	BBCH 78–81			
Águas da Prata SP, Brazil (2001) Monalisa	8 (7 7 7 7 7 7 7)	19	400	BBCH 51	0	Tuber	< 0.10
		19	400	BBCH 52	3	Tuber	< 0.10
		19	400	BBCH 57	7	Tuber	< 0.10
		19	400	BBCH 61	14	Tuber	< 0.10
		19	400	BBCH 69	21	Tuber	< 0.10
		19	400	BBCH 70–79			
		19	400	BBCH 79–92			
		19	400	BBCH 93			
	8 (7 7 7 7 7 7 7)	38	400	BBCH 51	0	Tuber	< 0.10
		38	400	BBCH 52	3	Tuber	< 0.10
		38	400	BBCH 57	7	Tuber	< 0.10
		38	400	BBCH 61	14	Tuber	< 0.10
		38	400	BBCH 69	21	Tuber	< 0.10
		38	400	BBCH 70–79			
		38	400	BBCH 79–92			
		38	400	BBCH 93			
See, Germany (1998) Selma	6 (6 7 7 7 7)	30	400	BBCH 65	0	Tuber	< 0.02
		30	400	BBCH 69	3	Tuber	< 0.02
		30	400	BBCH 75	7	Tuber	< 0.02
		30	400	BBCH 81	13	Tuber	< 0.02
		30	400	BBCH 85			
		30	400	BBCH 91			
Ursleben, Germany (1998) Secura	6 (7 7 7 8 7))	30	400	BBCH 33–35	0	Tuber	0.03
		30	400	BBCH 35	3	Tuber	0.04
		30	400	BBCH 35–51	6	Tuber	0.02
		30	400	BBCH 35–51	13	Tuber	0.02
		30	400	BBCH 46–48			
		30	400	BBCH 48–49			

Crop (Variety) POTATO	N (int)	Application Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop part	Residue (mg/kg)
Lembang, West Java, Indonesia (2000) Granola	16 (3 4 3 4 3 4 3 4 3 4 3 4 3 4 3)	12	500	BBCH 301	14	Tuber	0.04, 0.04, 0.03 (0.04)
		12	500	BBCH 303			
		12	500	BBCH 305			
		12	500	BBCH 307			
		12	500	BBCH 309			
		12	500	BBCH 402			
		12	500	BBCH 404			
		12	500	BBCH 406			
		12	500	BBCH 535			
		12	500	BBCH 635			
		12	500	BBCH 705			
		12	500	BBCH 725			
		12	500	BBCH 801			
		12	500	BBCH 805			
		12	500	BBCH 901			
		12	500	BBCH 903			
	8 (7 7 7 7 7 7 7)	12	500	BBCH 301	14	Tuber	< 0.02, < 0.02 [a], < 0.02 (< 0.02)
		12	500	BBCH 305			
		12	500	BBCH 309			
		12	500	BBCH 404			
		12	500	BBCH 535			
		12	500	BBCH 705			
		12	500	BBCH 801			
		12	500	BBCH 903			
	16 (3 4 3 4 3 4 3 4 3 4 3 4 3 4 3)	25	500	BBCH 301	14	Tuber	0.07, 0.06, 0.05 (0.06)
		25	500	BBCH 303			
		25	500	BBCH 305			
		25	500	BBCH 307			
		25	500	BBCH 309			
		25	500	BBCH 402			
		25	500	BBCH 404			
		25	500	BBCH 406			
		25	500	BBCH 535			
		25	500	BBCH 635			
		25	500	BBCH 705			
		25	500	BBCH 725			
		25	500	BBCH 801			
		25	500	BBCH 805			
		25	500	BBCH 901			
		25	500	BBCH 903			
	8 (7 7 7 7 7 7 7)	25	500	BBCH 301	14	Tuber	< 0.02, < 0.02 [a], < 0.02 [a] (< 0.02)
		25	500	BBCH 305			
		25	500	BBCH 309			
		25	500	BBCH 404			
		25	500	BBCH 535			
		25	500	BBCH 705			
		25	500	BBCH 801			
		25	500	BBCH 901			

[a] In the report, these values are calculated as 0.02 mg/kg by applying the correction factor from CGA210007 to express residues as acibenzolar-S-methyl. However, since the measured residues of CGA231007 were below the LOQ (< 0.02 mg/kg) it is not considered correct to convert them to positive residues of acibenzolar-S-methyl.

Cereal grains

Wheat

Trials on wheat were conducted in Argentina and Brazil.

For supervised trials on wheat conducted in Argentina acibenzolar-S-methyl was applied as a single foliar spray at 50 g ai/ha of a WG formulation. Samples of wheat grain were collected from the treated plots at normal commercial harvest in the range of 53–65 days after application. Samples were analysed for residues per the analytical method POPIT MET.081 rev.04 with an LOQ of 0.01 mg/kg. Samples from the trials were stored for a maximum of 517 days (17 months).

For the Brazilian trials, acibenzolar-S-methyl was applied three times as a WG formulation. Three foliar applications were made at 12.5 g ai/ha at an interval of 14 days. Samples of wheat grain were collected from the treated plots at 7, 14 and 21 DALA. Samples were analysed for residues per the validated analytical method POPIT MET.081 rev.11 with an LOQ of 0.01 mg/kg. Samples from the trials were stored for a maximum of 153 days (5 months).

Table 71 Residues of acibenzolar-S-methyl following foliar application to wheat in Argentina and Brazil (replicate samples) (de Gois 2008 10602, Casallanovo 2010 10036)

Crop (Variety) WHEAT	N (int)	Application Rate (g ai/ha)	Spray volume (L/ha)	Growth Stage at Application	DALA	Crop Part	Residue (mg/kg)
Bragado, Buenos Aires, Argentina (2006) Don Mario Onix	1	50	150	BBCH 39	53	Grain	0.07, 0.05 (0.06)
Miramar, Buenos Aires, Argentina (2006) Baguette 21	1	50	150	BBCH 39	65	Grain	0.02, 0.02 (0.02)
Pergamino, Buenos Aires, Argentina (2006) Guapo	1	50	150	BBCH 39	55	Grain	< 0.01, < 0.01 (< 0.01)
Engenheiro Coelho SP, Brazil (2009) IAC 370	3 (14 14)	12 12 12	150 150 150	BBCH 75–77 BBCH 83–85 BBCH 87–89	7 14 21	Grain Grain Grain	< 0.01 < 0.01 < 0.01
Bandeirantes PR, Brazil (2009) CD 114	3 (14 14)	12 12 12	150 150 150	BBCH 75 BBCH 85 BBCH 87–89	7 14 21	Grain Grain Grain	0.04 0.04 0.04
Carambeí, PR, Brazil (2009) Safira	3 (14 14)	12 12 12	150 150 150	BBCH 61–65 BBCH 75–77 BBCH 81–83	7 14 21	Grain Grain Grain	0.07 0.05 0.03
Itaberá SP, Brazil (2009) BRS 208	3 (14 14)	12 12 12	150 150 150	BBCH 71–73 BBCH 75–77 BBCH 83–85	7 14 21	Grain Grain Grain	< 0.01 < 0.01 < 0.01

PRIMARY FEED COMMODITIES OF PLANT ORIGIN

Fate on processing

Effects on the nature of the residues during processing

Adam (1999 0629) conducted a high-temperature aqueous hydrolysis study to determine the nature of any acibenzolar-S-methyl-derived residues under conditions representative of commercial processing: pasteurisation, baking, boiling, brewing, and sterilisation.

[^{14}C]acibenzolar-S-methyl was incubated in aqueous buffer solutions at a nominal concentration of 2.8 mg/L under three sets of conditions, each designed to simulate an appropriate process: 90 °C (pH 4, 20 minutes) to simulate pasteurisation, 100 °C (pH 5, 60 minutes), to simulate boiling, baking and brewing, and 120 °C (pH 6, 20 minutes) to simulate sterilisation.

Sterilised test solutions were heated to the appropriate temperature for the corresponding time and then allowed to cool before analysis. Total recovered radioactivity was measured for each test solution. Radioactive components were characterised by fractionation and co-chromatography with authenticated reference compounds using TLC and bio-imaging and/or HPLC. Control samples were prepared for each buffer and maintained at ambient temperature for 1 hour.

After incubation, total recoveries of applied radioactivity ranged from 99.9% to 103.5% for all samples. Acibenzolar-S-methyl was shown to be hydrolytically stable under representative processing conditions of 90 °C (pH 4, 20 minutes) and 100 °C (pH 5, 60 minutes). Mean amounts of acibenzolar-S-methyl remaining after incubation were 102.4% and 93.9%, respectively. No significant breakdown or reaction products were formed under these conditions.

Acibenzolar-S-methyl was shown to degrade under conditions of 120 °C (pH 6, 20 minutes). The mean amount of acibenzolar-S-methyl remaining after incubation was 51.4%. The hydrolysis product and known metabolite CGA210007 was found with mean values of applied radioactivity after incubation of 50.5%.

No other metabolites were found under any of the test conditions.

Table 72 Degradation on acibenzolar-S-methyl under conditions that simulate commercial processing

Test System (Compound)	(% AR)					
	pH 4, 90 ºC, 20 min (pasteurisation)		pH 5, 100 ºC, 60 min (boiling, baking, brewing)		pH 6, 120 ºC, 120 min (sterilisation)	
acibenzolar-S-methyl)	102.2	102.6	93.9	93.9	54.1	48.8
CGA210007	1.3	1.1	5.7	6.4	48.5	52.5

Oranges

Oranges from two trials that employed an exaggerated application rate as a soil drench were used for a processing study (Willard 2012a 50026).

The oranges were batch tub washed for 5 minutes. The washed oranges were transferred to the modified abrasive peeler for scarifying. Approximately 2.31 kg of oranges per batch were abraded for approximately 45 seconds to scarify the flavedo for oil recovery. The scarified fruit was weighed and retained for juice processing.

The collected oil-water emulsion was screened using an approximately 180 µm screen to separate any flavedo fragments from the oil-water emulsion. The scarified flavedo (+screen) was set aside for later addition to the shredded peel. The first run oil-water emulsion (–screen) was processed through the cream separator and centrifuge to separate the oil. The free oil was removed. The residual emulsion was then frozen overnight, thawed, centrifuged, and the oil removed. This oil was added to the free oil.

An aliquot of the scarified oranges was weighed and transferred to a juice extractor to recover the juice from the peel. The juice and peel recovered were weighed and Brix taken on the fresh juice. The collected juice was transferred to the pulper finisher and screened using an approximately 1.19 mm screen to remove vesicular membranes, seeds, segment membranes, and peel fragments from the juice. The collected rag and seeds were set aside for later addition to the shredded peel. A representative sample of the fresh juice was removed.

The peel from the juice extractor was shredded and was combined with the scarified flavedo from the scarification process and rag and seeds from the juice finisher extraction process to generate wet peel. Lime (approximately 95% CaO) was added to the wet peel and mixed for 17 minutes. If the pH was less than 8.0, additional lime and water was added and the peel was mixed again for an additional 15 minutes. The limed peel was pressed and the expressed liquid weighed, checked for pH and Brix.

The wet peel pulp was dried to below 10% moisture. The dried pulp was milled using a hammermill. A representative sample of the dried pulp was removed.

Table 73 Acibenzolar-S-methyl residues in orange and processed commodities with corresponding processing factors

Location, year, variety ORANGE	No. (int)	Rate (kg ai/ha)	DALA	Sample	Residues (mg/kg)	PF
Chuluota, FL, USA (2010) Hamlin (198 kg)	4 (28 27 27)	567 566 564 559	0	Fruit pre-processing Pulp (dried) Juice Oil Fruit (field)	0.018 0.014 (0.016) 0.072 < 0.01 < 0.01 0.017 0.017 (0.017)	4.5 < 0.625 < 0.625
Fresno, CA, USA (2010) Navel (197 kg)	4 (29 31 30)	560 558 560 563	0	Fruit pre-processing Pulp (dried) Juice Oil Fruit (field)	< 0.01 < 0.01 < 0.01 0.011 < 0.01 < 0.01 < 0.01	> 1.1 – –

Pears

A small-scale processing study was also carried out on pears harvested after foliar application (Kühne 2001f 0775).

Juice

Fruit were washed manually with cold water. Washed fruit were sliced and mashed fruit pressed with a juice press (1–2 MPa) to produce raw juice. Insoluble components in the raw juice were separated and the resulting natural turbid juice was pasteurized with a plat pasteurizer (85 °C, 2 min). The wet pomace was retained for processing into dry pomace.

Purée

Fruit with stalk removed were manually washed under cold water, and the washed fruit cut into halves, placed in a saucepan and covered with water. The contents were bought to the boil and cooked until the fruit was passable through a sieve (dry matter approximately 16.5% at this stage). When ready the fruit was sieved, and sugar added and the pH adjusted to 3.0–4.5 by addition of citric and ascorbic acids. Tins were filled with purée, closed and pasteurized (95 °C, 20 min).

Dry pomace

The wet pomace (after pressing of juice) was dried at 65 °C until a moisture content of less than 10% was achieved.

No residues above the method LOQ were detected in pears at harvest. Processing did result in residues above LOQ in pomace dry indicating concentration in this commodity. Based on results between the LOD and LOQ. Residues are reduced (PF < 1) for washed fruit, juice and purée and increased (PF> 1.2) in the case of pomace, wet and dry.

Table 74 Acibenzolar-S-methyl residues in pear and processed commodities with corresponding processing factors (Kühne 2001f 0775)

Location, year, variety	No.	Rate	DALA	Sample	Residues (mg/kg)	PF
PEAR		(kg ai/ha)				

Location, year, variety	No.	Rate	DALA	Sample	Residues (mg/kg)	PF
PEAR		(kg ai/ha)				
Stretti di Erachlea (VE) Italy (2000) Conference 4 batches, each 2-3.6 kg	8 (6 6 6 44 11 11 11)	100 100 100 100 100 100 100 100	21	Fruit pre-processing Fruit, washed Juice unpasteurized Juice pasteurized Pomace, wet Pomace, dry	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 0.01 0.012 0.013 0.011	
				Fruit pre-processing Fruit, washed Residue after filtration Purée	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	

The apparent PFs for pomace dry were 2.0, 3.2, 3.4 and 3.0 if the < LOQ residue value of 0.0039 mg/kg for fruit pre-processing is used in calculations.

Tomatoes

In a series of studies (Walser 1998egk 0509 0536 0525 Walser 1999p 0587) the distribution of residues on processing tomatoes following foliar application were studied.

In the two trials in Italy, during 1997, a WG formulation of acibenzolar-S-methyl was applied to tomatoes. Six applications were made to tomato plants at a rate of 25 g ai/ha, separated by an interval of nominally 7 days. At normal commercial harvest, 3 days after the final application, samples of treated and untreated tomato fruits were harvested and transported to the processing facility.

For the preparation of washed tomato fruit, the calyxes were first removed. The fruit were then washed by gentle movement in standing water for approximately 2 minutes. After draining, the tomatoes were cut into pieces with a knife to produce washed tomato fruit.

For the preparation of peeled tomato fruit, the calyxes were first removed. The fruit were then transferred to a vessel containing lukewarm water which after a few minutes enabled the peel to be carefully removed. After peeling, the tomatoes were cut into pieces with a knife to produce peeled tomato fruit.

For the juicing process, the calyxes were first removed. The fruit were then washed by gentle movement in standing water for approximately 2 minutes. After draining, the tomatoes were cut into pieces with a knife. The cut fruit were heated in water (100 mL per kg of fruit) to a temperature of 98–100 °C for 5 minutes to blanch them and halt enzymatic reactions. After blanching, the remaining pulp was passed through a sieve to yield separated juice and wet pomace. Sodium chloride (0.5 to 0.7% by weight relative to the amount of juice) was added to the raw juice. The juice was then pasteurised in cans by heating to 91 °C to produce pasteurised juice.

For the preparation of concentrate pulp/paste, the calyxes were first removed. The fruit were then washed by gentle movement in standing water for approximately 2 minutes. After draining, the tomatoes were cut into pieces with a knife. The cut fruit were heated in water (100 mL per kg of fruit) to a temperature of 98–100 °C for 4 minutes to blanch them and halt enzymatic reactions. After blanching, the remaining pulp was passed through a strainer to yield separated juice and wet pomace. The juice was concentrated to 45 to 31% dry weight by centrifugation and drying. The paste was then pasteurised by transfer into cans and heating to 92 °C to produce concentrated paste.

For the preparation of canned tomato fruit, the calyxes were first removed. The fruit were then transferred to a vessel containing lukewarm water which after a few minutes enabled the peel to be carefully removed. After peeling, the tomatoes were filled into cans and topped up with tomato juice. The cans containing the fruit were then pasteurised by heating to 93 °C to produce canned fruit.

Acibenzolar-S-methyl residues in washed fruit, peeled fruit, canned fruit and juice were like the level in the RAC before processing (0.06 mg/kg). Acibenzolar-S-methyl residues in washed fruit were 0.06 mg/kg, in peeled fruit 0.05 mg/kg, in juice 0.06 mg/kg and in canned fruit 0.05 mg/kg. Acibenzolar-S-methyl residues in concentrated paste/pulp were 0.20 mg/kg. The results and processing factors for processed samples are presented in Table 75.

Trials were also conducted in France in which a WG formulation of acibenzolar-S-methyl was applied to tomatoes. Six applications were made to tomato plants at a rate of 25 g ai/ha, separated by an interval of nominally 7 days. At normal commercial harvest, 3 days after the final application, samples of treated and untreated tomato fruits were harvested and transported to the processing facility.

For the juicing process, the green parts or any bruised fruit were removed. The fruit were then crushed before transfer to an automatic sieve for removal of juice from skin/seeds. Cooking salt was added to the raw juice (7 g/kg). The raw juice was then pasteurised by transfer into glass bottles and heating to 115 °C for 2 minutes to produce pasteurised juice.

For the preparation of concentrate (purée) the green parts or any bruised fruit were removed. The tomatoes were then cut into pieces and placed in a heated saucepan to reduce. When enough liquid phase was available, the brix degree was measured until it reached 11%. The contents were then transferred to an automatic sieve for removal of juice from skin/seeds. Cooking salt was added to the raw purée (4 g/kg). The raw purée was then pasteurised by transfer into glass bottles and heating to 115 °C for 2 minutes to produce purée.

For the preparation of preserved fruit, the green parts or any bruised fruit were removed. The fruit were then transferred to a vessel containing boiling water for thirty seconds to enable the peel to be carefully removed. Cooking salt and citric acid were added to previously-prepared tomato juice. The peeled tomatoes and juice were then placed into glass jars to simulate commercial canning (two thirds tomatoes to one third juice). The jars of peeled fruit and juice were then pasteurised by heating to 115 °C for 2 minutes to produce preserved fruit.

For the preparation of ketchup (sauce) the green parts or any bruised fruit were removed. The tomatoes were then cut into pieces and placed in a heated saucepan to reduce. When enough liquid phase was available, the brix degree was measured until it reached 11%. The contents were then transferred to an automatic sieve for removal of juice from skin/seeds and the raw ketchup was put back into the saucepan for a second reduction. The brix degree was measured until it reached 14%. The amount of puree obtained was weighed and then further ingredients added to produce a ketchup recipe; puree 72%, brown sugar 19%, cider vinegar 7% and salt 2%, all by weight. All ingredients were blended by whisking at elevated temperature. The raw ketchup was then pasteurised by transfer into glass bottles and heating to 115 °C for 2 minutes to produce ketchup.

Table 75 Acibenzolar-S-methyl residues in tomato and processed commodities with corresponding Processing factors

Country, Year, Trial	Commodity	Initial residue acibenzolar-S-methyl (mg/kg)	Processed residue acibenzolar-S-methyl (mg/kg)	PF
Italy 1997 2043/97	Tomato RAC	0.06	–	–
	Washed fruit		0.06	1.0
	Peeled fruit		0.05	0.83
	Juice		0.06	1.0
	Concentrated paste		0.20	3.33
	Canned fruit		0.05	0.83
Italy 1997 2043/97	Tomato RAC	0.06	–	–
	Washed fruit		0.04	0.67
	Peeled fruit		0.03	0.50
	Juice		0.04	0.067
	Concentrated paste		0.07	1.17
	Canned fruit		0.03	0.50

Country, Year, Trial	Commodity	Initial residue acibenzolar-S-methyl (mg/kg)	Processed residue acibenzolar-S-methyl (mg/kg)	PF
N France 1997 2360/97	Tomato RAC	0.04	–	–
	Juice		0.03	0.75
	Purée		0.07	1.75
	Ketchup		0.07	1.75
	Preserved fruit		0.02	0.50
S France 1997 2364/97	Tomato RAC	0.05	–	–
	Juice		0.04	0.80
	Purée		0.10	2.0
	Ketchup		0.10	2.0
	Preserved fruit		0.04	0.80

Processing factor = residue in processed commodity (mg/kg) / residue in RAC (mg/kg)

Table 76 Acibenzolar-S-methyl processing factors obtained from tomato processing studies

Commodity	Processing factors				
	Study 1	Study 2	Study 3	Study 4	Mean
Washed fruit	1.0	0.67	–	–	0.84
Peeled fruit	0.83	0.50	–	–	0.67
Tomato juice	1.0	0.67	0.75	0.80	0.81
Preserved fruit (canned)	0.83	0.50	0.50*	0.80*	0.66
Purée (paste) / concentrate	3.33	1.17	1.75	2.0	2.1
Ketchup	–	–	1.75	2.0	1.9

Livestock feeding studies

Dairy cow feeding study

The transfer of acibenzolar-S-methyl metabolites from feed to tissues and milk of dairy cows was studied by Walser (1998c 0465). Acibenzolar-S-methyl was administered orally to three groups of three Holstein cattle (3 yrs old; 451–612 kg bw) by gelatine capsule for 28 days. Mean daily feed consumption for the dose groups during the exposure period were 20.8–21.4 kg DM (hay, ad libitum and 16.4 kg/day feed). Mean daily milk yields for the dose groups during the exposure period were 21.1 to 23.6 kg/cow/day. Based on mean daily feed consumption, the exposure was equivalent to 0.25 (1×), 1.27 (5×) and 2.48 (10×) ppm in the feed or 5.3, 26.5 and 53 mg acibenzolar-S-methyl/cow/day. Milk was collected twice daily (am and pm sampling pooled) at nine intervals through the 28 days of dosing. Muscle (tenderloin, round steak, diaphragm), liver, kidney and fat (peri-renal, omental) samples were collected at sacrifice 20–22.2 hours after the last dose. The maximum frozen storage intervals were 49 days for milk while the maximum storage intervals for tissues were 97, 110, 35 and 90 days for liver, kidney, muscle and fat, respectively. Samples were analysed using several analytical methods: REM172.11 for milk, REM172.12 for tissues (muscle, fat, liver and kidney) and REM172.13 for blood samples.

The LOQ of the methods was 0.005 mg/kg for milk; 0.02 mg/kg for tissues and fat and 0.05 mg/kg for blood.

The methods were validated by fortification of samples of muscle, liver, kidney, fat, blood and milk spiked with known amounts of acibenzolar-S-methyl and carried through the procedure with the treated samples as recovery experiments. Recovery values for acibenzolar-S-methyl in milk and cow tissues ranged from 77% to 95% with relative standard deviations of 4% to 18%, indicating acceptability of the methods used in this study (Table 77).

Table 77 Recovery values for acibenzolar-S-methyl in milk and cattle tissues

Fortification level	Recovery (%)

(mg/kg)	Muscle	Fat	Liver	Kidney	Blood	Milk (8–26 days)	Milk (0–5 days)
0.02	86, 71, 74, 78	104, 89	92	99, 96	–	–	–
0.20	83, 70, 79	94, 94, 94	89	93	–	–	–
0.005	–	–	–	–	96, 95	91, 86, 83, 82, 101, 102, 94	51, 53, 58, 55
0.050	–	–	–	–	97	87, 85, 83, 86, 87, 81	91, 91, 83
Mean	77	95	94		93	82	
% RSD	8	6	4		–	18	

Even at the highest 10× dose level equivalent to 2.48 ppm, no residues of acibenzolar-S-methyl equivalents at or above the LOQ of 0.005 mg/kg were found in any of the milk samples; nor above the LOQ of 0.02 mg/kg in the liver, kidney, muscle and fat samples (Table 78).

Table 78 Residues of acibenzolar-S-methyl metabolites in milk and tissues of lactating cows

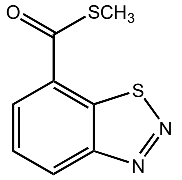
Sample	Dose group (ppm)	Residues
		(mg/kg)
Milk (0 pre-dose, 2, 5, 8, 11, 15, 19, 22, 26am, 26pm)	0.25 1.27 2.48	< 0.005 < 0.005 < 0.005
Tenderloin muscle	0.25 1.27 2.48	< 0.02 < 0.02 < 0.02
Round steak muscle	0.25 1.27 2.48	< 0.02 < 0.02 < 0.02
Diaphragm muscle	0.25 1.27 2.48	< 0.02 < 0.02 < 0.02
Perirenal fat	0.25 1.27 2.48	< 0.02 < 0.02 < 0.02
Omental fat	0.25 1.27 2.48	< 0.02 < 0.02 < 0.02
Liver	0.25 1.27 2.48	< 0.02 < 0.02 < 0.02
Kidney	0.25 1.27 2.48	< 0.02 < 0.02 < 0.02

APPRAISAL

Acibenzolar-S-methyl is a plant activator that stimulates the natural, inherent defence mechanisms of plants. Through this activation, control of *Erysiphe graminis* (powdery mildew of cereals) and *Mycosphaerella musci* (Black Sigatoka of banana) is achieved. This activation also results in partial control of *Septoria* spp. (side effect only) and *Puccinia* spp. (side effect only) in cereals. At the 47th Session of the CCPR (2015), it was scheduled for the evaluation as a new compound by the 2016 JMPR.

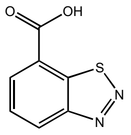
The Meeting received information on the metabolism of acibenzolar-S-methyl in lactating goats and laying hens, cotton, lettuce, sorghum, sunflower, rice, tomato, tobacco and wheat, as well as follow crops, methods of residue analysis, freezer storage stability, GAP information, supervised residue trials on citrus fruit (orange, grapefruit, lemon), pome fruit (apples, pears), stone fruit (apricot, peach), strawberries, bananas, kiwifruit, onions, brassica vegetables (cabbage, broccoli, mustard greens), cucurbits (cucumbers, melons, squash), tomatoes, leafy vegetables (lettuce, spinach), celery, potatoes and wheat as well as a livestock transfer study (lactating cow).

Acibenzolar-S-methyl is *S*-methyl 1,2,3-benzothiadiazole-7-carbothioate

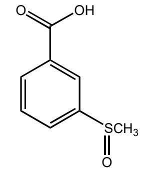


Metabolites referred to in the appraisal were addressed by their company codes:

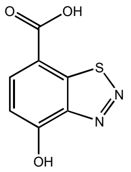
Acibenzolar acid
(CGA210007)



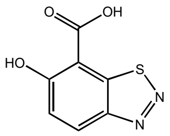
3-Methanesulfinyl-benzoic acid
(CGA379019)



4-OH acibenzolar acid
(CGA323060)



6-OH acibenzolar acid
(SYN546642)



Studies on the metabolism in plants and livestock and environmental fate all utilized [^{14}C-U-phenyl]-acibenzolar-S-methyl.

Plant metabolism

Acibenzolar-S-methyl is typically used for four different situations:

- as a seed treatment
- as a foliar application
- as a soil treatment for rice at transplanting
- application to the soil beneath a growing crop

The Meeting received plant metabolism studies with acibenzolar-S-methyl following seed treatment (sunflower, cotton, sorghum), foliar applications to lettuce, tobacco, tomato and wheat and soil treatment at transplanting rice.

Seed treatment

Residues in commodities at harvest of crops of cotton, sorghum and sunflower grown from seeds that were treated with [^{14}C]-acibenzolar-S-methyl prior to planting (0.0006-0.001 mg ai/seed cotton; 0.0065 mg ai/seed sorghum; 0.048 mg ai/seed sunflower), were low at 0.002-0.006 mg equiv/kg for cotton plants, stalks, fibre and seed, < 0.001-0.002 mg equiv/kg for sorghum forage, stover and grain and 0.002 mg equiv/kg for sunflower seeds.

Foliar application

Tomato

The metabolism of [^{14}C]-acibenzolar-S-methyl in tomato plants grown outdoors following three foliar sprays at 15 g ai/hL at 14 day intervals.

TRR found at harvest were 0.312 mg equiv/kg in tomato fruit (30 days after last application) and 0.72 mg equiv/kg in foliage (60 DALA). The ^{14}C recovered in surface washes of fruit decreased from 35% TRR (one hour after third application) to 1.4% TRR (one month after third application).

Extractability of ^{14}C residues with the solvent system (CH_3CN/H_2O) used was 97% of the TRR for tomato fruit at maturity, 30 DALA.

The metabolites identified in tomato fruit and foliage primarily resulted from initial hydrolysis of acibenzolar-S-methyl to give acibenzolar acid (CGA210007) followed by oxidation to give various metabolites hydroxylated at the phenyl ring. Acibenzolar-S-methyl was detected in foliage and fruit samples on the day of application and for up to a further 30 days, albeit at very low levels. At harvest, acibenzolar-S-methyl accounted for 0.8% TRR in fruit, free acibenzolar acid accounted for 8.1% TRR and conjugates of acibenzolar acid liberated by cellulase/mild base hydrolysis of the solvent extracts accounted for a further 56% TRR.

Lettuce

The metabolism of [^{14}C]-acibenzolar-S-methyl in lettuce plants grown outdoors following three foliar sprays at 35 g ai/hL (1 ×) at 7 day intervals. An additional set of plants were treated at 105 g ai/ha (3 ×). The first applications were made at the 7-9 leaf stage (BBCH 17-19).

TRR in lettuce plants harvested one week after final application were 1.0 mg equiv/kg (1 × rate) and 3.7 mg equiv/kg (3 × rate). In these samples, parent acibenzolar-S-methyl residues were 0.17 and 0.71 mg/kg, respectively. Surface rinses contained 20% (1 ×) and 23% (3 ×) of TRR, with penetrated residue amounting to 83% (1 ×) and 74% (3 ×) of the TRR (7 DALA).

Solvent (CH_3CN/H_2O) extracted $\geq 81\%$ of the TRR. Acibenzolar-S-methyl accounted for 17-19% TRR while free acibenzolar acid represented $\leq 5\%$ TRR. The solvent extracts were sequentially treated with 0.1N NaOH/cellulase to investigate the presence of conjugates. Based on the increase in acibenzolar acid on hydrolysis, conjugates of acibenzolar acid represented 12-20%TRR. The only other metabolite(s) present at levels >10% TRR were conjugates of 4-OH acibenzolar acid which represented 19-22% TRR. In contrast, free 4-OH acibenzolar acid only represented 0.9-1.1% TRR.

Tobacco

The metabolic fate of [^{14}C]-acibenzolar-S-methyl in tobacco plants maintained outdoors was examined following three foliar sprays, starting when plants were at the seven leaf stage, of 20, 50 and 100 g ai/ha with intervals of 21 and 34 days.

Translocation of the radioactivity into new grown leaves was low. Residues found 21 days after the first application were 0.60 and 0.031 mg equiv/kg in treated and new grown leaves respectively. At maturity, TRRs in tobacco plants were 1.4 mg equiv/kg in lower leaves, 0.43 mg eq./kg in upper leaves and 0.022 mg equiv/kg in stems.

Solvent (CH_3CN/H_2O) extracted $\geq 88\%$ of the TRR present in leaf samples collected 0 to 52 DALA.

Parent acibenzolar-S-methyl was present in low amounts in tobacco leaves 17–52 DALA (max. 6.1% TRR) with free acibenzolar acid present at 6.4–9.0% TRR. The major component of the ^{14}C residue was conjugates of acibenzolar acid which liberate acibenzolar acid on cellulase/0.1N NaOH treatment, accounting for a further 61% TRR.

Wheat

The metabolism of [^{14}C]-acibenzolar-S-methyl in wheat grown outdoors was studied following application to plants at the end of tillering at 50 g ai/ha. Total radioactive residues in shoots on the day of application were 1.8 mg equiv/kg, declining to 0.29 mg equiv/kg after 14 days. At harvest, 75 days after application, levels of ^{14}C in grain, husks and straw were 0.014, 0.23 and 0.33 mg eq/kg respectively.

Solvent (CH_3CN/H_2O) extracted ≥87% of the TRR present in immature plant parts (shoots) collected 0–14 days after application. Extraction of ^{14}C present at harvest (75 days after application) was low at 41% for grain, 39% for husks and 30% for straw.

Apart from on the day of application, acibenzolar-S-methyl was not detected in any of the samples. The metabolites identified primarily resulted from initial hydrolysis of acibenzolar-S-methyl to form acibenzolar acid followed by oxidation of the phenyl ring to give a range of hydroxy acibenzolar acid metabolites. Acibenzolar acid, and metabolites hydroxylated at the phenyl ring, can form conjugates with natural compounds such as sugars or bind to cell components such as proteins. Free acibenzolar acid accounted for 8.4, 12 and 14% TRR in grain, husks and straw respectively. Based on the increase in acibenzolar acid after mild base hydrolysis of the solvent extracts, conjugates of acibenzolar acid accounted for 15, 11 and 7.8% TRR for grain, husks and straw respectively.

The majority of the ^{14}C present in the solids after the initial solvent extraction (PES) were associated with natural products, 21% TRR for grain, 29% for husks and 36% TRR for straw; especially starch, protein, lignin and cellulose. The severe conditions utilized for further examination of ^{14}C residues in PES liberated additional acibenzolar acid, presumably bound to various natural components. The additional liberated acibenzolar acid accounted for a further 16, 17 and 23%TRR for grain, husks and straw respectively.

Soil treatment at transplanting

Rice

The metabolism of [^{14}C]-acibenzolar-S-methyl in greenhouse grown rice plants was studied following application to soil of a granular formulation to three-week old rice plants one day prior to transplanting at a rate equivalent to 200 g ai/ha. Plants with soil were transplanted into containers and flooded with water. Mature plants were harvested 119 days after application.

Residues in paddy water reached a maximum of 25% AR at 1–2 weeks after soil application and flooding, declining to 15% AR by 40 days after application (DAA). Thereafter the ^{14}C residues in paddy water declined rapidly to reach 0.4% AR by 60 DAA. Free acibenzolar acid was the major metabolite/degradate present in paddy water representing 32% TRR at 11 DAA and 57% TRR at 78 DAA.

One DAA, rice seedlings had taken up 1.3% AR, rising to 11.1% AR at maturity (harvest 119 DAA). At maturity, 0.2% AR was located in grain, 0.2% AR in husks and 11% AR in straw while 81% AR remained in the soil. Solvent (CH_3CN/H_2O) extracted only 6.4% TRR for grain, 33% TRR for husks and 41% TRR for straw. Parent acibenzolar-S-methyl was only detected in samples of leaves collected 1 DAA. The major metabolite identified in leaves collected 11-78 DAA was free acibenzolar acid at 6.7-11% TRR in leaves and at 1.7–10% TRR in rice commodities (grain, husks, straw) at harvest, 119 DAA.

The presence of conjugated residues in the solvent extracts was investigated. Following hydrolysis the proportion of unidentified ^{14}C decreased and there was a concomitant increase in the proportion of acibenzolar acid and to a lesser extent 3-methanesulfinyl benzoic acid. Based on the hydrolysis results, conjugated acibenzolar acid accounted for 38, 11 and 2.0% TRR in straw, husks and grain, respectively. Conjugates of 3-methanesulfinyl benzoic acid accounted for 5.3, 3.1 and 0.7% TRR in straw, husks and grain, respectively.

As the unextracted ^{14}C in plant samples was high, the PES were subject to harsh alkaline and acid conditions to assist further characterisation. Following hydrolysis of PES under harsh alkaline and acid conditions, the unextracted residues remaining in grain were reduced from 92% TRR to 39% TRR. While small amounts of additional acibenzolar acid were liberated (1.8–8.1% TRR), 3-methanesulfinyl benzoic acid was the major component released by the harsh treatment and accounted for 18% TRR in grain. Further investigation also revealed incorporation of ^{14}C into natural products, 24% TRR for grain, 31% TRR for husks and 17% TRR for straw.

In summary, the metabolism of acibenzolar-S-methyl by plants is well understood. Primary metabolic pathways of acibenzolar-S-methyl in plants included: 1) hydrolysis of the S-methyl group to form acibenzolar acid; 2) oxidation at the phenyl ring to form a range of hydoxy derivatives including 4-OH acibenzolar acid; 3) opening of the thiadiazole ring to form 3-methanesulfinyl benzoic acid; and 4) formation of conjugates, principally with sugars.

With the exception of 3-methanesulfinyl benzoic acid (CGA379019), all plant metabolites were also identified in the rat metabolism though the conjugate partners may differ.

Crop		Tomato	Tobacco	Lettuce	Wheat			Rice		
DALA		30	17-45	7	75	75	75	119	119	119
Matrix		Fruit	Leaf	Leaf	Grain	Husks	Straw	Grain	Husks	Straw
Acibenzolar-S-methyl		0.8	6.1	16.9-19.3						
Acibenzolar acid	Free	8.1	6.4-9.0	<5	8.4	12.1	14.4	1.7	3.7	10.2
	Conj	56.2	61.4	11.5-20.1	15.1	11.4	7.8	2.0	11.3	37.9
	Bound				15.8	17.4	23.1	1.9	1.8	8.1
4-OH acibenzolar acid	Free			0.9-1.1						
	Conj			19-21.5						
3-methanesulfinyl benzoic acid	Free							0.6	3.7	5.3
	Conj							0.7	3.1	5.3
	Bound							17.5	1.1	2.5

Animal metabolism

The plant metabolism studies show that livestock are unlikely to be exposed to parent acibenzolar-S-methyl. Rather, animals will be exposed to a range of metabolites that are mostly comprised of free and conjugated acibenzolar acid. Metabolism studies were made available to the Meeting that utilized dosing lactating goats and laying hens with acibenzolar-S-methyl. As acibenzolar-S-methyl is rapidly hydrolysed to acibenzolar acid, and conjugates of acibenzolar acid are readily cleaved to produce acibenzolar acid, the use of acibenzolar-S-methyl in the livestock metabolism studies is acceptable.

Lactating goats were orally dosed once daily for four consecutive days with [^{14}C]-acibenzolar-S-methyl at a dose equivalent to 12 ppm in the feed. The majority of the ^{14}C residues was recovered in the excreta (urine 64%AD, faeces 12% AD). For tissues, ^{14}C residues were highest in kidney, (0.28 mg equiv/kg), followed by liver (0.041 mg equiv/kg) with muscle (0.003 mg equiv/kg) and fat (0.002–0.003 mg equiv/kg) containing very low residues. TRR in milk reached 0.12 mg equivalents/kg before the end of dosing. Solvent (CH_3CN and CH_3CN/H_2O) extracted >86% of the TRR in milk and tissues. No intact acibenzolar-S-methyl was detected in tissues or milk. The majority of the residues were present as free (33–90% TRR) and soluble conjugated forms (2.3–22% TRR) of acibenzolar acid. Significant additional acibenzolar acid was released from muscle (27% TRR) when harsh extraction conditions were used on radioactivity in solids remaining after solvent extraction.

Laying hens were orally dosed once a day for four consecutive days with [^{14}C]-acibenzolar-S-methyl at a dose equivalent to 19 ppm in the feed. The majority of the ^{14}C residues was recovered in the excreta (87% AD). Radioactivity in eggs reached 0.001 mg equiv/kg, with average concentrations of 0.002 mg equiv/kg for yolk and 0.001 mg equiv/kg for egg whites. Mean levels of TRR were 0.90 mg equiv/kg in kidney, 0.33 mg equiv/kg in liver, 0.013 mg equiv/kg in breast muscle, 0.013 mg equiv/kg in peritoneal fat, and 0.045 mg equiv/kg in skin plus subcutaneous fat. Solvent (CH_3CN and CH_3CN/H_2O) extracted >82% of the TRR in eggs and tissues. No intact acibenzolar-S-methyl was detected in tissues or eggs. Free acibenzolar acid accounted for the majority of the residue (50–77%

TRR) with significant amounts of acibenzolar acid released from conjugates in egg white (18% TRR) when harsh extraction conditions were used.

In summary, the metabolism of acibenzolar-S-methyl in goats and laying hens is similar to metabolism in laboratory animals with acibenzolar acid (free and conjugated) the major component of the residue.

Environmental fate

The Meeting received information on soil aerobic metabolism, aqueous photolysis and aqueous hydrolysis properties of [^{14}C]-acibenzolar-S-methyl. Studies were also received on the behaviour of [^{14}C]-acibenzolar-S-methyl in a rotational crop situation.

The degradation of acibenzolar-S-methyl in soil maintained under aerobic conditions was rapid with acibenzolar acid and 6-OH acibenzolar acid, the major degradation products formed. While parent acibenzolar-S-methyl was degraded quickly in soils, the degradates formed are moderately to highly persistent. In the laboratory studies, soil DT_{50} values for parent acibenzolar-S-methyl ranged from 0.031 to 2.1 days while for acibenzolar acid DT_{50} values ranged from 4.1 to 91 days and for 6-OH acibenzolar acid 130 to >1000 days.

Acibenzolar-S-methyl was stable to hydrolysis in aqueous solutions at pH 5 and 7 but undergoes rapid hydrolysis at pH 9 and above suggesting hydrolysis plays a negligible role in its degradation under environmental conditions. The main hydrolysis degradate was acibenzolar acid which was stable at all pH values.

The soil photolysis of acibenzolar-S-methyl in dry and wet soils was investigated. Light enhanced the degradation of acibenzolar-S-methyl on both wet and dry soils. Acibenzolar acid was essentially stable on dry soils but moderately degraded in the irradiated wet soils. No other metabolites were reported to reach levels above 5% AR. Hydrolysis of acibenzolar-S-methyl to form acibenzolar acid was more rapid on wet compared to dry soils.

In a confined rotational crops study with lettuce, radish, maize and wheat, a plot of clay soil was treated with [^{14}C]-acibenzolar-S-methyl at the equivalent of 50 g ai/ha and crops sown 30, 113, 141 and 337 days after the soil application. Lettuce was sampled at 50% maturity and at full maturity (61 and 82 DAA), radish was sampled at 180 DAA, wheat samples were taken at 180, 370 and 414 DAA and maize was sampled at 50% and full maturity (404 and 498 DAA). The uptake of radioactivity in rotational crops was very low for lettuce, winter wheat, maize and radish: all residue levels in the crops were ≤ 0.001 mg equiv/kg.

In a separate confined rotational crop study a sandy loam soil was treated with [^{14}C]-acibenzolar-S-methyl at the equivalent of 421 g ai/ha and radish, wheat and mustard sown at 30, 60 and 210 days after application. The metabolism of acibenzolar-S-methyl in rotated crops was similar for all crop types and proceeded by hydrolysis of the S-methyl ester leading to formation of the major metabolite, acibenzolar acid which existed as polar conjugates that could be cleaved by treatment with cellulase plus NaOH. Subsequent hydroxylation of the phenyl ring gave metabolites 5-OH acibenzolar acid and 4-OH acibenzolar acid. Comparison with primary metabolism studies shows that the pathway in rotational crops is consistent with that in primary crops.

The major degradates in soil were acibenzolar acid and 6-OH acibenzolar acid, however, no 6-OH acibenzolar acid was detected in crops.

In summary, acibenzolar-S-methyl related residues in soil are unlikely to be observed at significant levels in rotational crops following application at maximum permitted seasonal rates of up to 332 g ai/ha for non-permanent crops.

Methods of Analysis

Methods have been reported in the scientific literature for the analysis of acibenzolar-S-methyl in food, including multi-residue methods. These literature methods do not involve a hydrolysis step and the residue measured is therefore parent compound (acibenzolar-S-methyl).

The metabolism of acibenzolar-S-methyl in crops results in a complex mixture of metabolites, most of which produce acibenzolar acid on base hydrolysis. Any non-metabolized parent acibenzolar-S-methyl that might be present would be converted to acibenzolar acid upon base hydrolysis. Consequently most of the methods developed to quantify acibenzolar-S-methyl residues in supervised trials on animal and plant commodities involve hydrolytic conversion of parent compound and metabolites to acibenzolar acid. This analyte is quantified and expressed in acibenzolar-S-methyl equivalents. LOQs are typically 0.01 mg/kg.

The common moiety methods all involve homogenisation and base hydrolysis (1N NaOH, typically 60–65 °C) followed by extraction of the hydrolysed samples with an organic/aqueous solvent mixture, typically CH_3OH. The main differences between methods involve clean-up conditions, instrumentation for quantification (HPLC-UV, HPLC-ECD, LC-MS/MS), and scale. Acibenzolar-S-methyl and acibenzolar acid are used as reference materials for fortification and method validation. In addition, radiovalidation studies demonstrated the acceptability of the extraction and hydrolysis used in the common moiety method as measured residues accounted for 75–110% of the residues of acibenzolar-S-methyl and acibenzolar acid (free and conjugated) identified in metabolism studies.

The methods involving hydrolysis are suitable for analysis of acibenzolar-S-methyl and acibenzolar acid (free and conjugated) in plant and animal matrices.

Multi-residue methods are currently not validated for the sum of acibenzolar-S-methyl and acibenzolar acid (free and conjugated).

A method was also made available for the analysis of 4-OH acibenzolar acid in lettuce and spinach. Following extraction with acidified acetonitrile:water, soluble conjugates were cleaved using cellulase and residues quantified using HPLC-UV (252 nm). The LOQ was 0.02 mg/kg.

Stability of pesticide residues in stored analytical samples

The Meeting received information on the stability of acibenzolar-S-methyl and acibenzolar acid in various matrices on freezer storage (-18 °C).

The periods of demonstrated stability cover the frozen storage intervals used in the residue studies on crops.

Residues of acibenzolar-S-methyl when measured by the common moiety method were stable in dry commodities (wheat grain) for 24 months and in high water commodities (cabbage, squash, lettuce, tomatoes, and turnip roots) and tobacco for 21 months. Residues were stable in banana for 24 months. Residues were stable in dry commodities (wheat grain) for 24 months and in high water commodities (cabbage, squash, lettuce, tomatoes, and turnip roots) and tobacco for 21 months.

The stability of acibenzolar-S-methyl in animal commodities was studied in the lactating cow residue transfer study with fortified samples stored for the same intervals as experimental samples. Acibenzolar-S-methyl, measured using the common moiety method, was stable in muscle for 87 days, kidney for 131 days, fat for 129 days and milk for 115 days. In liver, the amount remaining after 124 days was 51% of the spike concentration.

Definition of the residue

Following application of acibenzolar-S-methyl to crops the parent compound was generally only detected at short intervals after application and other than on the day of application, only as a relatively minor component of the ^{14}C residue in the case of lettuce (17–19% TRR); tomato (0.8% TRR) and tobacco (6.1% TRR). For the other crops investigated where the interval between application and sampling was longer, parent compound was not detected (cotton, rice, sorghum, sunflower and wheat).

In crops where levels of ^{14}C were sufficiently high to allow identification of metabolites, the major component of the ^{14}C could generally be attributed to acibenzolar acid free+conjugated+bound: wheat grain 8.4+15+16% TRR; wheat husks 12+11+17% TRR; wheat straw 14+7.8+23% TRR;

tomato fruit 8.1+56.2+0% TRR; tobacco leaf 9.0+61+0; lettuce <5+20+0% TRR; rice grain 1.7+2.0+1.9% TRR; rice husks 3.7+11+1.8% TRR and rice straw 10+38+8.1% TRR. The other components that exceeded 10% TRR were 4-OH acibenzolar acid free+conjugated in lettuce leaf (1.1+22% TRR) and 3-methanesulfinyl-benzoic acid free+conjugated+bound in rice grain (0.6+0.7+18% TRR) and rice straw (5.3+5.3+2.5% TRR).

Residues of acibenzolar-S-methyl and acibenzolar acid (free and conjugated) are unlikely to occur at significant levels in rotational (follow) crops.

There is no obvious single compound for use as a suitable marker for compliance. It is noted that the majority of the residue in crops is present as conjugates of acibenzolar acid that can easily be converted to acibenzolar acid on base hydrolysis. Acibenzolar-S-methyl is also converted to acibenzolar acid on base hydrolysis. As such a common moiety residue definition would allow residues to be monitored in all crops and derived commodities. Validated analytical methods are available for the determination of acibenzolar-S-methyl together with free and conjugated acibenzolar acid in crop matrices.

The Meeting decided the residue definition for compliance with MRLs in plants should be the sum of acibenzolar-S-methyl and acibenzolar acid, free and conjugated, expressed as acibenzolar-S-methyl.

In deciding which additional compounds should be included in the residue definition for risk assessment the Meeting considered the toxicological properties and likely occurrence of the candidates: acibenzolar acid (free and conjugated), 4-OH acibenzolar acid (free and conjugated) and 3-methylsulfinyl benzoic acid (free and conjugated). The toxicological properties of the various metabolites were considered. Acibenzolar acid has similar potency to the parent compound, while 4-OH acibenzolar acid is considered to be of no greater lower toxicity than the parent compound. Acibenzolar acid (free and conjugated) is the major residue in most plant commodities and should be included in the residue definition for risk assessment. The 4-OH acibenzolar acid metabolite (free and conjugated) was only significant in leafy vegetables (lettuce) where it occurred at half the level of the sum of parent compound and acibenzolar acid (free and conjugated). The Meeting agreed an adjustment factor of 1.5 could be applied to residues in leafy vegetables measured according to the residue definition for compliance to convert residues to the equivalent sum of acibenzolar-S-methyl, acibenzolar acid (free and conjugated) and 4-OH acibenzolar acid (free and conjugated). The Meeting agreed residues of 4-OH acibenzolar acid (free and conjugated) should be included in the residue definition for dietary risk assessment.

The metabolite 3-methanesulfinyl benzoic acid was only detected in rice and then only at low levels. No information was available on the toxicity of 3-methanesulfinyl benzoic acid. The Meeting agreed that as rice is not among the uses currently under consideration, residues of 3-methanesulfinyl benzoic acid are not expected and the compound does not need to be considered further. If uses on rice are considered in the future, a dietary risk assessment comparing exposures against the Cramer class TTC values should be conducted.

The Meeting decided the residue definition for dietary risk assessment in plants should be: *the sum of acibenzolar-S-methyl and acibenzolar acid, (free and conjugated) and 4-OH acibenzolar acid (free and conjugated), expressed as acibenzolar-S-methyl*

The plant metabolism studies show that livestock are unlikely to be exposed to parent acibenzolar-S-methyl. Livestock will be exposed to a range of metabolites that are mostly comprised of free and conjugated acibenzolar acid. As acibenzolar-S-methyl is rapidly hydrolysed to acibenzolar acid, and conjugates of acibenzolar acid are readily cleaved to produce acibenzolar acid, the use of acibenzolar-S-methyl in the livestock metabolism studies is acceptable.

In lactating goats no intact acibenzolar-S-methyl was detected in tissues or milk. The majority of the residues were present as free (33–90% TRR) and conjugated forms (2.3–22% TRR) of acibenzolar acid. In laying hens no intact acibenzolar-S-methyl was detected in tissues or eggs. Free acibenzolar acid accounted for the majority of the residue (50–77% TRR) with conjugates of acibenzolar acid only present at significant proportion of ^{14}C in egg white (18% TRR).

Analytical methods are available that determine residues of acibenzolar-S-methyl and acibenzolar acid, free and conjugated.

Based on the above, the Meeting decided the residue definition for animal commodities for compliance with MRLs and dietary risk assessment should be as follows:

sum of acibenzolar-S-methyl and acibenzolar acid, free and conjugated, expressed as acibenzolar-S-methyl

There is insufficient data to characterise whether the sum of residues in the residue definition (sum of acibenzolar-S-methyl and free and conjugated acibenzolar acid) is fat soluble. Total radioactive residues in muscle compared to fat and egg white compared to egg yolk suggest the residues, measured according to the residue definition might be higher in fat however, the data are inconclusive. The log K_{ow} for acibenzolar-S-methyl is 3.1 and the log K_{ow} values of acibenzolar acid and its conjugates are expected to be lower, suggesting the residue does not preferentially partition into fatty matrices.

On the weight of evidence, the Meeting decided the residue is not *fat soluble*.

Results of supervised residue trials on crops

Supervised residue trial data were available for acibenzolar-S-methyl on citrus (oranges, grapefruit, lemons), pome fruit (apples, pears), stone fruit (peaches, apricots), strawberries, bananas, kiwifruit, brassica vegetables (cabbage, broccoli, mustard greens), leafy vegetables (head lettuce, leaf lettuce, spinach, celery), cucurbits (cucumber, melon, squash), tomatoes, potatoes, and wheat.

The residue definition for exposure assessment includes 4-OH acibenzolar acid (free and conjugated). As residues of 4-OH acibenzolar acid (free and conjugated) are only expected in leafy crops and these residues were not measured in field trials, an adjustment factor of 1.5 (see above) was used to convert residues measured using the compliance definition to that for dietary risk assessment. The Meeting considered the factor would only be applied to the highest residues of leafy vegetables to estimate the relevant STMR and HR values required for dietary risk assessment.

Citrus fruits

The Meeting received supervised residue trial data for acibenzolar-S-methyl on citrus fruit from the USA. The critical GAP for citrus in the USA is applications to soil under trees at 112 g ai/ha with a PHI of 0 days. The maximum rate per year is 448 g ai/ha. In trials approximating critical GAP in the USA residues in <u>citrus fruit</u> were:

Oranges: (n = 10) < 0.01 (9), 0.01 mg/kg

Lemons: (n = 5) < 0.01 (5) mg/kg

Grapefruit: (n = 6): < 0.01 (6) mg/kg.

The Meeting noted that residues following soil application to citrus are generally ≤LOQ and decided to combine the data for oranges, lemons and grapefruit to estimate a group maximum residue level. The Meeting recommended a maximum residue level, STMR and HR of 0.015, 0.01 and 0.01 mg/kg respectively for citrus fruit.

Pome fruits – apples and pears

In Italy acibenzolar-S-methyl is approved for use on apples and pears. cGAP in Italy for apples is applications at 100 g ai/ha at 5–14 day intervals and a PHI of 7 days. In trials conducted in EU member states approximating critical GAP in Italy residues in <u>apples</u> were: (n = 16) < 0.01 (11), 0.01 (2), 0.03, 0.16, 0.17 mg/kg. The Meeting recommended maximum residue level, STMR and HR of 0.3, 0.01 and 0.17 mg/kg respectively for apples.

cGAP in Italy for <u>pears</u> is applications at 100 g ai/ha at intervals of 5–7 days (pre-flowering) and 14–28 days (fruiting) with a PHI of 14 days. In trials conducted in EU member states approximating critical GAP in Italy residues in pears were: (n = 4) < 0.02 (4) mg/kg. The Meeting considered four trials insufficient to estimate a maximum residue level for pears and as the GAPs for apples and pears were different, and did not consider extrapolation of the data on apples to pears.

Peaches

Supervised residue trial data for acibenzolar-S-methyl on peaches and apricots were made available. cGAP in Italy is applications at 75 g ai/ha at intervals of 7–14 days with a PHI of 7 days.

In trials conducted in the EU approximating critical GAP in Italy residues in peaches were (n = 7): 0.02, 0.02, 0.02, 0.05, 0.05, 0.05 and 0.09 mg/kg.

In trials conducted in the EU approximating critical GAP in Italy residues in apricots were (n = 4): 0.05, 0.07, 0.08, 0.13 mg/kg.

A Mann-Whitney U-test suggest the residues in apricots and peaches are from similar populations and the Meeting decided to combine the data to estimate a maximum residue level for the Codex sub-group peaches.

Residues in eleven trials on apricots and peaches were (n = 11): 0.02, 0.02, 0.02, 0.05, 0.05, 0.05, 0.05, 0.07, 0.08, 0.09, 0.13 mg/kg.

The Meeting recommended a maximum residue level, STMR and HR of 0.2, 0.05 and 0.13 mg/kg respectively for the sub-group peaches (includes apricots and nectarines).

Low growing berries, including strawberries

The Meeting received supervised residue trial data for acibenzolar-S-methyl on strawberries. Critical GAP in the USA on low growing berries including strawberries (USA subgroup 13-07G) is applications at 26 g ai/ha with a PHI of 0 days. The maximum rate per year is 210 g ai/ha. In trials approximating critical GAP in the USA residues in strawberries were: (n = 10) 0.02, 0.02, 0.03, 0.03, 0.04, 0.05, 0.06, 0.06, 0.07, 0.08 mg/kg.

The Meeting noted residues for strawberries can be extrapolated to Codex Subgroup 004E, low growing berries and recommended a maximum residue level, STMR and HR of 0.15, 0.045 and 0.08 mg/kg respectively.

Banana

The Meeting received supervised residue trial data for acibenzolar-S-methyl on banana from Colombia, Costa Rica, Ecuador, Guatemala, France (Martinique), Malaysia and Mexico. GAP in Guatemala is applications at up to 40 g ai/ha at 30-40 day intervals with a PHI of 0 days. In trials approximating critical GAP in Guatemala residues in unbagged bananas were: (n = 15): < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, 0.03, 0.04, 0.05 mg/kg. The Meeting recommended a maximum residue level of 0.06 mg/kg.

Residues in the edible portion (pulp) were: < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, 0.02, 0.03 mg/kg. The Meeting recommended a STMR and HR of 0.02 and 0.03 mg/kg respectively for banana (pulp).

The Meeting noted that residues in bagged bananas from 15 trials were all < 0.02 mg/kg for both whole fruit and pulp.

Kiwifruit

Acibenzolar-S-methyl is approved in Italy and NZ for use on kiwifruit.

cGAP in Italy is a foliar application at 6 ×75 g ai/ha with a 14 day interval or 6 ×100 g ai/ha with a 21 day interval and a PHI of 90 days. No trials matched cGAP of Italy.

cGAP in NZ is foliar or soil applications to actively growing plants at 4 ×100 g ai/ha (10 g ai/hL). Foliar sprays are not to be applied between flowering and harvest while the PHI for soil application is 14 days. The foliar application at flowering does not lead to residues in fruit at harvest.

In fourteen trials conducted in NZ that matched the GAP for soil application, residues were: (n = 14) < 0.01 (11) 0.01, 0.02, 0.02 mg/kg. The Meeting recommended a maximum residue level, STMR and HR of 0.03, 0.01 and 0.02 mg/kg respectively for kiwifruit.

Bulb onions

The Meeting received supervised residue trial data for acibenzolar-S-methyl on onions. cGAP in the USA for the US onion crop group 3-07A is applications at 35 g ai/ha at 7 – 10 day intervals with a PHI of 7 days. The maximum rate per year is 140 g ai/ha. In twelve trials that approximated critical GAP, residues were (n = 12): < 0.01, < 0.01, 0.01, 0.02, < 0.05 (7), 0.06 mg/kg. The Meeting recommended a maximum residue level, STMR and HR of 0.15, 0.05 and 0.06 mg/kg respectively for onions, bulb.

The Meeting noted residues on bulb onions can be extrapolated to garlic and shallots and also recommended a maximum residue level, STMR and HR of 0.15, 0.05 and 0.06 mg/kg respectively for garlic and shallots.

Brassica vegetables

Supervised residue trial data for acibenzolar-S-methyl on Brassica vegetables were available. cGAP in the USA for Brassica (cole) crops (USA group 5) is applications at 35 g ai/ha at intervals of 7 days with a PHI of 7 days. The maximum rate per year is 140 g ai/ha.

In trials conducted in the USA on cabbages the application rate (53 g ai/ha) was higher than the current GAP (35 g ai/ha) and the Meeting agreed to utilize the proportionality approach to estimate residues matching cGAP. Unscaled residues for cabbage heads with wrapper leaves were (n = 9): 0.08, 0.13, 0.19, 0.21, 0.31, 0.32, 0.39, 0.51, 0.58 mg/kg.

After scaling using a scaling factor of 0.66 (35/53), the following residues in cabbages were obtained: 0.05, 0.09, 0.13, 0.14, 0.20, 0.21, 0.26, 0.34, 0.38 mg/kg.

In trials conducted in the USA on broccoli the application rate was higher (53 g ai/ha) than the current GAP in the USA (35 g ai/ha) and the Meeting agreed to apply proportionality in assessing the data. Unscaled residues were (n = 6): 0.20, 0.31, 0.46, 0.47, 0.55, 0.62 mg/kg.

The scaling of residues (scaling factor 53/35 = 0.66) in broccoli to match cGAP results in the following: 0.13, 0.20, 0.30, 0.31, 0.36, 0.41 mg/kg.

GAP in the USA for Brassica vegetables and a group maximum residue level recommendation may be possible based on the data for cabbages and broccoli. The medians for the two datasets differed by less than a factor of five and the Meeting decided to recommend a group maximum residue level. In deciding which datasets to use for the recommendation, as a Mann-Whitney U-test indicated the populations were not different, it was decided to combine the datasets. The combined dataset is (n = 15): 0.05, 0.09, 0.13, 0.13, 0.14, 0.20, 0.20, 0.21, 0.26, 0.30, 0.31, 0.34, 0.36, 0.38, 0.41 mg/kg.

The Meeting recommended a maximum residue level, STMR and HR of 0.7, 0.315 (= 1.5 ×0.21) and 0.62 (= 1.5 ×0.41) mg/kg respectively for Brassica (cole or cabbage) vegetables, Head cabbages, Flowerhead brassicas.

Fruiting vegetables, Cucurbits

Supervised residue trial data for acibenzolar-S-methyl on cucurbit vegetables were available. cGAP in the USA for cucurbits (crop group 9) is applications at 35 g ai/ha at intervals of 7 days with a PHI of 0 days. The maximum rate per year is 280 g ai/ha.

In trials conducted in the USA on field grown cucumbers, melons and summer squash the application rate (68 to 71 g ai/ha) was higher than the current GAP (35 g ai/ha) and the Meeting agreed to utilize the proportionality approach (scaling factor 0.48 to 0.51) to estimate residues matching cGAP. Unscaled residues for cucumbers were (n = 11): 0.07, 0.12, 0.13, 0.14, 0.15, 0.17, 0.18, 0.28, 0.46, 0.48, 0.77 mg/kg.

After scaling the following residues in cucumbers were obtained: 0.04, 0.06, 0.07, 0.07, 0.08, 0.09, 0.09, 0.14, 0.23, 0.24, 0.39 mg/kg.

For melons, unscaled residues were (n = 12): 0.15, 0.25, 0.26, 0.31, 0.31, 0.33, 0.35, 0.60, 0.63, 0.83, 0.87, 0.98 mg/kg.

Scaled residues were: 0.08, 0.13, 0.13, 0.15, 0.16, 0.17, 0.18, 0.31, 0.32, 0.42, 0.44, 0.47 mg/kg.

For summer squash, unscaled residues were (n = 4): 0.04, 0.13, 0.14, 0.18 mg/kg.

Scaled residues in summer squash were: 0.02, 0.07, 0.07, 0.09 mg/kg.

GAP in the USA is for cucurbit vegetables and a group maximum residue level recommendation may be possible based on the data for cucumber, melons and summer squash. The medians for the three datasets differed by less than a factor of five and the Meeting decided to recommend a group maximum residue level. In deciding which datasets to use for the recommendation, as a Kruskal-Wallis H-test indicated the data were not from the same population it was decided to use the dataset with the highest residues, melons, to estimate a maximum residue level for the group.

Using the melon dataset (0.08, 0.13, 0.13, 0.15, 0.16, 0.17, 0.18, 0.31, 0.32, 0.42, 0.44, 0.47 mg/kg), the Meeting recommended a maximum residue level, STMR and HR of 0.8, 0.175 and 0.47 mg/kg respectively for fruiting vegetables cucurbits.

Tomato

Supervised residue trial data for acibenzolar-S-methyl on field grown tomato were available. GAP in the USA is applications at 26 g ai/ha at intervals of 7 days with a PHI of 14 days. No trials matched USA cGAP.

GAP in France is applications at 6 ×25 g ai/ha at intervals of 7days with a PHI of 3 days. In trials conducted in the EU approximating critical GAP in France residues in tomatoes were (n = 13): 0.04, 0.05, 0.05, 0.06, 0.06, 0.07, 0.09, 0.10, 0.10, 0.12, 0.13, 0.14, 0.15 mg/kg.

The Meeting recommended a maximum residue level, STMR and HR of 0.3, 0.09 and 0.15 mg/kg respectively for tomatoes.

Lettuce

Supervised residue trial data for acibenzolar-S-methyl on lettuce were available. GAP in the USA is applications at 4 ×35 g ai/ha at intervals of 7-10 days with a PHI of 7 days. The maximum rate per year is 332 g ai/ha. In trials conducted in the US approximating critical GAP residues in lettuce were:

Head lettuce (n = 6): 0.04, 0.04, 0.05, 0.06, 0.08, 0.10 mg/kg.

Leaf lettuce (n = 6): 0.04, 0.06, 0.10, 0.14, 0.14, 0.18 mg/kg.

The Meeting recommended a maximum residue level, STMR and HR of 0.2, 0.0825 (= 1.5 ×0.055) and 0.15 (= 1.5 ×0.10) mg/kg respectively for head lettuce.

The Meeting recommended a maximum residue level, STMR and HR of 0.4, 0.18 (= 1.5 ×0.12) and 0.27 (= 1.5 ×0.18) mg/kg respectively for leaf lettuce.

Spinach

Supervised residue trial data for acibenzolar-S-methyl on spinach were available.

GAP in France is applications at 3 ×12.5 g ai/ha with a PHI of 10 days. In trials conducted in the EU approximating critical GAP in France residues in spinach were (n = 7): 0.04, 0.06, 0.06, 0.11, 0.12, 0.16, 0.18 mg/kg.

Critical GAP in the USA is applications at 4 ×26 g ai/ha at intervals of 7-10 days with a PHI of 7 days. The maximum rate per year is 332 g ai/ha.

In trials conducted in the USA on spinach the application rate was higher (35 g ai/ha) than the current GAP and the Meeting agreed to utilize the proportionality approach (scaling factor 26/35 = 0.74) to estimate residues matching cGAP. The following scaled residues in spinach matched cGAP

Unscaled residues for spinach were: 0.12, 0.18, 0.21, 0.24, 0.26, 0.29, 0.29, 0.33, 0.48 mg/kg.

After scaling the following residues in spinach were obtained (n = 9): 0.09, 0.13, 0.16, 0.18, 0.19, 0.22, 0.22, 0.25, 0.36 mg/kg.

The Meeting noted residues matching cGAP were higher in the dataset from the US than France and decided to use these residue data to estimate a maximum residue level for spinach.

The Meeting recommended a maximum residue level, STMR and HR of 0.6, 0.285 (= 1.5 ×0.19) and 0.54 (= 1.5 ×0.36) mg/kg respectively for spinach.

Brassica leafy vegetables

Supervised residue trial data for acibenzolar-S-methyl on mustard greens were available. GAP in the USA for Brassica (cole) crops (USA group 5) is applications at 35 g ai/ha at intervals of 7 days with a PHI of 7 days. The maximum rate per year is 140 g ai/ha.

In trials conducted in the USA on mustard greens the application rate (53 g ai/ha) was higher than the current GAP and the Meeting agreed to utilize the proportionality approach (scaling factor 35/53 = 0.66) to estimate residues matching cGAP. Unscaled residues were: 0.16, 0.29, 0.59, 0.67, 0.76 mg/kg. The following scaled residues in mustard greens matched cGAP (n = 5): 0.11, 0.19, 0.39, 0.44, 0.50 mg/kg

The Meeting recommended a maximum residue level, STMR and HR of 1, 0.585 (= 1.5 ×0.39) and 0.795 (= 1.5 ×0.53) (highest individual sample) mg/kg respectively for mustard greens. As the use pattern includes all Brassica leafy vegetables, the meeting agreed to extrapolate the recommendations to all Brassica leafy vegetables (VL 0054).

Potato

Supervised residue trial data for acibenzolar-S-methyl on potato were available. GAP in Brazil is 6 ×12.5 g ai/ha at intervals of 7days with a PHI of 14 days. None of the trials matched cGAP (number of sprays, application rate) for Brazil.

Wheat

Supervised residue trial data for acibenzolar-S-methyl on wheat were available. Critical GAP in Brazil is 3 ×12.5 g ai/ha at intervals of 14 days with a PHI of 21 days. In trials conducted in the Argentina and Brazil approximating critical GAP in Brazil residues in wheat grain were (n = 4): < 0.01, < 0.01, 0.03 and 0.04 mg/kg. The Meeting considered the number of trials insufficient for the estimation of a maximum residue limit.

Fate of residues during processing

The Meeting received information on the fate of incurred residues of acibenzolar-S-methyl during the processing of oranges, pears and tomatoes. A study of the nature of the residue of acibenzolar-S-methyl under simulated processing conditions (pasteurization 20 minutes at 90 °C, pH 4, baking/brewing/boiling 60 minutes at 100 °C, pH 5, sterilization 20 minutes at 120 °C, pH 6) showed acibenzolar-S-methyl, if present, is hydrolytically stable under processing conditions representative of pasteurisation and baking/boiling/brewing; a significant degradation of the parent compound into acibenzolar acid (CGA210007) occurred under sterilisation conditions. A hydrolysis study demonstrated that acibenzolar acid (CGA210007), once formed, is stable to hydrolysis.

Summary of relevant processing factors calculated for the sum of acibenzolar-S-methyl and free and conjugated acibenzolar acid is provided below.

	Processed Fraction	Processing Factor	Best estimate PF	RAC STMR or median	STMR×PF = STMR-P
Oranges	Dried pulp	4.5	4.5	< 0.01	< 0.045
	Juice	< 0.625	< 0.625		< 0.0062
	Oil	< 0.625	< 0.625		< 0.0062
Pome fruit [A]	Dried pomace	2.0 3.0 3.2 3.4	3.1	< 0.01	< 0.031
Tomato	Peeled fruit	0.83 0.50	0.67	0.09	0.060
	Juice	0.67 0.75 0.80 1.0	0.78		0.070
	Preserved fruit	0.5 0.5 0.8 0.83	0.66		0.059
	Purée	1.17 1.75 2.0 3.33	1.88		0.169
	Ketchup	1.75 2.0	1.89		0.170

[A] Pfs are based on results for pear and are indicative only as the residue in the RAC used to calculate the PF was between LOD and LOQ.

The HR-P for peeled tomato fruit is 0.15 mg/kg × 0.67 = 0.10 mg/kg and for preserved (canned) tomato fruit is 0.15 mg/kg × 0.66 = 0.099 mg/kg.

Residues in animal commodities

Farm animal feeding studies

The Meeting received information on the residue levels in tissues and milk of dairy cows dosed with acibenzolar-S-methyl at the equivalent of 0.25, 1.27 and 2.48 ppm in the feed for 28 consecutive days.

Residues in milk were < 0.005 mg/kg and tissues < 0.02 mg/kg for the 2.48 ppm dose group for all samples.

A laying hens transfer study was not available.

Estimation of livestock dietary burdens

Dietary burden calculations for beef cattle and dairy cattle and poultry are provided below. The dietary burdens were estimated using the OECD diets listed in Appendix IX of the 2016 edition of the FAO Manual.

Potential cattle feed items include: cabbage wrapper leaves, kale, apple pomace and citrus pulp.

Summary of livestock dietary burden (ppm acibenzolar-S-methyl equivalents of dry matter diet)

	US-Canada		EU		Australia		Japan	
	Max	Mean	Max	mean	max	Mean	max	Mean
Beef cattle	0.005	0.005	0.71	0.53	0.01	0.01	-	-
Dairy cattle	0.005	0.005	0.72	0.53	1.4[AB]	1.0[CD]	-	-
Broilers	-	-	-	-	-	-	-	-
Layers	-	-	0.18[E]	0.13[F]	-	-	-	-

[A] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat

[B] Highest maximum dairy cattle dietary burden suitable for MRL estimates for mammalian milk

[C] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat.

[D] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.

[E] Highest maximum poultry dietary burden suitable for MRL estimates for poultry meat and eggs

[F] Highest mean poultry dietary burden suitable for STMR estimates for poultry meat and eggs

Animal commodity maximum residue levels

No residues were detected in milk and tissues of lactating dairy cows dosed at 2.5 ppm in the diet, 1.8 times the maximum livestock dietary burden. The storage stability of residues in liver was poor with 51% remaining after the freezer storage period of 124 days. However, the Meeting noted that as residues were not present in liver in animals dosed at 1.8 times the maximum livestock dietary burden, even if residues had declined by 50% on storage, no residues are expected to have been present in liver.

The Meeting estimated the following maximum residue levels: milk 0.01* mg/kg; meat (mammalian except marine mammals) 0.02* mg/kg, mammalian fat (except milk fat) 0.02* mg/kg and edible offal 0.02* mg/kg. The Meeting estimated the following STMR and HR values: mammalian meat 0 mg/kg; mammalian fat 0 mg/kg; mammalian edible offal 0 mg/kg and milk 0 mg/kg.

Although a laying hens transfer study was not available, in a metabolism study where hens were dosed at the equivalent of 19.1 ppm in the diet for four days residues of acibenzolar-S-methyl and free and conjugated acibenzolar acid were < 0.01 mg/kg in eggs, 0.29 mg/kg in liver, 0.01 mg/kg in muscle and 0.03 mg/kg in skin/fat.

At the maximum dietary burden for poultry (0.18 ppm), no residues above the LOQ for analytical methods are expected in eggs (< 0.01 ×0.14/19.1 = < 0.00007 mg/kg), liver (0.002 mg/kg), muscle (0.00007 mg/kg) and skin/fat (0.0002 mg/kg). The Meeting estimated the following maximum residue levels for poultry commodities: poultry meat 0.02* mg/kg; poultry edible offal 0.02* mg/kg, poultry fat 0.02* mg/kg and eggs 0.02* mg/kg. The Meeting estimated the following STMR and HR values: poultry meat 0 mg/kg; poultry fat 0 mg/kg; poultry edible offal 0 mg/kg and eggs 0 mg/kg.

RECOMMENDATIONS FURTHER WORK OR INFORMATION

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

Definition of the residue for compliance with MRL for animal and plant commodities and for dietary risk assessment for animal commodities: *Sum of acibenzolar-S-methyl and 1,2,3-benzothiadiazole-7-carboxylic acid (acibenzolar acid) (free and conjugates), expressed as acibenzolar-S-methyl*

Definition of residue (for dietary risk assessment for plants): *Sum of acibenzolar-S-methyl and 1,2,3-benzothiadiazole-7-carboxylic acid (acibenzolar acid), (free and conjugated) and 1,2,3-benzothiadiazole-4-hydroxy-7-carboxylic acid (4-OH acibenzolar acid) (free and conjugated), expressed as acibenzolar-S-methyl*

The residue is not fat soluble.

CCN	Commodity	Recommended Maximum residue level (mg/kg)		STMR or STMR-P mg/kg	HR or HR-P mg/kg
		New	Previous		
FP 0226	Apple	0.3		0.01	0.17
FI 0327	Banana	0.06		0.02	0.03
VB 0040	Brassica (cole or cabbage) vegetables, Head cabbages, Flowerhead brassicas	0.7		0.315	0.62
VL 0054	Brassica leafy vegetables	1		0.585	0.795
FC 0001	Citrus fruits	0.015		0.01	0.01
MO 0105	Edible offal (Mammalian)	0.02 *		0	0
PE 0112	Eggs	0.02 *		0	0
VC 0045	Fruiting vegetables, Cucurbits	0.8		0.175	0.47
VA 0381	Garlic	0.15		0.05	0.06
FI 0341	Kiwifruit	0.03		0.01	0.02
VL 0482	Lettuce, Head	0.2		0.0825	0.15
VL 0483	Lettuce, Leaf	0.4		0.18	0.27
	Low growing berries (including strawberries)	0.15		0.045	0.08
MF 0100	Mammalian fats (except milk fats)	0.02 *		0	0

CCN	Commodity	Recommended Maximum residue level (mg/kg)		STMR or STMR-P mg/kg	HR or HR-P mg/kg
		New	Previous		
MM 0095	Meat (from mammals other than marine mammals)	0.02 *		0	0
ML 0106	Milks	0.01 *		0	0
VA 0385	Onion, Bulb	0.15		0.05	0.06
FS 0247	Peaches (including nectarines and apricots)	0.2		0.05	0.13
PF 0111	Poultry fats	0.02 *		0	0
PM 0110	Poultry meat	0.02 *		0	0
PO 0111	Poultry, Edible offal of	0.02 *		0	0
VA 0388	Shallot	0.15		0.05	0.06
VL 0502	Spinach	0.6		0.285	0.54
VO 0448	Tomato	0.3		0.09	0.15
AB 0226	Apple pomace dry			0.031	
	Cabbage wrapper leaves (as animal feed)			0.21[A]	0.41[A]
AB 0048	Citrus pulp, dry			0.045	
	Kale (as animal feed)			0.39[A]	0.053[A]
JF 0048	Tomato juice			0.0702	
MW 0448	Tomato purée			0.169	
	Tomato canned			0.059	0.10

[A] sum of residues of acibenzolar-S-methyl and free and conjugated acibenzolar acid only, cabbage wrapper leaves based on values for brassica (cole or cabbage) vegetables.

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The 2016 JMPR established an Acceptable Daily Intake (ADI) of 0–0.08 mg/kg bw for acibenzolar-S-methyl.

The evaluation of acibenzolar-S-methyl resulted in recommendations for MRLs and STMR values for raw and processed commodities. Where data on consumption were available for the listed food commodities, dietary intakes were calculated for the 17 GEMS/Food Consumption Cluster Diets. The results are shown in Annex 3.

The IEDIs in the seventeen Cluster Diets, based on the estimated STMRs were 0–1% of the maximum ADI (0.08 mg/kg bw). The Meeting concluded that the long-term dietary exposure to residues of acibenzolar-S-methyl from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term dietary exposure

The 2016 JMPR established an Acute Reference Dose (ARfD) of 0.5 mg/kg bw for acibenzolar-S-methyl. The IESTI of acibenzolar-S-methyl for the commodities for which STMR, HR and maximum residue levels were estimated by the current Meeting are shown in Annex 4. The IESTI represented 0–10% of the ARfD (0–9% general population, 0–10% children 8 months–6 years old).

The Meeting concluded that the short-term dietary exposure to residues of acibenzolar-S-methyl resulting from uses that have been considered by the JMPR is unlikely to present a public health concern.

REFERENCES

Code	Author	Year	Title, Institute, Report reference
0050	Adam, D	1994	Hydrolysis of CGA 245704 under laboratory conditions. Syngenta Crop Protection AG, Basel, CH, 93DA01. GLP, not published. Syngenta File No CGA245704/0050
0226	Adam, D	1995	Soil Photolysis of ^{14}C labelled CGA 245704 under Laboratory Conditions. Syngenta Crop Protection AG, Basel, CH, 95DA01. GLP, not published. Syngenta File No CGA245704/0226
0629	Adam, D	1999	Hydrolysis of ^{14}C-Phenylring labelled CGA 245704 under processing conditions. Syngenta Crop Protection AG, Basel, CH, 99DA04. GLP, not published. Syngenta File No CGA245704/0629
10020	Amic, S	2010a	Acibenzolar-S-methyl—Residue Study on Apples in Northern France in 2009. Syngenta Crop Protection AG, Basel, CH, Eurofins–ADME Bioanalyses, Vergeze, FR, S09-01430-REG. GLP, not published. Syngenta File No A9180A/10020
10019	Amic, S	2010b	Acibenzolar-S-methyl—Residue Study on Apples in Southern France and Italy in 2009. Syngenta Crop Protection AG, Basel, CH, Eurofins–ADME Bioanalyses, Vergeze, FR, S09-01431. GLP, not published. Syngenta File No A9180A/10019
10040	Amic, S	2011a	Acibenzolar-S-methyl—Residue Study on Apples in Northern France and Germany in 2010. Syngenta Crop Protection AG, Basel, CH, Eurofins–ADME Bioanalyses, Vergeze, FR, S10-01531. GLP, not published. Syngenta File No A9180A_10040
10039	Amic, S	2011b	Acibenzolar-S-methyl—Residue study on apples in Southern France, Italy and Spain in 2010. Syngenta Crop Protection AG, Basel, CH, Eurofins–ADME Bioanalyses, Vergeze, FR, S10-01532. GLP, not published. Syngenta File No A9180A_10039
10563	Amic, S	2014	Acibenzolar-S-methyl—Residue Study on Kiwi in Spain, Portugal, Southern France and Italy in 2013. Syngenta Crop Protection AG, Basel, CH, Eurofins Agroscience Services Chem SAS, Vergèze, France, S13-02496. GLP, not published. Syngenta File No A9180A_10563
0340	Anonymous	1996	CGA 245704 (total), WG 50, banana (pulp, peel, bagged & unbagged) Malaysia. Syngenta Crop Protection AG, Basel, CH, 2186/95. GLP, not published. Syngenta File No CGA245704/0340
10325	Anonymous	2012a	Extraction of Acibenzolar-S-Methyl in Kiwifruits and Kiwifruit Leaves. Syngenta Crop Protection AG, Basel, CH, KB30588. Not GLP, not published. Syngenta File No CGA245704_10325
10326	Anonymous	2012b	Analysis of Total Acibenzolar-S-Methyl in Kiwifruits and Kiwifruit Leaves by LC-MS/MS. Syngenta Crop Protection AG, Basel, CH, KB30589. Not GLP, not published. Syngenta File No CGA245704_10326
10327	Anonymous	2012c	Method Validation: Determination of Total Acibenzolar-S-Methyl in Kiwifruits by LC-MS/MS. Syngenta Crop Protection AG, Basel, CH, KB30811. Not GLP, not published. Syngenta File No CGA245704_10327

Code	Author	Year	Title, Institute, Report reference
	Banerjee, K, Utture, S, Dasgupta, S, Kandaswamy, C, Pradhan, S, Kulkarni, S & Adsule, P	2012	Multiresidue determination of 375 organic contaminants including pesticides, polychlorinated biphenyls and polyaromatic hydrocarbons in fruits and vegetables by gas chromatography–triple quadrupole mass spectrometry with introduction of semi-quantification approach. Journal of Chromatography A 1270: 283–295.
0848	Baravelli, P	2004	Residue study with acibenzolar-S-methyl (Bion 50 WG) in or on peach in Italy 2003. Syngenta Crop Protection AG, Basel, CH, AgriParadigma S.r.l., Ravenna, Italy, AGRI002/03 GLP DEC AGRI003/03. GLP, not published. Syngenta File No CGA245704/0848
10028	Brandelli, A	1999	Rate of Degradation of (Phenyl-U-^{14}C)–CGA 245704 under Aerobic Laboratory Conditions in various Soils at 20°C. Syngenta Crop Protection AG, Basel, CH, Bioensaios Análises e Consultoria Brazil, BS-023. GLP, not published. Syngenta File No CGA245704_10028
	Buonaurio, R, Scarponi, L, Ferrara, M, Sidoti, P & Bertona, A	2002	Induction of systemic acquired resistance in pepper plants by acibenzolar-S-methyl against bacterial spot disease. European Journal of Plant Pathology 108: 41–49.
0129	Burkhard, N	1995	Henry`s law constant. Not Known. Syngenta Crop Protection AG, Basel, CH, Not GLP, not published. Syngenta File No CGA245704/0129
10036	Casallanovo, F	2010	Bion 500 WG—Magnitude of Acibenzolar-S-Methyl and CGA210007 mixed residues in wheat grains—Brazil, 2009-10. Syngenta Crop Protection AG, Basel, CH, M09139. GLP, not published. Syngenta File No A9180A_10036
	Christia, C, Bizani, E, Christophoridis, C & Fytianos, K	2015	Pesticide residues in fruit samples: comparison of different QuEChERS methods using liquid chromatography—tandem mass spectrometry. Environmental Science and Pollution Research 22:13167–13178
10098	Class, T & Bacher, R	2012	Acibenzolar-S-methyl—Independent Laboratory Validation of Residue Method GRM051.01A and GRM051.01B for the Determination of Acibenzolar-S-methyl in Crop Matrices by LC-MS/MS. Syngenta Crop Protection AG, Basel, CH, PTRL Europe, Ulm, Germany, B 2407 G. GLP, not published. Syngenta File No CGA245704_10098
0063	Das, R	1994a	Report on melting point/melting range. Syngenta Crop Protection AG, Basel, CH, 17288. GLP, not published. Syngenta File No CGA245704/0063
0064	Das, R	1994b	Report on general physico-chemical properties (pure active ingredient). Syngenta Crop Protection AG, Basel, CH, 17421. GLP, not published. Syngenta File No CGA245704/0064
0176	Das, R	1995a	Report on boiling point / boiling range. Syngenta Crop Protection AG, Basel, CH, 31115. GLP, not published. Syngenta File No CGA245704/0176
0120	Das, R	1995b	Report on general physico-chemical properties (technical grade active ingredient). Syngenta Crop Protection AG, Basel, CH, 31181. GLP, not published. Syngenta File No CGA245704/0120

Code	Author	Year	Title, Institute, Report reference
10602	de Gois, F	2008	Bion 500 WG—Magnitude of Residues Combined of Acibenzolar-S-Methyl and CGA210007 in Wheat Grain—Argentina, 2006–07. Syngenta Crop Protection AG, Basel, CH, S00106. Not GLP, not published. Syngenta File No A9180A_10602
	Eitzer, BD, Hammack, W & Filigenzi, M	2014	Interlaboratory comparison of a general method to screen foods for pesticides using QuEChERs extraction with high performance liquid chromatography and high resolution mass spectrometry. J. Agric. Food Chem., 62: 80–87.
0461	Enriquez, MA	1998	Determination of total residues of CGA 245704 as CGA 210007 in tobacco—Validation of the Method. Syngenta Crop Protection AG, Basel, CH, Battelle Memorial Institute, Geneva, CH, A-20-97-02. GLP, not published. Syngenta File No CGA245704/0461
0660	Eudy, L	1999	CGA-245704 And Cyromazine—Magnitude of the Residues in or on Crop Group 5: Brassica (Cole) Leafy Vegetables. Syngenta Crop Protection AG, Basel, CH, 42-97. GLP, not published. Syngenta File No CGA245704/0660
	Fillâtre, Y, Rondeau, D, Jadas-Hécart, A & Communal, PY	2010	Advantages of the scheduled selected reaction monitoring algorithm in liquid chromatography/electrospray ionization tandem mass spectrometry multi-residue analysis of 242 pesticides: a comparative approach with classical selected reaction monitoring mode. Rapid Communications in Mass Spectrometry 24: 2453–2461
50007	Findak, D	2009	[Phenyl-U-^{14}C] Acibenzolar-S-Methyl—Nature of the Residue in Cotton. Syngenta Crop Protection AG, Basel, CH, Ricerca Biosciences, LLC, Concord, OH, USA, Excel Research Services, Inc., Fresno, USA, 022502, ERS 28049. GLP, not published. Syngenta File No CGA245704/50007
0038	Formica, G	1993	Determination of total residues of CGA 245704 as CGA 210007 by high performance liquid chromatography (HPLC) in cereals, including validation. Syngenta Crop Protection AG, Basel, CH, REM 172.02. GLP, not published. Syngenta File No CGA245704/0038
0175	Formica, G	1995a	Determination of total residues of CGA 245704 as CGA 210007 by high performance liquid chromatography, plant material and milk. Syngenta Crop Protection AG, Basel, CH, REM 172.11. GLP, not published. Syngenta File No CGA245704/0175
0154	Formica, G	1995b	CGA 245704 method validation (^{14}C-extractability and accountability) in wheat (grain, straw, husk). Syngenta Crop Protection AG, Basel, CH, 117/95. GLP, not published. Syngenta File No CGA245704/0154
0155	Formica, G	1995c	CGA 245704: Validation of REM 172.02 for tobacco leaves. Syngenta Crop Protection AG, Basel, CH, 125/95. GLP, not published. Syngenta File No CGA245704/0155
0199	Formica, G	1995d	Determination of total residues of CGA 245704 as CGA 210007 by high performance liquid chromatography, meat and eggs. Syngenta Crop Protection AG, Basel, CH, REM 172.12. GLP, not published. Syngenta File No CGA245704/0199
0254	Formica, G	1995e	Validation of method REM 172.13 for the recoveries of CGA 245704 and CGA 210007 from blood and urine. Syngenta Crop Protection AG, Basel, CH, 155/95. GLP, not published. Syngenta File No CGA245704/0254
0222	Formica, G	1995f	CGA 245704, WG 50, Tomatoes, Switzerland. Syngenta Crop Protection AG, Basel, CH, 2065/95. GLP, not published. Syngenta File No CGA245704/0222

Code	Author	Year	Title, Institute, Report reference
0223	Formica, G	1995g	CGA 245704, WG 50, Tomatoes, Switzerland. Syngenta Crop Protection AG, Basel, CH, 2066/95. GLP, not published. Syngenta File No CGA245704/0223
0297	Formica, G	1996a	Validation of the extractability and accountability of the analytical method REM 172.11 for tomatoes, Syngenta Crop Protection AG, Basel, CH, 157/95, GLP, not published, Syngenta File No CGA245704/0297
0248	Formica, G	1996b	Stability of total residues of CGA 245704 in wheat grain under freezer storage condition, Syngenta Crop Protection AG, Basel, CH, 175/93, GLP, not published, Syngenta File No CGA245704/0248
0301	Formica, G	1996c	CGA 245704 (total), WG 50 (A9180), banana (pulp & peel, bagged & unbagged), Costa Rica. Syngenta Crop Protection AG, Basel, CH, 2181/95. GLP, not published. Syngenta File No CGA245704/0301
0315	Formica, G	1996d	CGA 245704 (total), WG50, banana (pulp & peel, bagged & unbagged), Malaysia. Syngenta Crop Protection AG, Basel, CH, 2187/95. GLP, not published. Syngenta File No CGA245704/0315
0070	Fuldner, HH	1994	Report on density of solids. Syngenta Crop Protection AG, Basel, CH, PP-94-14P-DES. GLP, not published. Syngenta File No CGA245704/0070
10056	Gemrot, F	2011a	Acibenzolar-S-methyl—Analytical Method (GRM051.01B) for the Determination of Acibenzolar-S-methyl in Crops. Final Determination by LC-MS/MS. Syngenta Crop Protection AG, Basel, CH, Eurofins–ADME Bioanalyses, FR, GRM051.01B. Not GLP, not published. Syngenta File No CGA245704_10056
10043	Gemrot, F	2011b	Acibenzolar-S-methyl—Validation of Analytical Method GRM051.01A for the Determination of Acibenzolar-S-methyl in Crops. Syngenta Crop Protection AG, Basel, CH, Eurofins–ADME Bioanalyses, Vergeze, FR, S10-00680-REG. GLP, not published. Syngenta File No CGA245704_10043
0073	Geoffroy, A	1994	Report on vapour pressure curve. Syngenta Crop Protection AG, Basel, CH, PP-93/31P.VPC. GLP, not published. Syngenta File No CGA245704/0073
	González-Curbelo, MA, Lehotay, SJ, Hernández-Borges, J & Rodríguez-Delgado, MA	2014	Use of ammonium formate in QuEChERS for high-throughput analysis of pesticides in food by fast, low-pressure gas chromatography and liquid chromatography tandem mass spectrometry. Journal of Chromatography A 1358: 75–84.
78570	Grunenwald, MC	1998	Analytical Method for the Determination of Total Residues of CGA245704 as CGA210007 in Tobacco, Leafy Vegetables, and Fruiting Vegetables by Column Switching High Performance Liquid Chromatography. Syngenta Crop Protection AG, Basel, CH, AG-671A. GLP, not published. Syngenta File No 78570
10300	Haller, B	2012	Determination of residues of acibenzolar-S-methyl and CGA 210007 in kiwifruit following applications of Actigard as foliar and soil application. Syngenta Crop Protection AG, Basel, CH, Peracto Pty Ltd, Devonport, Australia, SYN-NZ-1102. GLP, not published. Syngenta File No CGA245704_10300

Code	Author	Year	Title, Institute, Report reference
0464	Hayworth, C	1998a	Analytical method for the determination of total residues of CGA 245704 as CGA 210007 in tobacco, leafy vegetables and fruiting vegetables by column switching high performance liquid chromatography. Syngenta Crop Protection AG, Basel, CH, 671. GLP, not published. Syngenta File No CGA245704/0464
0486	Hayworth, C	1998b	Validation of Draft Analytical Method AG-671 for the Determination of Total Residues of CGA-245704 as CGA-210007 in Tobacco, Leafy Vegetables and Fruiting Vegetables by Column Switching HPLC. Syngenta Crop Protection AG, Basel, CH, ABR-97117. GLP, not published. Syngenta File No CGA245704/0486
0487	Hayworth, C	1998c	Final Report / Analytical Report. Syngenta Crop Protection AG, Basel, CH, ABR-97139. GLP. not published. Syngenta File No CGA245704/0487
0716	Hayworth, C	1999a	Stability of CGA 245704 and CGA 210007 in crops and processed fractions under freezer storage conditions. Syngenta Crop Protection AG, Basel, CH, 422-97. GLP, not published. Syngenta File No CGA245704/0716
0672	Hayworth, C	1999b	CGA 245704—Magnitude of the Residues in or on Crop Group 4: Leafy Vegetables. Syngenta Crop Protection AG, Basel, CH, 156-98. GLP, not published. Syngenta File No CGA245704/0672
0485	Hughes, DL	1998	Independent Laboratory Validation of Novartis Method AG 671. Syngenta Crop Protection AG, Basel, CH, Covance Laboratories, Madison WI, USA, 6117-380. GLP, not published. Syngenta File No CGA245704/0485
	Ishibashi, M, Izumi, Y, Sakai, M, Ando, T, Fukusaki, E & Bamba, T	2015	High-throughput simultaneous analysis of pesticides by supercritical fluid chromatography coupled with high-resolution mass spectrometry. J. Agric. Food Chem., 2015, 63 (18), pp 4457–4463
0074	Jakel, K	1993	Report on dissociation constant in water. Syngenta Crop Protection AG, Basel, CH, PP-93/31P.DCW. GLP, not published. Syngenta File No CGA245704/0074
0481	Kennedy, ET	1998	14C-CGA-245704: Uptake and Distribution of Residues in Field Grown Confined Rotational Crops. Syngenta Crop Protection AG, Basel, CH, ABR-98002. GLP, not published. Syngenta File No CGA245704/0481
0460	Kiffe, M	1998	Metabolism of CGA 245704 in Greenhouse Grown Lettuce after Treatment with [Phenyl-(U)-14C] Labelled Material. Syngenta Crop Protection AG, Basel, CH, 96MK02PR1. GLP, not published. Syngenta File No CGA245704/0460
10062	Kind, B	2011	Acibenzolar-S-methyl—Calculation of Kinetic Modelling and Trigger Endpoints in Soil from Laboratory Study Data according to FOCUS Kinetics Guidelines. Syngenta Crop Protection AG, Basel, CH, Rifcon, Heidelberg, Germany, R11216-2. Not GLP, not published. Syngenta File No CGA245704_10062
0370	Krauss, J	1996	Behaviour and metabolism of CGA 245704 in greenhouse grown paddy rice after granular application of (phenyl-(U)-^{14}C) labelled material. Syngenta Crop Protection AG, Basel, CH, 9/96. GLP, not published. Syngenta File No CGA245704/0370

Code	Author	Year	Title, Institute, Report reference
10047	Kuehne, M	2011	CGA245704—Determination of the physico-chemical property surface tension of an aqueous solution. Syngenta Crop Protection AG, Basel, CH, Harlan Laboratories Ltd., Itingen, CH, D28930. GLP, not published. Syngenta File No CGA245704/10047
0782	Kuhne, RO	2001a	Residue Study with Acibenzolar-S-Methyl (CGA 245704) in or on Pears in Greece. Syngenta Crop Protection AG, Basel, CH, 2079/00. GLP, not published. Syngenta File No CGA245704/0782
0781	Kuhne, RO	2001b	Residue Study with Acibenzolar-S-Methyl (CGA 245704) in or on Pears in Greece. Syngenta Crop Protection AG, Basel, CH, 2080/00. GLP, not published. Syngenta File No CGA245704/0781
0780	Kuhne, RO	2001c	Residue Study with Acibenzolar-S-Methyl (CGA 245704) in or on Pears in Italy. Syngenta Crop Protection AG, Basel, CH, 2098/00. GLP, not published. Syngenta File No CGA245704/0780
0779	Kuhne, RO	2001d	Residue Study with Acibenzolar-S-Methyl (CGA 245704) in or on Pears in Italy. Syngenta Crop Protection AG, Basel, CH, 2099/00. GLP, not published. Syngenta File No CGA245704/0779
0777	Kuhne, RO	2001e	Residue Study with Acibenzolar-S-Methyl (CGA 245704) in or on Pears in Italy. Syngenta Crop Protection AG, Basel, CH, 2100/00. GLP, not published. Syngenta File No CGA245704/0777
0775	Kuhne, RO	2001f	Residue Study with Acibenzolar-S-Methyl (CGA245704) in or on Pears in Italy. Syngenta Crop Protection AG, Basel, CH, 2101/00. GLP, not published. Syngenta File No CGA245704/0775
0769	Kuhne, RO	2001g	Residue Study with Acibenzolar-S-Methyl (CGA 245704) in or on Bananas in Martinique. Syngenta Crop Protection AG, Basel, CH, 2152/00. GLP, not published. Syngenta File No CGA245704/0769
0770	Kuhne, RO	2001h	Residue Study with Acibenzolar-S-Methyl (CGA 245704) in or on Bananas in Martinique. Syngenta Crop Protection AG, Basel, CH, 2153/00. GLP, not published. Syngenta File No CGA245704/0770
	Kwon, H, Lehotay, SJ & Geis-Asteggiante, L	2012	Variability of matrix effects in liquid and gas chromatography—mass spectrometry analysis of pesticide residues after QuEChERS sample preparation of different food crops. Journal of Chromatography A 1270: 235–245.
	Kwon, Hye-Young, Kim, Chan-Sub, Park, Byung-Jun, Jin, Yong-Duk, Son, Kyung-Ae, Hong, Su-Myeong, Lee, Je-Bong & Im, Geon-Jae	2011	Multiresidue analysis of 240 pesticides in apple and lettuce by QuEChERS sample preparation and HPLC-MS/MS analysis. The Korean Journal of Pesticide Science 15: 417–433.
50229	Lin, K	2011	Acibenzolar-S-Methyl—Analytical Method (GRM051.05A) for Determination of Residues of Acibenzolar-S-Methyl as CGA210007 in Crop Commodities by LC-MS/MS. Syngenta Crop Protection AG, Basel, CH, GRM051.05A. Not GLP, not published. Syngenta File No CGA245704_50229

Code	Author	Year	Title, Institute, Report reference
	López, MG, Fussell, RJ, Stead, SL, Roberts, D, McCullagh, M & Rao, R	2014	Evaluation and validation of an accurate mass screening method for the analysis of pesticides in fruits and vegetables using liquid chromatography—quadrupole-time of flight—mass spectrometry with automated detection. Journal of Chromatography A 1373: 40–50
10099	Mackenzie, R	2012	Acibenzolar-S-methyl—Calculation of Kinetic Endpoints from Laboratory Study Data according to FOCUS Kinetics Guidelines (including the soil metabolites CGA210007 and SYN546642). Syngenta Crop Protection AG, Basel, CH, RAJ0940B. Not GLP, not published. Syngenta File No CGA245704_10099
10303	Maslowski, K	2010	Determination of combined residues of acibenzolar-s-methyl (CGA245704) and its metabolite CGA210007 in crop samples by LC-MS/MS. Syngenta Crop Protection AG, Basel, CH, POPIT MET.081.Rev11. Not GLP, not published. Syngenta File No CGA245704_10303
10086	Miner, P	2012	Acibenzolar-S-Methyl—[^{14}C]-Acibenzolar-S-Methyl: Rate and Route of Degradation under Aerobic Laboratory Conditions in Multiple Soils. Syngenta Crop Protection AG, Basel, CH, Ricerca Biosciences, LLC, USA, 026254-1-1. GLP, not published. Syngenta File No CGA245704_10086
0205	Muller, W	1995	Report on spectra. Syngenta Crop Protection AG, Basel, CH, 36232. GLP, not published. Syngenta File No CGA245704/0205
10544	Munro, P	2013	ACTIGARD elicitor. Determination of acibenzolar-s-methyl residues in kiwifruit after foliar and soil applications. Syngenta Crop Protection AG, Basel, CH, Peracto New Zealand Limited, Pukekohe, New Zealand, SY1236. Not GLP, not published. Syngenta File No A9180A_10544
	Myresiotis, C, Vryzas, Z & Papadopoulou-Mourkidou, E	2014	Enhanced root uptake of acibenzolar-S-methyl (ASM) by tomato plants inoculated with selected *Bacillus* plant growth-promoting rhizobacteria (PGPR). Applied Soil Ecology 77: 26-33]
0299	Nicollier, G	1996a	Metabolism of CGA 245704 in greenhouse grown tomatoes after treatment with [phenyl-(U)-^{14}C] labelled material. Syngenta Crop Protection AG, Basel, CH, 01/96. GLP, not published. Syngenta File No CGA245704/0299
0266	Nicollier, G	1996b	Behaviour and metabolism of CGA 245704 in greenhouse grown tobacco after foliar spray application of [phenyl-(U)^{14}C]CGA 245704. Syngenta Crop Protection AG, Basel, CH, 02/96. GLP, not published. Syngenta File No CGA245704/0266
	Okihashi, M, Kitagawa, Y, Obana, H, Tanaka, Y, Yamagishi, Y, Sugitate, K, Saito, K, Kubota, M, Kanai, M, Ueda, T, Harada, S & Kimura, Y	2007	Rapid multiresidue method for the determination of more than 300 pesticide residues in food. Food 1: 101-110.

Code	Author	Year	Title, Institute, Report reference
	Pang, G-F, Cao, Y-Z, Fan, C-L, Jia, G-Q, Zhang, J-J, Li, X-M, Liu, Y-M, Shi, Y-Q, Li, Z-Y, Zheng, F, Lian, Y-J	2009	Analysis Method Study on 839 Pesticide and Chemical Contaminant Multiresidues in Animal Muscles by Gel Permeation Chromatography Cleanup, GC/MS, and LC/MS/MS. Journal of AOAC International, 92:S1-S72(72)
	Pérez-Ortega, P, Lara-Ortega, FJ, García-Reyes, JF, Beneito-Cambra, M, Gilbert-López, B, Martos, NR & Molina-Díaz A	2016	Determination of over 350 multiclass pesticides in jams by ultra-high performance liquid chromatography time-of-flight mass spectrometry (UHPLC-TOFMS). Food Analytical Methods 9: 1939-1957.
0247	Phaff, R	1996	Rate and quantum yield of the direct phototransformation of CGA 245704 under laboratory conditions in water. Syngenta Crop Protection AG, Basel, CH, 94RP03. GLP, not published. Syngenta File No CGA245704/0247
	Pihlström, T, Blomkvist, G, Friman, P, Pagard, U & Österdahl, B-G	2007	Analysis of pesticide residues in fruit and vegetables with ethyl acetate extraction using gas and liquid chromatography with tandem mass spectrometric detection Anal Bioanal Chem 389: 1773–1789.
50167	Quistad, G, LaMar, J & Kovatchev, A	2011a	[^{14}C]Acibenzolar-S-Methyl—Uptake and Metabolism in Sunflower. Syngenta Crop Protection AG, Basel, CH, PTRL West, Inc., Hercules, USA, 2085W. GLP, not published. Syngenta File No CGA245704/50167
50168	Quistad, G, LaMar, J & Kovatchev, A	2011b	[^{14}C]Acibenzolar-S-Methyl—Uptake and Metabolism in Grain Sorghum. Syngenta Crop Protection AG, Basel, CH, PTRL West, Inc., Hercules, USA, 2086W. GLP, not published. Syngenta File No CGA245704/50168
10305	Roncato, C	2003	Determination of combined residues of Acibenzolar-S-methyl (CGA245704) and its metabolite CGA210007 in potato and tomato. Syngenta Crop Protection AG, Basel, CH, POPIT MET.003. Rev 01. Not GLP, not published. Syngenta File No CGA245704_10305
10014	Roncato, C, Boscolo, P & Regina, S	2003	Bion 500WG—Magnitude of Acibenzolar-S-Methyl residues in potato—Brazil, 2000–01 (3 Trials). Syngenta Crop Protection AG, Basel, CH, M00134. GLP, not published. Syngenta File No A9180A_10014
0183	Rümbeli, R	1995	Metabolism of [Phenyl-(U)-^{14}C]CGA 245704 after Multiple Oral Administration to Lactating Goats. Syngenta Crop Protection AG, Basel, CH, 8/95. GLP, not published Syngenta File No CGA245704/0183
0349	Rümbeli, R	1996	Metabolism of [Phenyl-(U)-^{14}C]CGA 245704 after Multiple Oral Administration to Laying Hens. Syngenta Crop Protection AG, Basel, CH, 5/96. GLP, not published Syngenta File No CGA245704/0349

Code	Author	Year	Title, Institute, Report reference
0143	Ryser, M	1995	Report on surface tension of aqueous solutions. Syngenta Crop Protection AG, Basel, CH, PP-95/11T.SUR. GLP, not published. Syngenta File No CGA245704/0143
0644	Sack, S	1999a	Residue Study with Acibenzolar-S-Methyl (CGA 245704 and Copper as Copper Hydroxide (ASF 321) in or on Tomatoes in Italy. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland, 2032/98. GLP. not published. Syngenta File No CGA245704/0644
0640	Sack, S	1999b	Residue Study with Acibenzolar-S-Methyl (CGA 245704) and Copper as Copper Hydroxide (ASF 321) in or on Tomatoes in Italy. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland, 2033/98. GLP. not published. Syngenta File No CGA245704/0640
0643	Sack, S	1999c	Residue Study with Acibenzolar-S-Methyl (CGA 245704 and Copper as Copper Hydroxide (ASF 321) in or on Tomatoes in Greece. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland, 2128/98. GLP. not published. Syngenta File No CGA245704/0643
0642	Sack, S	1999d	Residue Study with Acibenzolar-S-Methyl (CGA 245704 and Copper as Copper Hydroxide (ASF 321) in or on Tomatoes in Greece. Syngenta Crop Protection AG, Basel, CH, 2129/98. GLP, not published. Syngenta File No CGA245704/0642
0725	Sack, S	2000a	Residue study with acibenzolar-S-methyl (CGA 245704) in or on pears in Italy. Syngenta Crop Protection AG, Basel, CH, 2102/99. GLP, not published. Syngenta File No CGA245704/0725
0726	Sack, S	2000b	Residue study with acibenzolar-S-methyl (CGA 245704) in or on pears in Italy. Syngenta Crop Protection AG, Basel, CH, 2103/99. GLP, not published. Syngenta File No CGA245704/0726
0740	Sack, S	2000c	Residue Study with Acibenzolar-S-Methyl (CGA 245704) and Metalaxyl-M (CGA 329351) in or on Spinach in Italy. Syngenta Crop Protection AG, Basel, CH, 2032/99. GLP, not published. Syngenta File No CGA245704/0740
0719	Sack, S	2000d	Residue study with acibenzolar-S-methyl (CGA 245704) and metalaxyl-M (CGA 329351) in or on spinach in Italy. Syngenta Crop Protection AG, Basel, CH, 2033/99. GLP, not published. Syngenta File No CGA245704/0719
0741	Sack, S	2000e	Residue Study with Acibenzolar-S-Methyl (CGA 245704) and Metalaxyl-M (CGA 329351) in or on Spinach in Italy. Syngenta Crop Protection AG, Basel, CH, 2034/99. GLP, not published. Syngenta File No CGA245704/0741
0723	Sack, S	2000f	Residue study with acibenzolar-S-methyl (CGA 245704) and metalaxyl-M (CGA 329351) in or on spinach in Switzerland. Syngenta Crop Protection AG, Basel, CH, 2044/99. GLP, not published. Syngenta File No CGA245704/0723
0742	Sack, S	2000g	Residue Study with Acibenzolar-S-Methyl (CGA 245704) and Metalaxyl-M (CGA 329351) in or on Spinach in Switzerland. Syngenta Crop Protection AG, Basel, CH, 2045/99. GLP, not published. Syngenta File No CGA245704/0742
0724	Sack, S	2000h	Residue study with acibenzolar-S-methyl (CGA 245704) and metalaxyl-M (CGA 329351) in or on spinach in Switzerland. Syngenta Crop Protection AG, Basel, CH, 2046/99. GLP, not published. Syngenta File No CGA245704/0724

Code	Author	Year	Title, Institute, Report reference
0753	Sack, S	2001a	Residue Study with Acibenzolar-S-Methyl (CGA 245704) and Metalaxyl-M (CGA 329351) in or on Spinach in France (North). Syngenta Crop Protection AG, Basel, CH, 2062/99. GLP, not published. Syngenta File No CGA245704/0753
0752	Sack, S	2001b	Residue Study with Acibenzolar-S-Methyl (CGA 245704) and Metalaxyl-M (CGA 329351) in or on Spinach in France (South). Syngenta Crop Protection AG, Basel, CH, 2063/99. GLP, not published. Syngenta File No CGA245704/0752
0768	Salvi, M	2001	Residue Study with Acibenzolar-S-Methyl (CGA245704) and Mancozeb (ASF 21) in or on Potatoes in Indonesia. Syngenta Crop Protection AG, Basel, CH, ADME–Bioanalyses, Vergeze, France, 504/00. GLP, not published. Syngenta File No CGA245704/0768
	Scarponi, L, Buonaurio, R & Martinetti, L	2001	Persistence and translocation of a benzothiadiazole derivative in tomato plants in relation to systemic acquired resistance against *Psuedomonas syringae* pv tomato. Pest Management Science 57:262-268.
0626	Smith, JA	1999a	Determination of Residues of CGA 245704 and the Metabolite CGA 210007 in Potatoes. Syngenta Crop Protection AG, Basel, CH, gr 29498. GLP, not published, Syngenta File No CGA245704/0626
0627	Smith, JA	1999b	Determination of Residues of CGA 245704 and the Metabolite CGA 210007 in Potatoes. Syngenta Crop Protection AG, Basel, CH, gr 28298. GLP, not published. Syngenta File No CGA245704/0627
0061	Stulz, J	1993a	Report on water solubility. Syngenta Crop Protection AG, Basel, CH, 17289. GLP, not published. Syngenta File No CGA245704/0061
0062	Stulz, J	1993b	Report on octanol/water partition coefficient. Syngenta Crop Protection AG, Basel, CH, 16548. GLP, not published. Syngenta File No CGA245704/0062
0060	Stulz, J	1994	Report on solubility in organic solvents. Syngenta Crop Protection AG, Basel, CH, 21794. GLP, not published. Syngenta File No CGA245704/0060
467403	Thompson, D	2008	Acibenzolar—Magnitude of the Residue on Onion (Dry Bulb). IR-4 Project, North Brunswick, USA. IR-4 Project, North Brunswick, USA, 09090. GLP, not published. Syngenta File No 467403
50155	Thompson, D	2010	Acibenzolar—Magnitude of the Residue on Strawberry. IR-4 Project, North Brunswick, USA. IR-4 Project, North Brunswick, USA, 07817. GLP, not published. Syngenta File No CGA245704_50155
4782	Timothy, AJ	1999	CGA 215944 and CGA 245704—Magnitude Of The Residues In Or On Crop Group 9: Cucurbit Vegetables. Syngenta Crop Protection AG, Basel, CH, 131-98. Not GLP, not published. Syngenta File No CGA215944/4782
10070	Valli, F & Baravelli, P	2003a	Residue study with acibenzolar-S-methyl (Bion 50 WG) in or on apple in Italy, degradation curve determination—2002 Italy. Syngenta Crop Protection AG, Basel, CH, AGRI 2000, Bologna, Italy, 0226R/27R DEC, 0228R/29R HAR. GLP, not published. Syngenta File No A9180A_10070
0849	Valli, F & Baravelli, P	2003b	Residue study with acibenzolar-S-methyl (Bion 50 WG) in or on peach in Italy 2002. Syngenta Crop Protection AG, Basel, CH, AGRI 2000, Bologna, Italy, GLP245704BPC. GLP, not published. Syngenta File No CGA245704/0849

Code	Author	Year	Title, Institute, Report reference
0149	Walser, M	1995a	Behavior and Metabolism of [U-^{14}C-Phenyl]-CGA 245704 in Spring Wheat. Syngenta Crop Protection AG, Basel, CH, 9/94. GLP, not published. Syngenta File No CGA245704/0149
0148	Walser, M	1995b	Outdoor Confined Accumulation Study on Rotational Crops after Bareground Soil Application of [U-^{14}C-Phenyl]-CGA 245704. Syngenta Crop Protection AG, Basel, CH, 2/95. GLP, not published. Syngenta File No CGA245704/0148
0550	Walser, M	1998a	Validation of Method REM 172.11 for the Determination of CGA 245704 (as CGA 210007) in Fruits of Mango. Syngenta Crop Protection AG, Basel, CH, 216/98. GLP, not published. Syngenta File No CGA245704/0550
0466	Walser, M	1998b	Validation of Method REM 172.12 App. 1—Validation by analysis of fortified specimens (fat, liver, kidney) and determination of recoveries. Syngenta Crop Protection AG, Basel, CH, 172/97. GLP, not published. Syngenta File No CGA245704/0466
0465	Walser, M	1998c	Residues in milk, blood and tissues (muscle, fat, liver, kidney) of dairy cattle resulting from a feeding of three levels of CGA 245704 (Acibenzolar-S-methyl). Syngenta Crop Protection AG, Basel, CH, 111/97. GLP, not published. Syngenta File No CGA245704/0465
0508	Walser, M	1998d	CGA 245704 + ASF 321 (Copper hydroxide), WG 41.25, A-9883 A, Tomatoes, Italy. Syngenta Crop Protection AG, Basel, CH, 2042/97. GLP, not published. Syngenta File No CGA245704/0508
0509	Walser, M	1998e	CGA 245704 + ASF 321 (Copper hydroxide), WG 41.25, A-9883 A, Tomatoes, Italy. Syngenta Crop Protection AG, Basel, CH, 2043/97. GLP, not published. Syngenta File No CGA245704/0509
0534	Walser, M	1998f	CGA 245704 + ASF 321 (Copper hydroxide), WG 41.25, A-9883 A, Tomatoes, France(N). Syngenta Crop Protection AG, Basel, CH, 2359/97. GLP, not published Syngenta File No CGA245704/0534
0536	Walser, M	1998g	CGA 245704 + ASF 321 (Copper hydroxide), WG 41.25, A-9883 A, Tomatoes (fruit, juice, ketchup, puree and canned fruits/preserves), France(N). Syngenta Crop Protection AG, Basel, CH, 2360/97. GLP, not published. Syngenta File No CGA245704/0536
0535	Walser, M	1998h	CGA 245704 + ASF 321 (Copper hydroxide), WG 41.25, A-9883 A, Tomatoes, France(N). Syngenta Crop Protection AG, Basel, CH, 2361/97. GLP, not published. Syngenta File No CGA245704/0535
0521	Walser, M	1998i	CGA 245704, WG 50, A-9180 A, Tomatoes, France(N). Syngenta Crop Protection AG, Basel, CH, 2362/97. GLP, not published. Syngenta File No CGA245704/0521
0524	Walser, M	1998j	CGA 245704 + ASF 321 (Copper hydroxide), WG 41.25, A-9883 A, Tomatoes, France(S). Syngenta Crop Protection AG, Basel, CH, 2363/97. GLP, not published. Syngenta File No CGA245704/0524
0525	Walser, M	1998k	CGA 245704 + ASF 321 (Copper hydroxide), WG 41.25, A-9883 A, Tomatoes (fruits, juice, ketchup, puree and canned fruits/preserves), France(S). Syngenta Crop Protection AG, Basel, CH, 2364/97. GLP. not published. Syngenta File No CGA245704/0525

Code	Author	Year	Title, Institute, Report reference
0523	Walser, M	1998l	CGA 245704 + ASF 321 (Copper hydroxide), WG 41.25, A-9883 A, Tomatoes, France (South). Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland, 2365/97. GLP. not published. Syngenta File No CGA245704/0523
0522	Walser, M	1998m	CGA 245704, WG 50, A-9180 A, Tomatoes, France (South). Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland, 2366/97. GLP. not published. Syngenta File No CGA245704/0522
0609	Walser, M	1999a	Determination of metabolite CGA 323060 by HPLC, Lettuce, Spinach. Syngenta Crop Protection AG, Basel, CH, REM 172.14. GLP, not published. Syngenta File No CGA245704/0609
0572	Walser, M	1999b	Validation of Method REM 172.14 for the Determination of CGA 323060 (metabolite of CGA 245704) in Lettuce and Spinach. Syngenta Crop Protection AG, Basel, CH, 218/98. GLP, not published. Syngenta File No CGA245704/0572
0569	Walser, M	1999c	Storage stability study of total residues of CGA 245704 in fortified bananas (whole fruits) under freezer storage conditions. Syngenta Crop Protection AG, Basel, CH, 154/95. GLP, not published. Syngenta File No CGA245704/0569
0588	Walser, M	1999d	CGA 245704, SC 500, A-9625 A, Bananas, Costa Rica. Syngenta Crop Protection AG, Basel, CH, 2087/98. GLP, not published. Syngenta File No CGA245704/0588
0589	Walser, M	1999e	CGA 245704, SC 500, A-9625 A, Bananas, Costa Rica. Syngenta Crop Protection AG, Basel, CH, 2088/98. GLP, not published. Syngenta File No CGA245704/0589
0590	Walser, M	1999f	CGA 245704, SC 500, A-9625 A, Bananas, Costa Rica. Syngenta Crop Protection AG, Basel, CH, 2089/98. GLP, not published. Syngenta File No CGA245704/0590
0591	Walser, M	1999g	CGA 245704, SC 500, A-9625 A, Bananas, Ecuador. Syngenta Crop Protection AG, Basel, CH, 2090/98. GLP, not published. Syngenta File No CGA245704/0591
0592	Walser, M	1999h	CGA 245704, SC 500, A-9625 A, Bananas, Ecuador. Syngenta Crop Protection AG, Basel, CH, 2091/98. GLP, not published. Syngenta File No CGA245704/0592
0593	Walser, M	1999i	CGA 245704, SC 500, A-9625 A, Bananas, Ecuador. Syngenta Crop Protection AG, Basel, CH, 2092/98. GLP, not published. Syngenta File No CGA245704/0593
0594	Walser, M	1999j	CGA 245704, SC 500, A-9625 A, Bananas, Colombia. Syngenta Crop Protection AG, Basel, CH, 2093/98. GLP, not published. Syngenta File No CGA245704/0594
0595	Walser, M	1999k	CGA 245704, SC 500, A-9625 A, Bananas, Colombia. Syngenta Crop Protection AG, Basel, CH, 2094/98. GLP, not published. Syngenta File No CGA245704/0595
0596	Walser, M	1999l	CGA 245704, SC 500, A-9625 A, Bananas, Mexico. Syngenta Crop Protection AG, Basel, CH, 2095/98. GLP, not published. Syngenta File No CGA245704/0596

Code	Author	Year	Title, Institute, Report reference
0597	Walser, M	1999m	CGA 245704, SC 500, A-9625 A, Bananas, Guatemala. Syngenta Crop Protection AG, Basel, CH, 2098/98. GLP, not published. Syngenta File No CGA245704/0597
0598	Walser, M	1999n	CGA 245704, SC 500, A-9625 A, Bananas, Guatemala. Syngenta Crop Protection AG, Basel, CH, 2099/98. GLP, not published. Syngenta File No CGA245704/0598
0634	Walser, M	1999o	Residue Study with Acibenzolar-S-methyl (CGA 245704), Copper as Copper Hydroxide (ASF 321) in or on Tomatoes in Switzerland (2 Trials). Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland, 2078/98. GLP. not published. Syngenta File No CGA245704/0634
0587	Walser, M	1999p	CGA 245704, and ASF 321, WG 41.25, A-9883 C, Tomatoes, Spain. Syngenta Crop Protection AG, Basel, CH, 2045/98. GLP, not published. Syngenta File No CGA245704/0587
50026	Willard, T	2012a	A9180A—Magnitude of the Residues in or on Oranges, Grapefruit and Lemons as Representative Commodities of Citrus Fruits, Group 10. Syngenta Crop Protection AG, Basel, CH, 110.041. GLP, not published. Syngenta File No A9180A_50026
50017	Willard, T	2012b	A9180A—Magnitude of the Residues in or on Several Vegetable Crops from Side-by-Side Bridging Trials to Compare Application Types and Adjuvants. Syngenta Crop Protection AG, Basel, CH, 110.040. GLP, not published. Syngenta File No A9180A_50017
50020	Willard, T	2012c	Acibenzolar-S-Methyl WG A9180A_Magnitude of the Residues in or on Cantaloupe and Honeydew. Syngenta Crop Protection AG, Basel, CH, 110.043. GLP, not published. Syngenta File No A9180A_50020