BENALAXYL (155)

First draft prepared by Dr W Donovan, United States Environmental Protection Agency, Washington, DC, USA

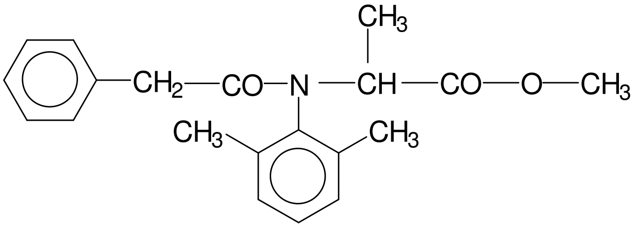
EXPLANATION

Benalaxyl [methyl N-phenylacetyl-N-2,6-xylyl-DL-alaninate] is a broad-spectrum phenylamide fungicide. Residue and analytical aspects of benalaxyl were evaluated by the JMPR in 1986, 1988, 1992, and 1993. It was evaluated for toxicological review by JMPR 2005. The ADI for benalaxyl was established at 0–0.07 mg/kg bw and an acute reference dose of 0.1 mg/kg bw was selected for women of childbearing age. This compound was listed in the Periodic Re-Evaluation Program at the 40th Session of the CCPR for periodic review by the 2009 JMPR.

Residue studies were submitted by the manufacturer to support the use of benalaxyl in or on a variety of fruits and vegetables.

IDENTITY

ISO common name:	Benalaxyl
IUPAC name:	methyl N-phenylacetyl-N-2,6-xylyl-DL-alaninate
Chemical Abstract name:	methyl N-(2,6-dimethylphenyl)-N-(phenylacetyl)-DL-alaninate
CAS No.:	71626-11-4
CIPAC No.:	416
Manufacturer’s experimental name:	Galben
Molecular Formula:	$C_{20}H_{23}NO_3$
Structural Formula:	



Molecular Weight:	325.4 g/mol

Physical and chemical properties

Pure active ingredient (except as noted)

Physical-Chemical Property	Test material purity and specification	Results	Reference
Melting point	Technical active ingredient	76.8 °C	Costantini G. *et al.* 1995. Report No: 94/1087.C
Relative Density		1.181 g/mL at 20 °C	Costantini G. *et al.* 1995. Report No:94/1087.B
Vapour pressure		Extrapolated: 5.72×10^{-4} Pa for 20 °C 7.08×10^{-4} Pa for 28 °C 19.6×10^{-4} Pa for 50 °C	Costantini G. *et al.* 1995. Report No:94/1087.B
Henry's law constant		Henry's law constants at 20 °C: 6.50×10^{-3} Pa/m^3/mol	Costantini G. *et al.* 1995. Report No:94/1087.B
Description of the physical state and colour of the ai		Active substance, pure: white crystalline solid	Costantini G. *et al.* 1995. Report No:94/1087.B
Solubility of purified active substance in water		In distilled water: pH 6.1 28.6 mg/L at 20 °C	Costantini G. *et al.* 1995. Report No:94/1087.B
Solubility in organic solvents	Technical material	[g/kg at 22 °C] n-heptane 19.4 xylene > 250 acetone > 250 ethylacetate > 250 1,2-dichloroethane > 250 Methanol > 250	Costantini G. *et al.* 1995. Report No:94/1087.B
n-Octanol/water partition coefficient		pH 6.1 log Pow 3.54 at 20 °C	Costantini G. *et al.* 1995. Report No:94/1087.B
Hydrolysis rate at pH 4, 7 and 9		The experimental half-lives of the test substance at pH 9 were 86 days (25 °C) and 157 days (20 °C).	Masoero M., Crisippi T. 1982

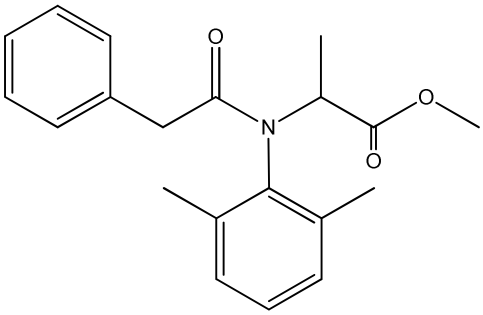
Formulations

The main formulation type is a wettable powder (WP), although a SC formulation is also registered in France. Benalaxyl is typically co-formulated with a variety of other pesticides including mancozeb, chlorothalonil, folpet, cymoxanil, and copper compounds.

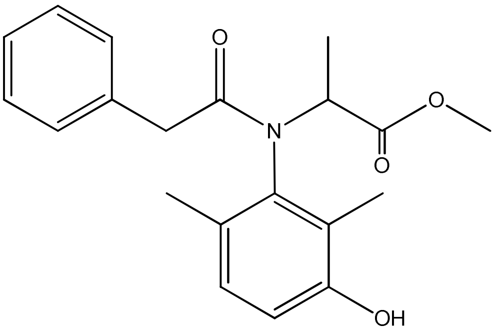
METABOLISM AND ENVIROMENTAL FATE

The structures and identification labels used for compounds found in plant and animal metabolism studies are shown below. Likewise, Figure 1 presents a combined metabolic profile based on the results of these studies.

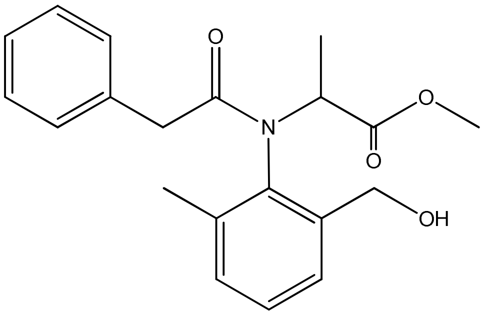
Metabolites indentified in the plant and animal metabolism studies.



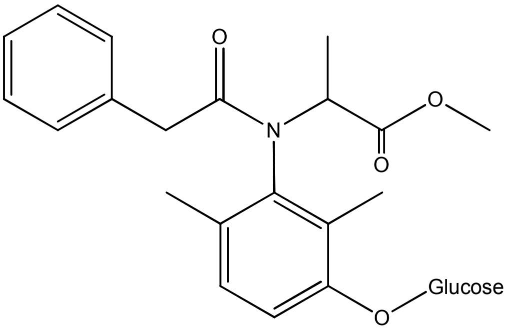
Benalaxyl [Galben]



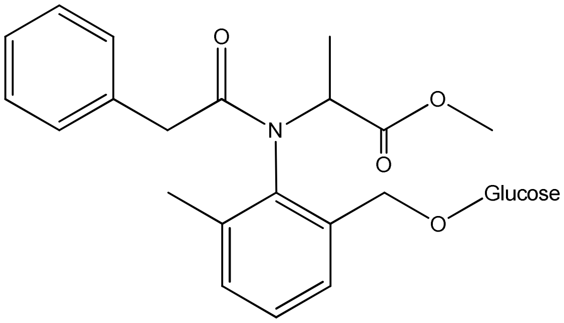
GX5c (Plants)



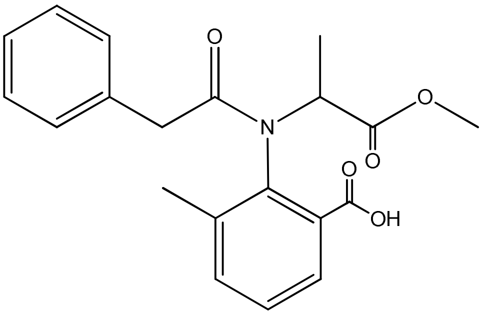
GX5a and GX5b (Plants), G8 and G14 (Animals)



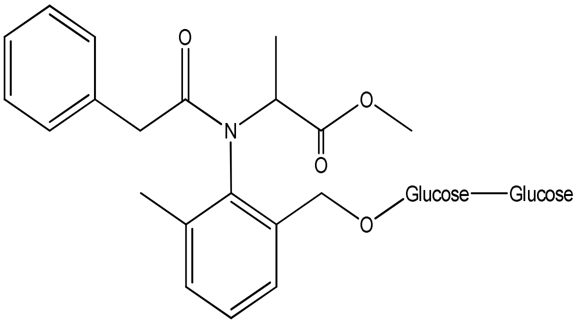
GX1c (Plants)



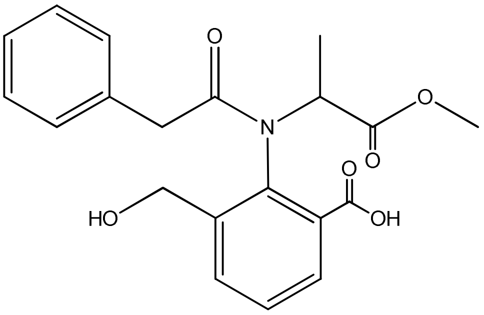
GX1a and GX1b (Plants)



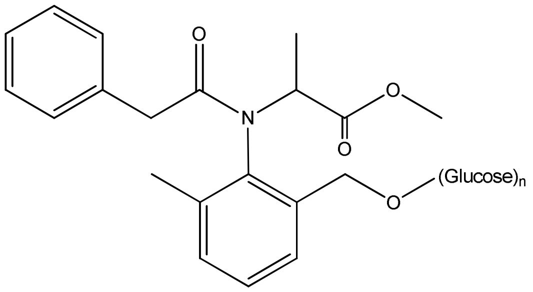
G7a and G7b (Animals)



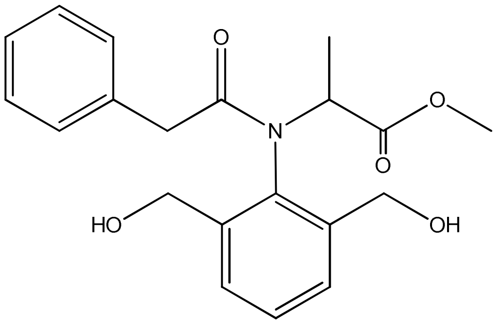
GX6 (Plants)



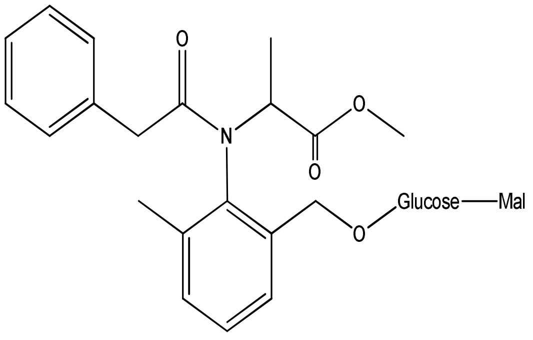
G6 (Animals)



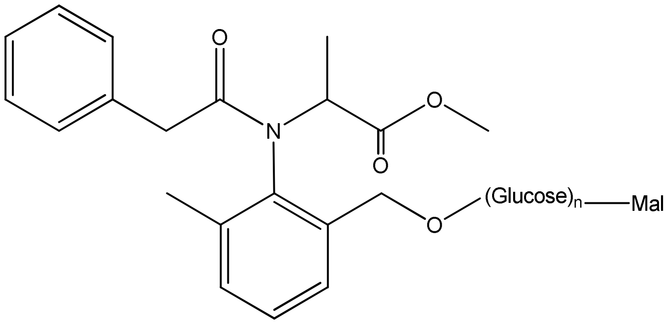
GX4 (Plants)



M4 (Plants and Animals)



GX11 (Plants)



GX16 (Plants)

Figure 1 Metabolites indentified in the plant and animal metabolism studies.

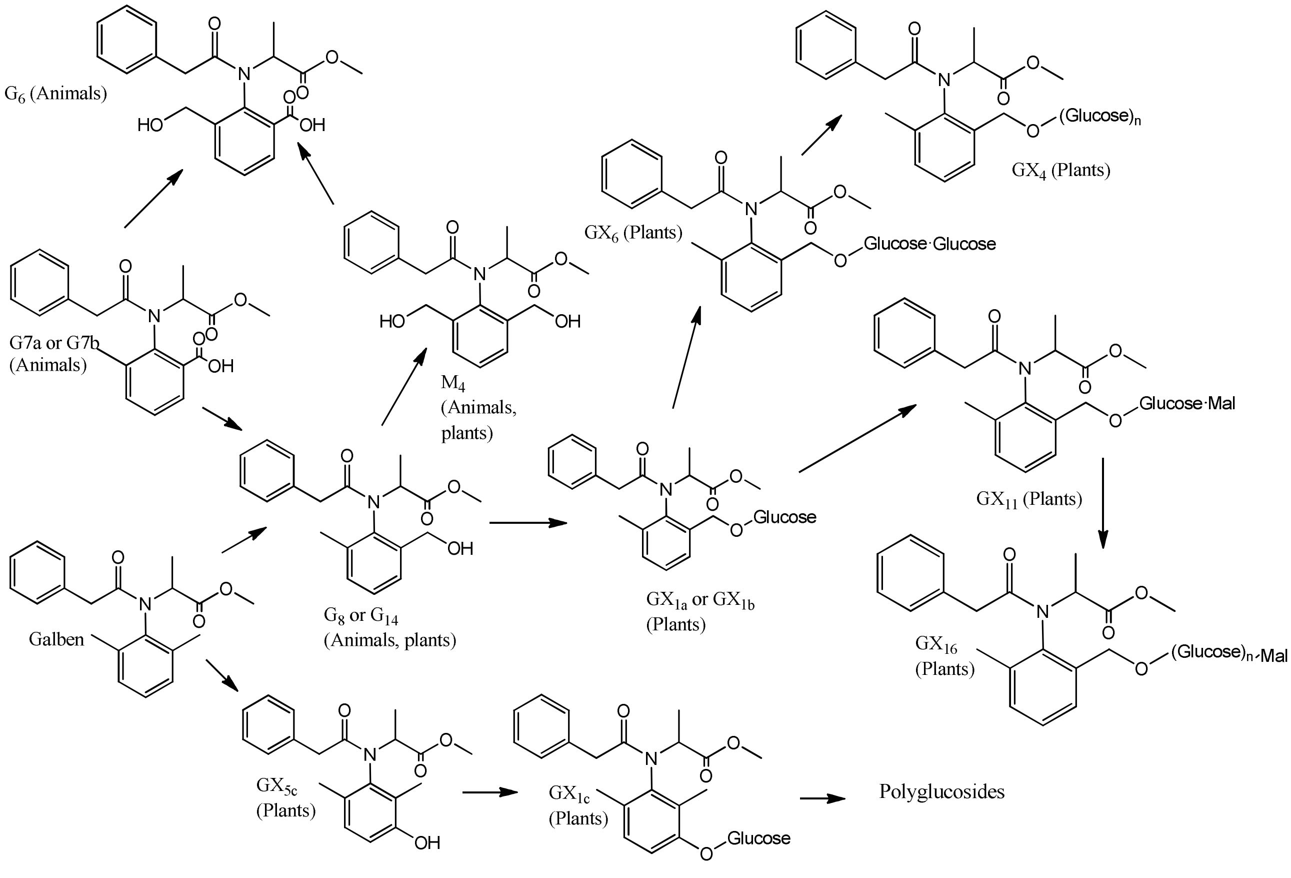


Figure 2 Metabolic profile of benalaxyl in plants and animals

Animal metabolism

Two livestock metabolism studies in goat and hen were made available to the Meeting. It was found that approximately 80% of ^{14}C-labelled material was excreted via urine, faeces or milk. In both the goat and the hen residue levels were very low; in milk residue levels found were less than 0.01 mg/kg. All metabolites found in the goat and hen were also found in the rat (WHO, 2006)[1].

Metabolism, distribution and expression of residues in Lactating goats

Two lactating goats weighing 53.6 and 34.5 kg were dosed twice daily for seven consecutive days with gelatine capsules containing [^{14}C]Benalaxyl at a dose level of 36.1 mg/kg diet/day for Goat 1 and 41.8 mg/kg diet/day for Goat 2. Milk, blood, urine and faeces were collected over a 10 hour period. Tissues and gut contents were assayed for total ^{14}C-residues, and then metabolic profiles were determined for selected samples. Total ^{14}C-residues of ^{14}C-labelled material in the milk fat, tissues, gut contents and faeces were determined by oxidative combustion; the levels of total unextracted ^{14}C-residue remaining after solvent extraction were also determined by oxidative combustion. ^{14}C-residue levels in milk, urine and expired carbon dioxide were determined by direct radioassay. Radioactivity was measured with a Beckman LS9800 Liquid Scintillation System. The chromatographic profiles of samples were determined by radio-TLC. Radioactivity in urine, liver and kidney was extracted and analysed before and after enzyme hydrolysis.

Benalaxyl treatment had no effects on the feed consumption, milk production or on the general health of the goats. Eighty seven percent (urine: 47%; faeces: 40%; milk: < 0.02%) and seventy nine percent (urine: 39%; faeces: 40%; milk: 0.02%) of the total dose was excreted by Goat 1 and Goat 2, respectively. Tissues and gut contents contained 4.6% of the total dose at slaughter. The data on blood concentration for both goats showed the peak level (0.123 mg/kg for Goat 1; 0.178 mg/kg for Goat 2) to be reached approximately 45 minutes after dosing. The residue levels in milk were very low (< 0.01 mg/kg) and remained at the same level throughout the entire treatment period; the highest values obtained were 0.0069 mg/kg for Goat 1 and 0.0109 mg/kg for Goat 2. Recoveries of ^{14}C-residues as % of total dose and as mg/kg in milk, urine and faeces are shown in Table 1.

Table 1 Radioactivity in goat milk, urine and faeces

	Goat 1		Goat 2	
	% of total dose	mg/kg	% of total dose	mg/kg
Milk	0.017	0.0051-0.0069	0.023	0.0063-0.0109
Urine	47.367	25.6-50.6	38.879	19.3-44.0
Faeces	40.355	5.72-10.4	39.742	5.15-14.3
Total	87.739	-------	78.644	------

Recoveries of ^{14}C-residues as % of total dose and as mg/kg in tissues and gut contents are shown in Table 2.

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[1] WHO, Pesticide residues in food — 2005 Joint FAO/WHO Meeting on Pesticide Residues EVALUATIONS 2005, Part II — Toxicological, 2006, p. 39

Table 2 Radioactivity in goat tissues and gut contents at slaughter

	Goat 1		Goat 2	
Tissues and gut content	% of total dose	mg/kg	% of total dose	mg/kg
Blood	0.061	0.069	0.059	0.105
Fat	0.002	< 0.01	0.005	0.027
Heart	0.001	0.021	0.001	0.032
Kidney	0.013	0.360	0.009	0.374
Liver	0.119	0.631	0.144	1.140
Muscle	0.042	< 0.01	0.047	0.017
Large intestine content	3.322	14.6	3.282	13.1
Small intestine content	0.538	4.47	0.747	6.49
Stomach content	0.492	0.649	0.267	0.473
Total	4.591	-----	4.566	-----

The ^{14}C-residue in tissues were low with liver (0.631 and 1.140 mg/kg) and kidney (0.360 and 0.374 mg/kg) the only two tissues containing residues greater than 0.1 mg/kg at slaughter.

The metabolic profiles obtained for the samples were as shown in Table 3. Thin-layer chromatography (TLC) analysis of enzyme-hydrolysed extracts showed that the ^{14}C-residues in urine were mainly glucuronide and/or sulphate conjugates of the hydroxylated metabolites G8 and G14; most of the radioactive material in the urine appeared to be polar conjugates which remained at the origin in high amounts and were isolated successively with different mixtures of solvents. The faeces contained benalaxyl (12.9%) and small amounts (< 5%) of G6, G7A, G7B and G14. Concerning the unidentified compounds in the faeces extracts, one of them comprised 11% of the total residue and all others comprised less than 9%.

Table 3 Radioactivity in goat tissues and gut contents at slaughter

	Urine		Faeces	Liver		Kidney	
	a	b	a	a	b	a	b
Percent Total Radioactivity Recovered (conc)	99.90 (50 mg/kg)	96.60 (50 mg/kg)	70.10 (8 mg/kg)	46.90 (0.296 mg/kg)	65.30 (0.412 mg/kg)	100.70 (0.363 mg/kg)	105.50 (0.350 mg/kg)
Galben	----	----	12.9		1.2		----
Galben Acid	0.1	5.3	----		----		----
G8	0.6	25.6	5.5		5.6		21.3
G14	----	8.2	----		6.2		14.9
G7A	0.7	3.1	2.0		2.8		1.8
G7B	0.8	2.3	1.4		----		----
G6	0.5	3.7	4.3		----		2.0
Unidentified	2.2	23.8	31.8		20.8		30.6
Remained at origin	95	24.6	12.3		34.7		27.4

[a] - unhydrolysed samples;

[b] - Hydrolysed samples

For liver samples, the extractability was poor and difficulties were encountered during the TLC analysis; the chromatography was poor due to the overloading of chromatographic peaks as a consequence of the very low levels of radioactivity and it was impossible to clearly correlate the bands with the reference standards for the kidney; similar difficulties were encountered during TLC analysis but the results were better and the remaining ^{14}C-residue was fractionated into two bands comprising 14 and 15%. The ^{14}C-residue levels in other tissues were too low for metabolite identification.

In conclusion, these results indicate that the major metabolites of benalaxyl in tissues are glucuronide and/or sulphate conjugates of the hydroxylated metabolites G8 and G14. Three unidentified polar species, each comprising about 10%, were present in the kidney hydroxylates along with two unidentified less polar species of approximately 14%.

Based on the above, a metabolic pathway for the metabolism of benalaxyl in the lactating goat is proposed: benalaxyl is oxidized giving rise to both G8 and G14 hydroxymethyl derivatives. The G8 and G14 are further oxidised to form the G7A and G7B carboxy derivatives. The G6 hydroxymethylcarboxy metabolite is a further oxidation product. Conjugation appears to occur with all the above compounds except benalaxyl. It can be concluded that oxidation followed by conjugation is the principal route of benalaxyl degradation.

Determination of the metabolic fate in Hens

Ten laying hens with an average weight of 1.3 kg were dosed once daily for fourteen days with gelatine capsules containing [^{14}C]benalaxyl at a dose level of 59.6 mg/kg diet/day. Eggs and excreta were collected at specific intervals. Tissue samples were collected at slaughter, which was 14 hours after the last dose. All samples were assayed for total ^{14}C-residue and then the metabolic profiles were determined for selected samples. Total ^{14}C-residues of ^{14}C-labelled material in the eggs, tissues and excreta were determined by oxidative combustion. Radioactivity was measured by a Beckman LS 9800 Liquid Scintillation System. Chromatographic profiles of samples were determined by radio-TLC. The ^{14}C-residues in liver, kidney, blood and egg yolk were extracted and analysed before and after enzyme hydrolysis.

Benalaxyl did not adversely affect the feed consumption or egg production of the tested hens. Eighty one percent of the total administered dose of [^{14}C]benalaxyl was found in excreta following dosing. Radioactivity in tissues and eggs are shown in Table 4.

Table 4 Radioactivity in excreta during dosing and in tissues and eggs at slaughter

	% of total dose	mg/kg
Excreta	81.50	21-44
Blood		0.51
Breast muscle		0.04
Fat		0.04
Gizzard		0.08
Heart		0.10
Kidney		0.72
Liver		1.36
Skin		0.08
Thigh muscle		0.05
Egg white		0.02-0.05
Egg yolk		0.30

The ^{14}C-residues in egg whites were considered too low (0.02-0.05 mg/kg) for metabolite identification work. The residue levels in egg yolks reach a plateau level of about 0.3 mg/kg after 9 days of dosing. After enzyme hydrolysis, 63% of the total residue in egg yolk was extractable. The ^{14}C-residues levels were low for tissues. Liver, kidney and blood contained the highest residue levels. The levels in other tissues were 0.1 mg/kg or less and were considered too low for the further metabolite identification work. For liver and kidney, respectively, 53 and 62% of the total residue were extracted after enzyme hydrolysis. Non-polar species were present in both extracts, but comprised less than 5% of the total ^{14}C-residue. For blood, 55% of the total radioactivity was extractable after enzyme hydrolysis and most of these residues (30–40%) consisted of polar material: 4% were G8/G7B and 9% benalaxyl.

About 85% of the ^{14}C-residue in excreta were extractable and comprised low levels of benalaxyl, benalaxyl acid, G6, G7A, G7B and several unidentified species, which remained at the origin; however, each comprised less than 10% of the total residue. The metabolic profile in hen samples is shown in Table 5.

Table 5 Extraction efficiency and metabolites detected in hen samples (%of total radioactivity) [a]

	Egg yolk	Liver		Kidney	Blood	Excreta
Percent Total Radioactivity Recovered (conc)	62.2 (0.22 mg/kg)	53.1 (0.69 mg/kg)	99.3 (5.40 mg/kg)	61.5 (0.45 mg/kg)	55.4 (0.29 mg/kg)	85.2 (37.8 ppm)
Galben	----	----		----	8.9	11.8
Galben Acid	----	----		----	----	1.4
G8/G7B	----	----		----	3.6	5.4
G7A	----	----		----	----	1.8
G6	20.5	----		----	----	5.6
Unidentified	22.7	14.9		13.0	11.3	13.7
Remained at origin	19.8	38.3		48.5	31.7	39.9

[a] Egg yolk, kidney and blood samples were enzyme hydrolysed before analysis; for liver, 1st column values are related with samples enzyme hydrolysed and 2nd column values are related with enzyme hydrolysed fortified liver samples.

Based on the studies described above, a metabolic pathway for the metabolism of benalaxyl in laying hens was proposed. Benalaxyl is oxidized giving rise to both G8 and G14 hydroxymethyl derivatives; G8 and G14 are further oxidized to form G7A and G7B carboxy derivatives. The G6 hydroxymethylcarboxy metabolite is a further oxidation product. Since enzyme hydrolysis increased the levels of extractable ^{14}C-residue in the tissues and egg yolk, conjugation probably occurs. It is likely that oxidation followed by conjugation is the principal route of benalaxyl metabolism.

Based on goat and laying hens metabolism studies, a metabolic pathway for benalaxyl in farm animals may be proposed. Benalaxyl is oxidised giving G8 and G14 hydroxymethyl derivatives. The G8 and G14 are further oxidized to form the G7A and G7B carboxy derivatives. The G6 hydromethylcarboxy metabolite is a further oxidation product. Conjugation appears to occur with all the compounds. Since enzymatic hydrolysis increased the levels of extractable ^{14}C-residue in the tissues and egg yolk, conjugation probably occurs.

Thus it is likely that oxidation followed by conjugation is the main route of benalaxyl metabolism.

The metabolism in rats of benalaxyl is not significantly different from metabolism in goats and hens.

Plant metabolism

The Meeting received metabolism studies with benalaxyl on the following plants: grape, tomato, and potato. Benalaxyl metabolism was relatively consistent in these matrices: oxidation followed by conjugation with glucose took place at several sites of the benalaxyl compound.

Absorption and Metabolism in Grapes, Wine Making and Residues in Wine

Two field-grown vines (cv. Dolcetto) were used for the experiment, with grapes in the stage of darkening in colour. A solution of water+acetone+tween 20 (80+20+0.02) containing 24 g as/hL [^{14}C]benalaxyl was sprayed on the vines on September 8th, 1980. After the treatment, the plants were kept under a polyethylene cover. Bunches of grapes were sampled 3 hours and 8, 15, and 24 days after the treatment, washed with acetone, homogenized with $NaHCO_3$ and CH_3CN, and extracted with hexane and ethyl ether. The radioactivity was determined by liquid scintillation spectrometer; ^{14}C compounds contained in the extracts were detected by auto-radiography and were quantified by scraping the bands of TLC plates. The ripe grapes (harvested 24 days after treatment) were crushed and the must inoculated with yeast in order to increase alcoholic fermentation.

There is a rapid fall of ^{14}C on the outside of the fruit, with a consequent progressive increase of its concentration inside, reaching 50% of the total radioactivity 3 days after treatment (see Table 6).

Table 6 Distribution of radioactivity in grape vine leaves (outside and inside)

Days after treatment	^{14}C outside+inside	Total ^{14}C outside		Total ^{14}C inside	
	mg/kg	%	mg/kg	%	mg/kg
3 hours	3.54	85.31	3.02	14.69	0.52
3	3.73	49.06	1.83	50.94	1.90
8	1.84	23.37	0.43	76.63	1.41
15	2.04	23.04	0.47	76.96	1.57
24	2.59	12.35	0.32	87.65	2.27

Compounds on the surface and within the grapes:

Most of the ^{14}C on the fruit surface consists of benalaxyl *per se*. As shown in the Table 7, 3.46 mg/kg and 1.34 mg/kg of the ^{14}C found 3 hours and 24 days after the last treatment in grapes were benalaxyl *per se*. Benalaxyl is subjected to a metabolic process which produces mainly two compounds: GX1 and GX6, the first being the most prevalent. The remaining residue in the aqueous acidic phase was below 6% of the total ^{14}C.

Table 7 Distribution and Identification of the Radioactivity in Grapes Expressed as Benalaxyl

Days after treatment	Benalaxyl		GX1		GX5a		GX5b		GX5c		GX6		Aqueous acidic phase	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
3 hours	97.74	3.46	0	< 0.01	0	< 0.01	0	< 0.01	0	< 0.01	0	< 0.01	2.26	0.08
3	93.30	3.48	2.41	0.09	0.80	0.03	0.27	0.01	0.27	0.01	0.54	0.02	2.41	0.09
8	78.81	1.45	9.78	0.18	2.17	0.04	1.63	0.03	1.09	0.02	2.72	0.05	3.80	0.07
15	66.18	1.35	17.65	0.36	2.45	0.05	1.96	0.04	0.98	0.02	4.90	0.10	5.88	0.12
24	51.74	1.34	25.10	0.65	2.70	0.07	2.70	0.07	1.93	0.05	10.81	0.28	5.02	0.13

The wine-making process greatly reduced the residues of benalaxyl and of its ^{14}C metabolites. Only 36.19% of the ^{14}C present in the grapes was transferred to wine. A considerable amount of benalaxyl was adsorbed in the solid parts (peel, grapes stones, and lees), see Table 8; GX1 and other metabolites also remain incorporated in solid parts, although to a lesser extent.

Benalaxyl and metabolites GX4, GX7, GX8 (formed during the alcoholic fermentation), GX1 GX5a-b-c, and GX6 are also present in wine. The total concentration of benalaxyl and of its ^{14}C metabolites in the wine is 0.8 mg/kg. The most prevalent residue is metabolite GX1 (0.3 mg/kg) while the benalaxyl concentration is 0.15 mg/kg; the concentration of the other metabolites is much lower than 0.1 mg/kg.

Table 8 Distribution and Identification of Radioactivity in Wine and Peel, Grape-Stones, and Lees (24 days after treatment)

Plant Product	^{14}C Total	Benalaxyl	GX1	GX4	GX5a	GX5b	GX5c	GX6	GX7	GX8	^{14}C unextracted
Wine (%)	36.19	6.70	13.46	0.96	1.64	1.78	1.48	4.13	1.33	3.15	1.56
mg/kg		0.15	0.30	0.02	0.04	0.04	0.03	0.09	0.03	0.07	0.03
Peel, grape-stones, lees (%)	63.81	40.67	8.76	0.00	1.97	1.64	1.96	4.68	0.68	2.66	0.79

In conclusion, benalaxyl penetrates into the grapes, where it undergoes significant metabolism. The ripe grapes (harvested 24 days after treatment) contain not only benalaxyl, but also GX1 and GX6 in greater concentrations and GX5a, GX5b, and GX6 in lower concentrations. During the wine-making process, benalaxyl and its metabolites remain primarily adsorbed in the solid parts which sediment during fermentation. The wine contains not only benalaxyl (0.15 mg/kg) but also GX1 (0.3 mg/kg) and seven other metabolites in very low concentrations (<< 0.1 mg/kg).

Identification of Some Metabolites in Vine Leaves

This study investigates the chemical structure of metabolite GX1. Fourteen grape plants (cv. Tokay) growing in pots were used for the test. The plants (divided in two groups of 10 and 4, respectively) were sprayed 5 times at 4 day intervals with a water+acetone+tween 20 (80+20+0.02) solution where 0.3% [^{14}C]benalaxyl (labelled in the alpha position of the ester moiety) having a specific activity of 1 µCi/mg or 0.3% unlabelled benalaxyl had been dissolved.

The glucoside was extracted from grape vine leaves and then analysed.

The characterization of GX1 was made by TLC, by GLC (of derivatised compound), and by hydrolysis with β-glucosidase. The identification was performed by NMR and by MS/GC-MS of the metabolite and its aglicons (free or derivatised).

After hydrolysis by β-glucosidase, GX1 decomposes almost completely in three substances. These results show that the compound consists of three glycosides (GX1a, GX1b, and GX1c) which give origin to three aglycones (GX5a, GX5b, and GX5c) with GX5a and GX5b predominating.

Hydrolysis of GX1 with HCl (0.5 N for 24 hours, at 60 °C) released glucose, as determined by TLC. GX5a and GX5b correspond to two pairs of diastereoisomers of benalaxyl with the methyl aromatic ring oxidized to CH_2OH. The GX5c corresponds to benalaxyl with an OH group on the anilinic ring.

In conclusion, sprayed over grape vine leaves, benalaxyl is absorbed and converted through oxidative and glucose conjugation reactions into GX5a, GX5b, and GX5c; and GX1a, GX1b, and GX1c, respectively. GX1a and GX1b are the most significant metabolites. Since the conjugation reaction with glucose proceeds rapidly, there is no accumulation of GX5a, GX5b, or GX5c.

Identification of the GX6 metabolite extracted from grape vine leaves

This study was performed in order to identify GX6 metabolite extracted from grape leaves. Fifteen grape plants growing in pots were used for the test; 2 mL of water-acetone-tween 20 (80+20+0.02) suspension containing 24 g as/hL of [^{14}C]benalaxyl (specific activity 1.30 µCi/mg) were sprayed on each plant; 4 applications were performed.

The metabolite GX6 was extracted from grape leaves and then hydrolysed by β-glucosidase and analysed by LSC and TLC.

Under the hydrolysing action of β-glucosidase, GX6 gave rise primarily to the GX5a and GX5b metabolites, already isolated and characterized as products of the enzymatic hydrolysis of the GX1 metabolite in vine leaves.

After hydrolysis, traces of GX6 and small amounts of GX1 were found. Consequently, it appears that GX6 comes from further conjugation of GX1 with another glucose molecule.

In conclusion, both enzymatic (with β-glucosidase) and chemical hydrolysis demonstrated that the GX6 metabolite extracted from vine leaves is formed by conjugation of GX1 glucoside with glucose.

Isolation and identification of the main metabolites in grapes and wine

This study was designed to isolate and characterize the main benalaxyl metabolites found both in grapes and wine. A solution of water+acetone+tween20 (80+20+0.02) containing 24 g as/hL of [^{14}C]benalaxyl (specific activity 18.45 µCi/mg) was sprayed on the vines. After treatment, the plants were kept under a polyethylene cover in order to avoid loss of active substance from rainfall.

The ripe grapes were collected 22 days after the treatment and extracted. The grapes, used for wine-making process test were also collected and crushed. The must was inoculated with yeast *Saccharomices cerevisiae* to promote the alcoholic fermentation and then analysed.

The metabolites GX1 and GX6 were identified by co-chromatography with the metabolites already identified in wine leaves. GX1 and GX6 were also hydrolysed with β-glucosidase and their aglycones were analysed by TLC.

GX5a, GX5b, and GX5c metabolites were found both in grapes and wine. GX5a and GX5b are diastereoisomers produced by oxidation of either one of benalaxyl's aromatic methyl groups.

GX1 metabolite is the main metabolite in both grapes and wine. Though TLC analysis shows GX1 as a single spot, it is actually formed by three compounds which are originated by GX5a, GX5b, and GX5c and glucose conjugation.

GX6 residues in both grapes and wine represent quantitatively the secondary metabolite. Under the hydrolysing action of β-glucosidase, it gives rise to GX5a and GX5b; the percentages of such hydrolysed products are reported in Table 9.

Table 9 Hydrolysis of ^{14}C-GX6 with β-glucosidase

	% ^{14}C referred to ^{14}C used	
Compound	From grapes	From wine
GX6	2.00	3.62
GX1	8.08	6.90
GX5a	73.86	75.06
GX5b	11.43	12.35
Others	4.63	2.07

GX6 comes from the diastereoisomer couples GX1a and GX1b glucosides which in turn are formed from the diastereoisomer couples GX5a and GX5b.

In conclusion, benalaxyl is first hydroxylated to GX5a, GX5b, and GX5c; these metabolites then conjugated with glucose giving rise to 3 GX1 glucosides. Subsequent conjugation with glucose transforms GX1 into GX6.

Absorption and Metabolism in Vine Leaves

Grape vine plants (cv. Cannaiolo) were used for the test, operating in a box under 1000 and 5000 lux lighting conditions, with a photo period of 16 hours. [^{14}C]benalaxyl (0.03%) (specific activity 2.74 µCi/mg) dissolved in a water+acetone+tween 20 (80+20+0.02) was sprayed on the vine leaves. Leaves were collected 1, 2, 3, 4, 7, 10, and 16 days after the treatment and were then analysed. The radioactivity was assayed by LSC, with the metabolites containing ^{14}C characterized by co-chromatography, the radio-labelled compounds were revealed by autoradiography, and quantified by measuring the radioactivity of the various areas of silica gel after scraping from the chromatoplates.

In both of the tests with 1000 and 5000 lux, the percentage of ^{14}C and of benalaxyl on the outside of the leaf falls rapidly.

The percentage of benalaxyl inside the leaf reaches the maximum after 7 and 2 days, respectively, in the tests performed at 1000 and 5000 lux, while the highest concentration (mg/kg) is found after 3 and 1 day, respectively.

Benalaxyl undergoes significant metabolism within the leaf, giving rise to numerous compounds containing ^{14}C. The primary metabolite, GX1, has been isolated from leaves and analysed by GLC and GLC/MS. The precursor is probably the metabolite GX5, which is present in lesser amounts. The benalaxyl-acid deriving from hydrolysis of benalaxyl's ester group has been identified among the various metabolites, but only in the samples taken 10 and 16 days after the treatment.

In conclusion, the study with ^{14}C has shown that benalaxyl rapidly penetrates into the vine leaves; once inside, it is metabolized forming mainly the glucoside GX1.

The behaviour of the residue was similar to what seen in grapes, but the metabolite GX1 was detected in higher concentration than the active ingredient (63% of the residue was GX1 and 20% was benalaxyl at 16 days after the treatment).

Distribution and metabolism in tomato fruits

For this study, tomato plants (grown in outdoor pots) were used. When most of the fruits were at the stage of darkening in colour, the plants were sprayed using [^{14}C]benalaxyl M formulation (benalaxyl 8% and mancozeb 65%) at the rate of 25g benalaxyl/hL. The fruits were picked 1, 7, 14, 21, 28, and 35 days after the treatment and washed.

The total residue on the outside of the fruit ranged from 0.79 mg/kg one day after last treatment to 0.096 mg/kg 35 days after last treatment; see Table 10.

Table 10 Distribution of the Total Radioactivity in tomato fruit

Days	Total (outside+inside) mg/kg	Outside					
		^{14}C		benalaxyl		Others	
		%	mg/kg	%	mg/kg	%	mg/kg
1	0.854	91.87	0.785	90.70	0.775	1.17	0.01
7	0.487	83.65	0.407	81.02	0.395	2.63	0.012
14	0.591	72.06	0.426	69.29	0.410	2.77	0.016
21	0.254	65.64	0.167	62.02	0.158	3.62	0.009
28	0.196	64.79	0.127	59.69	0.117	5.10	0.01
35	0.165	58.36	0.096	53.86	0.089	4.50	0.007

The percentage of radioactivity inside the fruits increases progressively (Table 10). In addition to monoglucosides GX1a and GX1b, the polar products GX4, GX6, GX11, GX12, and GX16 are also present.

When tested by chromatographic analysis, the metabolite GX6 extracted from tomatoes corresponds to the di-glucoside previously isolated and characterized in grape vine leaves. The metabolite GX11 when incubated at 80 °C for 24 hours, hydrolyses and produces the metabolite GX1. Moreover it releases a product having chromatographic characteristics corresponding to those of malonic acid. Also the metabolites GX12 and GX16 are quickly transformed into stable polar compounds; in particular GX16 is transformed in GX4; it can also be assumed that GX12 corresponds to glucosides presumably bound to malonic acid.

Table 11 Distribution and Identification of the Radioactivity in Tomato Fruit

Days	Inside															
	^{14}C		benalaxyl		GX4		GX6		GX11		GX12		GX16		Unextracted	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
1	8.13	0.069	6.57	0.056	n.d.	< 0.001	0.02	< 0.001	0.98	0.008	n.d.	< 0.001	0.04	< 0.001	0.23	0.002
7	16.35	0.080	6.34	0.031	0.06	< 0.001	0.17	0.001	7.00	0.034	0.41	0.002	0.55	0.003	0.75	0.004
14	27.94	0.165	10.68	0.063	0.24	0.001	0.20	0.001	8.46	0.050	1.03	0.006	2.42	0.015	1.73	0.01
21	34.36	0.087	5.55	0.014	0.47	0.001	0.43	0.001	14.09	0.036	2.48	0.006	4.45	0.011	2.28	0.006
28	35.21	0.069	7.52	0.015	0.46	0.001	0.24	0.001	14.63	0.029	2.62	0.005	3.42	0.007	2.37	0.004
35	41.64	0.069	6.44	0.011	0.85	0.001	0.37	0.001	14.11	0.023	4.44	0.007	6.50	0.011	3.28	0.005

The total radioactivity detected inside the fruit is under 0.2 mg/kg at all time points, corresponding mostly to conjugated products (i.e., glucosides and malonil-glucosides); the total concentration of unidentified products is under 0.02 mg/kg at all times. It appears that benalaxyl is oxidized on a methyl of the xylyl ring, resulting in a formation of the diastereoisomers GX5a and GX5b, which are subsequently conjugated with glucose forming GX1a and GX1b.

Potato Plant and Residues in the Tubers

Potato plants were grown in large pots and sprayed with 25 g as/hL of [^{14}C]benalaxyl dissolved in water-acetone-tween20 (80+20+0.02). After treatment and during the trial, the plants were placed outdoors to grow, protected by polyethylene covers. Tubers were collected 26 days after treatment.

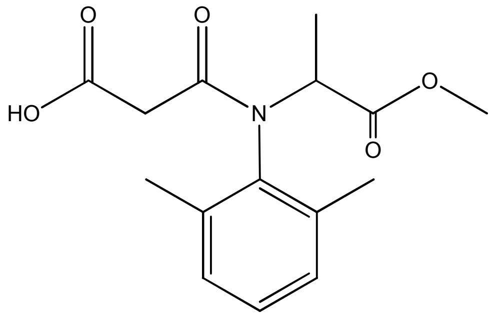
No significant radioactivity was found in tubers (Table 12) indicating that benalaxyl sprayed on the leaves of potato plants or present in the soil (due to dripping after spraying) does not transfer to the tubers either as active ingredient or as metabolites containing ^{14}C.

Table 12 Amount of radioactivity (mg/kg) in potato tubers

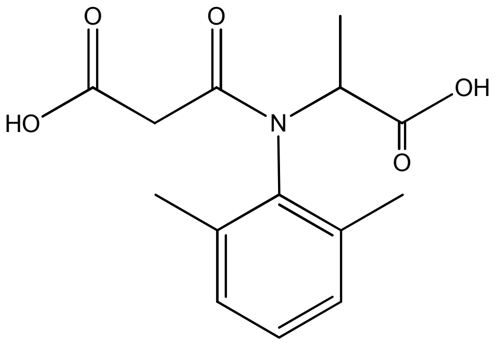
Days	^{14}C-total	Methanol	Methanol R.	Residue	Aqueous phase
5	0.002	0.002	< 0.001	< 0.001	< 0.001
12	0.004	0.003	0.001	< 0.001	< 0.001
19	0.005	0.005	< 0.001	< 0.001	< 0.001
26	0.002	0.002	< 0.001	< 0.001	< 0.001

ENVIRONMENTAL FATE IN THE SOIL

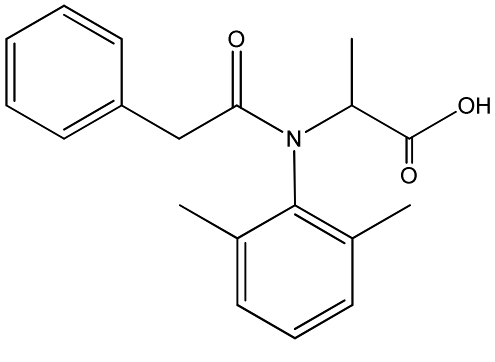
The Meeting received information on aerobic degradation of benalaxyl in soil and field dissipation studies performed in Italy and Germany. Figure 3 presents the structures and identification labels used for compounds found in the soil studies.



Compound A: methyl-N-(2,6-xylyl)-N-malonyl alaninate



Compound B: N-(2,6-xylyl)-N-malonyl alanine



Benalaxyl acid (Galben-acid): N-(2,6-xylyl)-N-(phenylacetyl) alanine

Figure 3 Metabolites identified in the soil studies.

Aerobic degradation

The aerobic degradation of [^{14}C-α position of the ester moiety] benalaxyl was investigated in sieved silt loam soil (017, Santi *et al.*, 1982a). The soil had 2.3% of organic content and a pH of 6.75. [^{14}C]benalaxyl was applied at a rate of 50 mg ai/kg. Sieved soil samples (100 g) were placed in 300 mL Erlenmeyer flasks and moistened to 40% of water-holding capacity (WHC). The flasks, stoppered with hydrophobic cotton, were incubated under aerobic conditions at 22 ± 2 °C in the dark.

Soil samples were collected 7, 14, 28, 56, 77, 98 and 133 days after treatment. Soil extracts were analysed by one dimensional TLC, co-chromatographed with standards and by GLC or NMR. Unextractables were combusted in a sample oxidizer. Radioanalysis was carried out by LSC and autoradiography.

Benalaxyl degraded very slowly in the first 28 days after treatment suggesting a lag phase followed by a steady degradation until the end of the incubation period (133 days after treatment). By this time the radioactivity associated with benalaxyl represented only 11.7% of the applied radioactivity (AR). The DT_{50} of benalaxyl taking into account the lag period was estimated to be 77 days. Excluding the initial lag phase of 28 days acclimation/adaptation period, during which very little benalaxyl degradation occurred, a shorter DT_{50} of 42 days was estimated by a linear/log regression analysis.

Table 13 shows distribution of radioactivity and metabolites at different sampling times. Two main degradation products were identified in soil extracts. Compound A, identified as methyl-N-malonyl-N-(2,6-xylyl)alaninate and Compound B, identified as N-malonyl-N-(2,6-xylyl)alanine with maximum soil concentrations after treatment at 133 days (31% AR) and 98 days (34.1% AR), respectively. Benalaxyl acid, identified as phenylacetyl-N-2,6-xylyl-DL-alanine was found with maximum soil concentration at 28 days (4.9% AR). In the first period (1-28 day), this is the only metabolite present in soil. Then, from day 56 to 133, the other two metabolites (compound A and compound B) are detectable. Bound residues increased to 18.8% AR at the end of the study, mostly found in the acid wash and fulvic acid fractions. The detected compounds, including benalaxyl were found associated with bound residues in soil.

Throughout the study, radioactivity recovery from soil was always higher than 93%, ranging between 93.7–100.8% AR. This indicates that no relevant evolution of $^{14}CO_2$ and of volatile labelled compounds occurred.

Table 13 Radioactivity distribution in an aerobic degradation study of benalaxyl in soil

Time (days)	% Applied radioactivity						
	Compound A	Compound B	Benalaxyl acid	Methanol extract	Bound residues	Benalaxyl	total
7	< 0.1	< 0.1	0.8	-	-	97	99
14	< 0.1	< 0.1	2.3	-	-	96	100
28	< 0.1	< 0.1	4.9	-	-	94	101
56	6.6	6.3	2.7	0.2	11	73	99
77	16	16	1.8	0.2	13	48	96
98	24	34	1.1	0.2	16	20	95
133	31	31	1.0	0.2	19	12	94

The structures of the ^{14}C-metabolites identified show that benalaxyl in soil undergoes an intense oxidative as well as hydrolytic process. The proposed degradation pathway of benalaxyl in soil under aerobic conditions is shown in Figure 4.

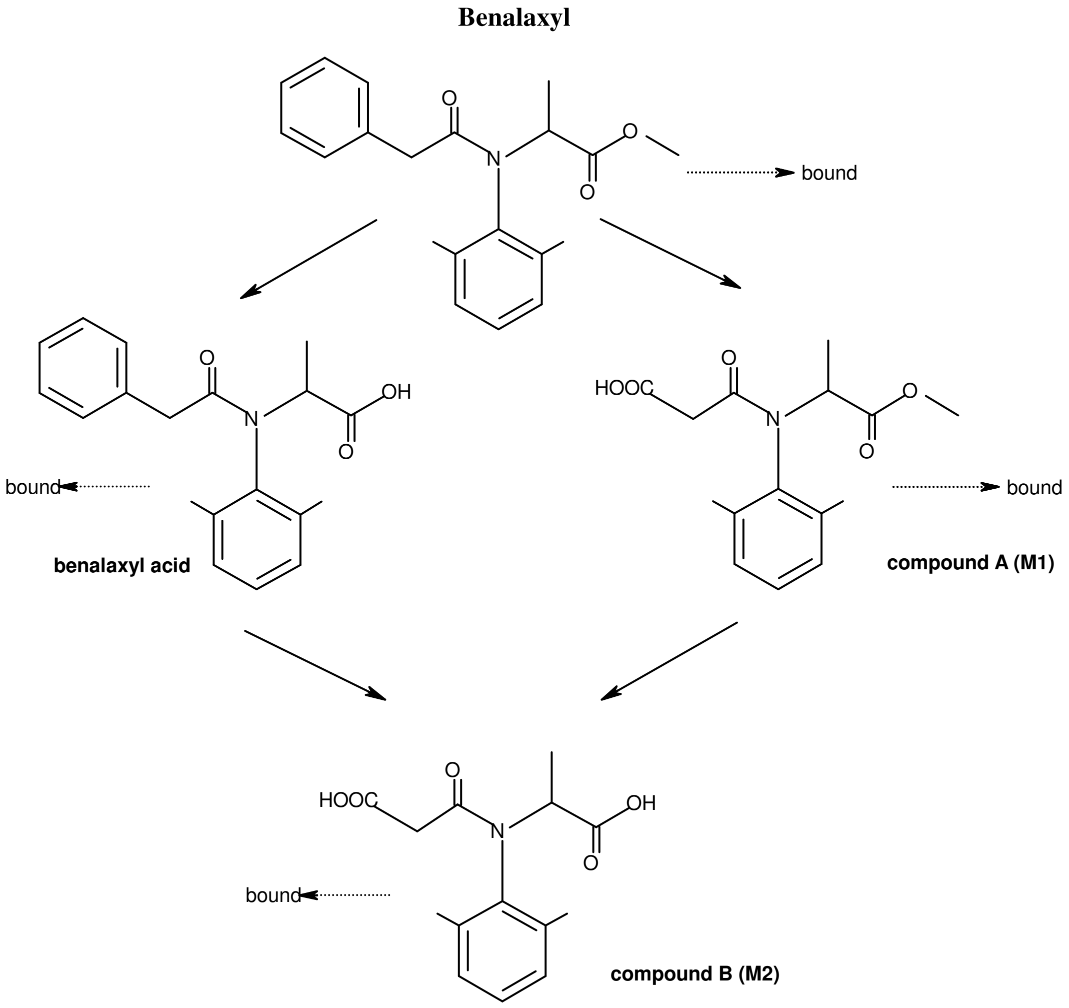


Figure 4 Proposed metabolic pathway of benalaxyl in soil under aerobic conditions

The rate of benalaxyl degradation under aerobic conditions was further studied in four Italian farmland soils (019, Masoero *et al.*, 1981a): Locate Triulzi (loam/sandy loam, pH 5.6, 1.7% organic carbon), Linate (loam, pH 6.7, 1.3% organic carbon), Cantonazzo (clay loam, pH 7.3, 1.7% organic carbon) and Badia Polesine (sandy loam, pH 6.9, 2.2% organic carbon).

Benalaxyl was applied at rates of 5 or 50 mg ai/kg soil (dry weight) to previously sieved fresh soil samples (equivalent to 100 g dry weight) moistened to 40% or 69% WHC. Incubation was performed under aerobic conditions at 22 ± 2 °C in the dark into 300 mL glass Erlenmeyer flasks, closed with a cotton-wool plug. Sterilized soil samples were also tested. Soil extracts were analysed by HPLC with a UV detector at 254 nm.

In order to show that the degradation rate may depend on microbial growth and adaptation mechanism, one soil was previously treated with benalaxyl, then allowed to degrade before benalaxyl was applied again. The tested incubation conditions, sampling intervals and DT_{50} values are shown in Table 14.

Table 14 Degradation rates at different incubation conditions in the Italian soils tested

Soil	Incubation conditions	Sampling dates (DAT)	DT_{50} (days)
Locate Triulzi (loam/sandy	50 mg ai/kg soil 40% WHC	0,7,14,28,56,70,105,143	100

Soil	Incubation conditions	Sampling dates (DAT)	DT_{50} (days)
loam)	5 mg ai/kg soil 40% WHC	0,7,14,28,56,70,105,143	82
	5 mg ai/kg soil 69% WHC	0,56,68 or 0,7,18,29,56,68	56
	5 mg ai/kg soil 69% WHC sterilized soil	0,84,112,212	> 300
Linate (loam)	50 mg ai/kg soil 40% WHC	0,7,14,27,56,79,91,139	77
	5 mg ai/kg soil 40% WHC	0,21,56,73,98	37
Cantonazzo (clay loam)	50 mg ai/kg soil 40% WHC	0,4,14,28,56,81,116,158	87
	5 mg ai/kg soil 40% WHC	0,21,56,73,98	36
	5 mg ai/kg soil 40% WHC soil previously treated with benalaxyl at the same rate (initial concentration was restored after 108 days of incubation)	0*, 25*, 54* *:+ 108 days	21
Badia Polesine (sandy loam)	50 mg ai/kg soil 40% WHC	0,7,14,28,56,81,116,158	88
	5 mg ai/kg soil 40% WHC	0,6,17,28,56,67,108,143	85

Benalaxyl declined in soil following a two step first order kinetics suggesting an initial lag phase of about 60 to 80 days. At the application rate of 50 mg ai/kg soil, DT_{50} values ranged between 77 and 100 days. At 5 mg ai/kg soil, DT_{50} values ranged from 36 to 85 days.

In sterilized soil, degradation was much slower with a DT_{50} greater than 300 days. In the soil previously treated with 5 mg ai/kg soil, degradation after the second application was faster suggesting microbial adaptation. It was concluded that the degradation rate of benalaxyl in the soil essentially depends on the presence of micro-organisms.

In laboratory-based aerobic soil degradation of benalaxyl, two major metabolites, methyl-N-malonyl-N-(2,6-xylyl)alaninate and N-malonyl-N-(2,6-xylyl)alanine, have been found at levels higher than 10% AR. Aerobic soil degradation of these compounds was studied in three German standard soils (020, Scacchi and Pizzingrilli, 1997a and 021, Scacchi and Pizzingrilli, 1997b).

The degradation of [^{14}C]-methyl-N-malonyl-N-(2,6-xylyl)alaninate was investigated in three fully characterized German standard soils (020, Scacchi and Pizzingrilli, 1997a): Speyer 2.1 (sand), Speyer 2.2 (loamy sand) and Speyer 2.3 (sandy loam). The soil characteristics are summarized in Table 15. The soil was treated at the rate of 0.199 mg ai/kg.

Table 15 Characteristics of Speyer 2.1, 2.2 and 2.3 soils

Soil		SP-2.1	SP-2.2	SP-2.3
Particle size distribution	0.05-2 mm (sand)	90	80	63
	0.002-0.05 mm (silt)	8.0	14	27
	< 0.002 mm (clay)	2.5	6.6	9.5
USDA classification		sand	loamy sand	sandy loam
pH (0.01 M $CaCl_2$)		6.0	6.1	6.6
Organic Carbon (%)		0.59	2.3	1.2
Cation Exchange Capacity (meq/100 g)		4.0	9.0	9.0
Microbial biomass C (mg C/100g dry weight)	(initial value)	20	57	28
	(final value)	20	50	15
WHC (gH_20/100g)		29	48	37

Soil samples (equivalent to 50 g dry weight) were placed into 250 mL glass Erlenmeyer flasks and mixed to a total amount of water corresponding to 40% WHC. Flasks were sealed with a glass stopper, incubated in the dark at 20 °C and fluxed with moistened carbon dioxide-free air that was passed in turn through a KOH trap to collect evolved CO_2.

Soil samples, collected 0, 10, 20, 40, 60, 80 and 100 days after treatment, were extracted with different solvent mixtures (acetone-water, acetone-HCl) and the extractable radioactivity was determined by LSC. Unextracted radioactivity in soil was quantified by LSC after combustion. Soil extracts were analysed by TLC. Identification of metabolites was performed by co-TLC with reference standard compounds. Evolved $^{14}CO_2$ contained in CO_2 traps was quantified by LSC. DT_{50} and DT_{90} values were estimated by regression analysis.

Unchanged [^{14}C]-methyl-N-malonyl-N-(2,6-xylyl)alaninate decreased in soil to 26%, 45% and 49% at Day 100 for SP-2.1, SP-2.2 and SP-2.3 soils, respectively. DT_{50} and DT_{90} values (calculated according to a first order kinetics of degradation) were 49 and 163, 86 and 285, 91 and 301 days for SP-2.1, SP-2.2 and SP-2.3 soils, respectively (Table 16).

Table 16 DT_{50} and DT_{90} values for [^{14}C]-methyl-N-malonyl-N-(2,6-xylyl)alaninate in the tested German soils

Soil	SP-2.1	SP-2.2	SP-2.3
DT_{50} (days)	49	86	91
Order	first	first	First
r^2	0.994	0.990	0.982
DT_{90} (days)	163	285	301

At least four degradation compounds of [^{14}C]-methyl-N-malonyl-N-(2,6-xylyl)alaninate were found in all tested soils. [^{14}C]-N-malonyl-N-(2,6-xylyl) alanine, which increased to 11.7–22.7% AR at Day 100, was the major degradation compound found. The others were not identified since none of them represented individually more than 6.3% AR during the study period.

The extracted radioactivity ranged from 101%, 101% and 103% on Day 0 to 58%, 64% and 68% on Day 100, while the unextracted radioactivity (soil residue) increased with time from 0.19%, 0.28% and 0.22% on Day 0 to 14%, 17% and 13% on Day 100 for soil SP-2.1, SP-2.2 and SP-2.3, respectively. $^{14}CO_2$ reached 25%, 15% and 16% AR at Day 100, for the three soils respectively. Mass balance was always higher than 95%.

Similarly to [^{14}C]-methyl-N-malonyl-N-(2,6-xylyl)alaninate, the degradation of [^{14}C]-N-malonyl-N-(2,6-xylyl)alanine was also investigated in three fully characterized German standard soils SP-2.1, SP-2.2 and SP-2.3 (021, Scacchi and Pizzingrilli, 1997b), which were treated at the rate of 0.190 mg ai/kg. The experimental conditions, sampling intervals and analysis were the same as described for the [^{14}C]-methyl-N-malonyl-N-(2,6-xylyl)alaninate degradation study.

Unchanged [^{14}C]-N-malonyl-N-(2,6-xylyl)alanine decreased in soil to 35%, 50% and 54% at Day 100 for SP-2.1, SP-2.2 and SP-2.3 soils, respectively. DT_{50} and DT_{90} values (calculated according to a first order kinetics of degradation) were 66 and 219, 106 and 351, 113 and 374 days for SP-2.1, SP-2.2 and SP-2.3 soils, respectively (Table 17).

Table 17 DT_{50} and DT_{90} values for [^{14}C]-N-malonyl-N-(2,6-xylyl)alanine in the tested German soils

Soil	SP-2.1	SP-2.2	SP-2.3
DT_{50} (days)	66	106	113
Order	first	First	First
r^2	0.977	0.955	0.922
DT_{90} (days)	219	351	374

No major degradation compounds were found in any of the soils tested. The main degradation product was represented by the volatile $^{14}CO_2$, which reached the maximum levels at Day 100 of 26%, 25% and 19% AR for SP-2.1, SP-2.2 and SP-2.3 soils, respectively.

The extracted radioactivity ranged from 100%, 101% and 98% on Day 0 to 56%, 63% and 67% on Day 100, while the unextracted radioactivity increased with time from 0.60%, 2.5% and 2.1% on Day 0 to 16%, 10% and 13% on Day 100 for SP-2.1, SP-2.2 and SP-2.3 soils, respectively. Mass balance was always higher than 95%.

Rotational Crops

The Meeting received confined rotational crop studies using radiolabelled benalaxyl showing very low levels of residues in the following crops (lettuce, tomato, carrot, and wheat) even after application at highly exaggerated rates (~10×). Based on the behaviour of benalaxyl in soil and the findings in the confined rotational crop studies, it is highly unlikely that residues above the limit of quantitation would occur in succeeding crops. A brief description of the studies and their results follows.

Lettuce

[^{14}C-α position of ester moiety] benalaxyl (≥ 98% radiochemical purity; 0.6 µCi/mg specific activity), formulated as an emulsifiable concentrate (240 g as/L) was applied to 12 concrete pots (0.45 m × 0.25 m × 0.20 m) filled with a silt loam soil at a rate of 2.25 kg as/ha (101, Guarnieri & Valcamonica, 1984). Thirty day old lettuce seedlings were transplanted to the pots after soil ageing under natural conditions for 30, 120 days and 1 year.

Transplanted lettuce were analysed at different times for the determination of residues absorbed by plant uptake. The residue profiles show no association between the ageing time of benalaxyl and translocation of residues from soil to lettuce. The highest residue levels were detected in the first 15–20 days after transplantation but in other samples of lettuce, the extractable (0.02–0.11 mg/kg) and non-extractable (< 0.001–0.01 mg/kg) residues show no accumulation and low translocation of benalaxyl into lettuce grown as a rotational crop.

Tomato

This study is similar to the previous study in all aspects except that 35 day old tomato seedlings were transplanted to soil pots after soil ageing under natural conditions for 30, 120 days and 1 year (102, Guarnieri & Valcamonica, 1984). The results showed no accumulation and very little translocation of benalaxyl from soil to tomato plants. The amounts detected were 0.02-0.04 mg/kg benalaxyl equivalents after a 33 day plant back period, 0.02–0.10 mg/kg benalaxyl equivalents after a 111 day plant back period, and 0.01–0.02 mg/kg benalaxyl equivalents after 363 day plant back period.

Carrot

This study is similar to the previous study in all aspects except that carrots were sown directly in the soil pots after soil ageing under natural conditions for 30, 120 days and 1 year (103, Guarnieri & Valcamonica, 1985). In the tap-roots of all samples, which is the marketable part, the residues were are 0.02–0.06 mg/kg in carrots harvested 70–80 days after sowing (30 day plant back interval) and 0.01–0.02 mg/kg in carrots harvested 112–115 days after sowing (120 day plant back interval).

Wheat

This study is similar to the previous study in all aspects except that wheat seeds were sown in soil pots after soil ageing under natural conditions for 30, 120 and 295 days (104, Guarnieri & Valcamonica, 1985). At the 30 day plant back interval, wheat grain samples harvested 272 days after soil treatment were reported to contain < 0.16 mg/kg benalaxyl equivalents, while wheat straw samples were found to have < 0.24 mg/kg benalaxyl equivalents. The same concentrations of benalaxyl residues were reported at the 120 and 295 day plant back intervals as well.

Photolysis

The Meeting received photolysis studies of benalaxyl in water. [^{14}C]benalaxyl (≥ 98% radiochemical purity; 100 kBq/mg specific activity) was irradiated under natural sunlight conditions in a distilled sterilized buffer solution at pH 7 and test concentration of 10 mg ai/L for up to 64 days (099, Guarnieri & Valcamonica, 1984). After 64 days, 60%AR was still present as benalaxyl. At least 15 different compounds were recorded but none of them represented individually more than 5.0% of the applied radioactivity and therefore were not identified. No degradation of benalaxyl was observed under dark conditions. The study was conducted during June – August, 1984 in Milan, Italy.

In a separate experiment, the degree of photolytic degradation and the quantum yield of benalaxyl were determined by irradiation with xenon light at 306 ± 12 nm at 20 °C (100, Rudel, 1996). The absorption coefficients of benalaxyl in the relevant wavelength range around 300 nm were very low (approximately 5–10 mol L^{-1} cm^{-1}). Just 2% benalaxyl degradation was found after 5 days and 3% degradation after 10 days. Degradation products could not be detected. The quantum yields of the photodegradation as estimated from the 5 days and 10 days-irradiations were both 0.01. Thus, benalaxyl may be considered a photolytically stable compound.

Field dissipation studies

The Meeting received results of field dissipation studies conducted in Italy and Germany.

In one study conducted in Italy (029, Masoero *et al*., 1981b), benalaxyl was applied once as a 250 WP formulation at the rate of 3.3 kg ai/ha to a bare soil plot of 3 m^2 and incorporated manually into the top 7.5 cm immediately after the treatment. The soil (loam, pH 6.75, 2.3% of organic content, 36% WHC) had no history of applications with benalaxyl or analogous products. The soil was maintained uncultivated during the study. Soil sampling was performed at 0, 10, 20, 28, 36, 50, 63 and 112 days after treatment. Cylinder cores of soil (depth more than 30 cm) were taken and then divided into three parts: surface (0–10 cm), medium (10–20) and deep layer (20–30 cm). Extraction of benalaxyl was performed using a methanol-water mixture followed by purification by preparative TLC. Analysis was performed by HPLC (LOQ of 0.2 mg/kg).

Benalaxyl remained confined to the upper 10 cm layer of soil; it was not detected in the two lower layers (below 10 cm) during the study. The dissipation of benalaxyl in soil followed 1st order kinetics, with an estimated DT_{50} of 49 days. In Table 18, benalaxyl residues in soil at different sampling times and soil depths are summarized expressed as % of initial concentration.

Table 18 Field dissipation of benalaxyl in a study conducted in Italy

Sampling time	% of initial concentration in the upper layer		
	Upper soil layer (0-10 cm)	Middle soil layer (10-20 cm)	Deeper soil layer (20-30 cm)
0	100	n.d.	n.d.
10	84	n.d.	n.d.
20	75	n.d.	n.d.
28	58	n.d.	n.d.
36	61	n.d.	n.d.
50	49	n.d.	n.d.
63	13	n.d.	n.d.
112	< 9.8	n.d.	n.d.

n.d. = not detected

Field dissipation trials in Germany were conducted at four sites: Bad Oldesloe; Moorfleet; Klein Offenseth and Verliehausen with different soil properties (Table 19) and somewhat different climate (030, Jonas, 1993a and 031, Bieber, 1993). At each location, a trial area of 25 m^2 was treated once with benalaxyl at the rate of 241 g ai/ha. The product was applied to the bare soil, which was kept free from plants during the study period. At all locations, soil samples were collected 0, 1, 4, 8, 12, 18 and 24 weeks after the treatment. Three further samplings were done after 49, 57 and 66 weeks at the locations Klein Offenseth and Verliehausen, at which benalaxyl residues still exceeded 10% of the initial amount after 24 weeks of the treatment. The samples were divided into two layers: 0–10 cm and 10–20 cm at each sampling time. Each sample was analysed for benalaxyl and the two main soil metabolites: methyl-N-malonyl-N-(2,6-xylyl)alaninate (compound A) and N-malonyl-N-(2,6-xylyl)alanine (compound B).

Table 19 Characteristics of field soils used in the German field dissipation studies

Location	Bad Oldesloe	Moorfleet Hamburg	Klein-Offenseth	Verliehausen
Particle size distribution (%)				
< 0.020 mm	28	72	12	30
0.020 - 0.060 mm	17	11	9.2	39
0.060 - 0.200 mm	26	6.5	32	16
0.200 - 0.600 mm	21	7.4	41	13
0.600 - 2.000 mm	8.6	3.5	6.0	2.6
pH value (0.01 M $CaCl_2$)	6.8	6.7	4.4	5.3
Organic carbon (%)	3.1	3.5	3.8	1.4
WHC (%)	53	62	51	51
Microbial biomass (mg C/100 g soil)				
Initial	41	36	18	32
After 24 weeks	50	60	21	9
After 66 weeks	-	-	27	40

Table 20 provides a summary of DT_{50} and DT_{90} values obtained in the field dissipation studies at the 4 different locations in Germany. More details are provided below.

Table 20 Summary of degradation parameters for benalaxyl obtained in the field dissipation studies at the 4 different locations in Germany

Location	Bad Oldesloe	Moorfleet Hamburg	Klein-Offenseth	Verliehausen
DT_{50} (Conf. Interval.)	20 (14-27)	25 (18-33)	98 (82-115)	71 (61-80)
DT_{90} (Conf. Interval.)	67 (46-88)	84 (60-109)	326 (271-380)	235 (204-266)
Kinetics	first order	first order	first order	first order

At the Bad Oldesloe location (Table 21), benalaxyl degraded rapidly in soil from mean levels of 0.130 mg/kg in the 0–10 cm soil layer on day 0 to 0.009 mg/kg soil on day 87. In the 10–20 cm soil layer, levels not higher than 0.005 mg/kg were found. Compound A and compound B were detected in both soil layers up to day 87. Compound A was the most important with a maximum residue level of 0.041 mg/kg on day 87 in the 0–10 cm soil layer. The residues were not determined in the 129 day samples because the benalaxyl residue was < 10% of the initial concentration at the previous sampling (on day 87). For benalaxyl, a first order DT_{50} of 20 days and DT_{90} of 67 days were estimated (Table 20).

Table 21 Residues of benalaxyl and its metabolites (in mg/kg) in Bad Oldesloe soil

Days	Benalaxyl		Compound A		Compound B	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
0	0.130	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
7	0.152	0.005	< 0.001	0.003	< 0.001	< 0.001

Days	Benalaxyl		Compound A		Compound B	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
28	0.074	0.001	0.003	< 0.001	< 0.001	< 0.001
59	0.017	0.005	0.032	0.006	0.006	< 0.001
87	0.009	0.003	0.041	< 0.001	0.009	0.003
129	ND	ND	ND	ND	ND	ND

ND = not determined because benalaxyl residues were <10% of the initial content on Day 87

At the Moorfleet location (Table 22), benalaxyl degraded rapidly in soil from mean levels of 0.147 mg/kg in the 0–10 cm soil layer on day 0 to 0.004 mg/kg soil on day 126. In the 10–20 cm soil layer, levels not higher than 0.005 mg as/kg soil were found. Compound A was detected in the 0–10 cm soil layer with maximum of 0.041 mg/kg soil found on day 56 and 84 and it was still present at the last sampling time (day 126) at a level of 0.021 mg/kg soil. Compound B was only detected in the upper soil layer on sampling days 56 to 126 with maximum levels of 0.006 mg/kg soil on day 56 and 84. In the lower soil layer, compound A was found with maximum of 0.008 mg/kg soil on day 7, whereas compound B was never found at detectable levels in the 10–20 cm layer. For benalaxyl, a first order DT_{50} of 25 days and DT_{90} of 84 days were estimated (Table 20).

Table 22 Residues of benalaxyl and its metabolites (in mg/kg) in Moorfleet soil

Days	Benalaxyl		Compound A		Compound B	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
0	0.147	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
7	0.077	0.002	< 0.001	0.008	< 0.001	< 0.001
29	0.079	0.003	0.005	0.002	< 0.001	< 0.001
56	0.042	0.003	0.041	0.006	0.006	< 0.001
84	0.014	0.005	0.041	0.006	0.006	< 0.001
126	0.004	0.001	0.021	0.003	0.003	< 0.001

At the Klein Offenseth location (Table 23), benalaxyl decreased from mean levels of 0.133 mg/kg in the 0–10 cm soil layer on day 0 to 0.005 mg/kg soil on day 456. In the 10–20 cm soil layer, maximum levels of 0.010 mg/kg soil were found on day 56. Compound A and compound B were found sporadically in the soil, with maximum levels found in the upper soil layer of 0.012 and 0.006 mg/kg soil, respectively, on days 84 and 126. For benalaxyl, a first order DT_{50} of 98 days and DT_{90} of 326 days were estimated (Table 20).

Table 23 Residues of benalaxyl and its metabolites (in mg/kg) in Klein Offenseth soil

Days	Benalaxyl		Compound A		Compound B	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
0	0.133	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
7	0.077	0.004	0.002	< 0.001	< 0.001	< 0.001
29	0.077	0.004	< 0.001	< 0.001	< 0.001	< 0.001
56	0.055	0.010	0.001	< 0.001	< 0.001	< 0.001
84	0.077 0.055	0.002 0.003	0.012	< 0.001	0.006	< 0.001
126	0.090 0.051	0.003	0.012	< 0.001	0.006	< 0.001
168	0.032	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
340	0.008	0.001	0.003	0.003	< 0.001	0.003
392	0.007	0.001	< 0.001	0.003	< 0.001	< 0.001
456	0.005	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

At the Verliehausen location (Table 24), benalaxyl decreased from mean levels of 0.094 mg/kg in the 0–10 cm soil layer on day 0 to 0.001 mg/kg soil on day 461. In the lower soil layer, maximum benalaxyl levels of 0.015 mg/kg soil were found on day 7. Undetectable residues are found from day 168 on. Compound A and compound B are found sporadically with maximum levels of 0.006 and 0.019 mg/kg soil, respectively, on day 342 in the 0–10 cm and 10–20 cm soil layers. For benalaxyl, a first order DT_{50} of 71 days and DT_{90} of 235 days were estimated (Table 20).

Table 24 Residues of benalaxyl and its metabolites (in mg/kg) in Verliehausen soil

Days	Benalaxyl		Compound A		Compound B	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
0	0.094 0.073	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
7	0.078 0.062	0.015	< 0.001	< 0.001	< 0.001	< 0.001
28	0.106 0.086	0.012	< 0.001	< 0.001	< 0.001	< 0.001
56	0.088	0.001	< 0.001	< 0.001	< 0.001	< 0.001
85	0.042	0.003	< 0.001	< 0.001	< 0.001	< 0.001
126	0.040	0.005	< 0.001	< 0.001	< 0.001	< 0.001
168	0.025	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
342	0.006	< 0.001	0.006	0.006	0.019 [a]	0.019 [a]
398	0.002	< 0.001	< 0.001	< 0.001	< 0.001	0.003
461	0.001	< 0.001	0.003	0.003	0.006 [a]	0.006 [a]

[a] values not consistent with the general trend of the decline curve

RESIDUE ANALYSIS

Analytical methods

The Meeting received information on a single-residue analytical method for benalaxyl in samples of plant and animal origin (033, Zini, 1999b).

For fruit and vegetable commodities, homogenized samples (30 g) are extracted with acetone (100 mL). The extract is filtered, acetone is removed by evaporation, and the aqueous residue is placed in a separatory funnel. Water (80 mL), dichloromethane (100 mL) and saturated sodium chloride solution (25 mL) are added. The mixture is shaken for 5 min. After phase separation, the organic layer is filtered through a layer of anhydrous sodium sulphate and evaporated to dryness. For clean-up, the residue after evaporation is redissolved in 2 mL of *n*-hexane-acetone (1:1, v/v) and cleaned up on an alumina column. The elution fraction containing benalaxyl is evaporated to dryness, redissolved in ethyl acetate, and analysed by GC-NPD.

For animal tissues, homogenized samples (30 g) are extracted with acetone (100 mL). In the case of fat, Cellite (10 g) is added to the samples to facilitate the extraction, the extract is filtered and evaporated to dryness, followed by the same clean-up and determination procedure as described above. For other tissues, the extracts are filtered, and acetone is removed by evaporation. The aqueous residue is placed in a separatory funnel and re-extracted with dichloromethane with addition of saturated sodium chloride solution. After phase separation, the organic layer is filtered through a layer of anhydrous sodium sulphate, evaporated to dryness, redissolved in *n*-hexane, and re-extracted with acetonitrile. The acetonitrile layer is evaporated to dryness, followed by the same clean-up and determination procedure as described above for fruits and vegetables.

For eggs and milk, homogenized samples (20 g for eggs, 25 g for milk) are added on the top of an Extrelut column and let to be absorbed by the column, which is then eluted by of *n*-hexane (75 mL). The eluant is evaporated to dryness, followed by the same clean-up and determination procedure as described above for fruits and vegetables.

The method was validated for grape, lettuce, bovine milk, bovine meat and poultry eggs with LOQ of 0.02 mg/kg. The recoveries for plant and animal matrices were in the range of 81–102% and 73–110%, respectively, with RSDs < 10% (Table 25). An independent laboratory validation was also conducted with similar results. The method was used in the supervised trials on plant commodities evaluated by this Meeting with concurrent recoveries within the range of 80–120% and RSD < 10% (Table 26).

Table 25 Validation data for benalaxyl analysis in selected plant and animal commodities

Matrix	Fortification level (mg/kg)	n	Recovery (%)		RSD (%)
			Mean	Range	
Grape (bunches)	0.019	5	91	82 – 99	7.8
	0.22	5	90	81 – 96	6.0
Lettuce	0.016	3	97	94 – 102	4.3
	0.16	3	100	98 – 100	1.3
Milk (bovine)	0.017	5	102	97 – 105	3.7
	0.22	5	97	91 – 103	4.3
Meat (bovine)	0.019	5	87	73 – 98	12
	0.22	5	99	88 – 110	8.7
Poultry eggs	0.019	5	84	77 – 89	5.7
	0.19	5	83	77 – 89	6.1

Table 26 Concurrent recoveries obtained for benalaxyl in plant commodities evaluated by this Meeting

Matrix	Fortification levels (mg/kg)	n	Recovery (%)		RSD (%)	Ref.
			Mean	Range		
Grape (bunches)	0.017 - 1.0	5	104	99 - 112	5.0	043
Onion	0.017 - 1.0	7	98	87 - 108	7.9	056
Melon pulp	0.066 - 1.7	9	96	92 - 103	3.4	064
Melon peel	0.066 - 1.7	9	99	92 - 105	4.0	064
Tomato	0.019 - 1.1	5	100	96 - 106	4.9	073
Lettuce	0.017 - 3.3	8	101	94 - 110	5.5	084
Potato	0.019 - 1.2	6	104	99 - 108	3.9	092

Stability of pesticide residues in stored analytical samples

The Meeting received information on the stability of benalaxyl in freezer-stored samples of grapes (039, Zini, 1996), grape must and marc (040, Zini, 1998), potato (041, Zini, 1996) and tomato (042, Zini, 1996). The samples were fortified at different concentration levels and stored at -20 °C for up to 3 years (1096–1110 days). Two replicates were analysed at each sampling interval using the method reported by Zini (033, Zini, 1999b). The concurrent recoveries were in the range of 98–100%, with RSDs of 4.0–6.4%. The results are summarized in Tables 27–30, showing that benalaxyl was stable under freezer-storage conditions during the tested storage interval of 3 years.

Table 27 Stability of benalaxyl residues in grape bunches stored at -20 °C

Freezer storage period (days)	Residue remaining (%) at fortification levels:	
	0.75 mg/kg	0.075 mg/kg
0	100	100
33	100	102
70	110	109
118	98	110
186	102	106
368	95	97
732	99	96
1096	97	106

Table 28 Stability of benalaxyl residues in grape must and marc stored at -20 °C

Freezer storage period (days)	Residue remaining (%) at fortification levels:			
	Must		Marc	
	1.2 mg/kg	0.02 mg/kg	1.0 mg/kg	0.12 mg/kg
0	92	89	102	109
30	99	95	95	107
62	107	96	92	106
91	98	105	106	97
183	97	96	95	103
365	98	93	106	101
741	92	89	92	88
1098	100	99	95	99

Table 29 Stability of benalaxyl residues in potato tubers stored at -20 °C

Freezer storage period (days)	Residue remaining (%) at fortification levels:	
	0.67 mg/kg	0.067 mg/kg
0	102	97
32	102	96
61	110	102
94	100	108
202	97	105
356	96	100
732	97	102
1096	99	97

Table 30 Stability of benalaxyl residues in tomato stored at -20°C

Freezer storage period (days)	Residue remaining (%) at fortification levels:	
	0.67 mg/kg	0.067 mg/kg
0	104	94
30	100	99
62	101	102
90	100	97
179	102	103
370	98	104
732	100	105
1110	102	99

USE PATTERN

Benalaxyl is applied primarily as a foliar spray of a WP formulation (SC formulation is applied to grapes in France) and has registered uses in or on a variety of plant commodities. The GAPs are summarized by commodity and country in Table 31.

Table 31 List of Registered uses

CROP	COUNTRY	FORMULATION TYPE	APPLICATION				PHI days
			METHOD	RATE (kg ai/ha)	SPRAY CONC. (kg ai/hL)	Max Number	
Grapes	France	WP	Spray	0.20		4	28
	France	SC	Spray	0.15		4	28
	Brazil	WP	Spray	0.24		4	7

CROP	COUNTRY	FORMULATION TYPE	APPLICATION				PHI days
			METHOD	RATE (kg ai/ha)	SPRAY CONC. (kg ai/hL)	Max Number	
	Italy	WP	Spray	0.20		4	20
Lettuce	Italy	WP	Spray	0.24		3	15
	Spain	WP	Spray	0.20		2	15
Melon	Italy	WP	Spray	0.20		3	7
	Italy	WP	Spray	0.24		2	10
	Spain	WP	Spray	0.20		3	7
Onions	Brazil	WP	Spray	0.24		4	7
	France	WP	Spray	0.20		3	28
	Cyprus	WP	Spray	0.20		3	14
	Italy	WP	Spray	0.20		3	20
	Spain	WP	Spray	0.20		3	15
Potatoes	Brazil	WP	Spray	0.24		2	7
	France	WP	Spray	0.24		4	7
	Italy	WP	Spray	0.24		4	7
Tomatoes	Brazil	WP	Spray	0.24		4	7
	France	WP	Spray	0.24		4	14
	Italy	WP	Spray	0.24		4	7
	Spain	WP	Spray	0.24		4	3
Watermelon	Italy	WP	Spray	0.20		2	7
	Spain	WP	Spray	0.24		3	7

RESIDUES RESULTING FROM SUPERVISED TRIALS

The Meeting received supervised trial data for benalaxyl uses on grapes, lettuce, melons and water melons, onion, potato and tomato. In most trials, benalaxyl was applied as a foliar spray using WP (wettable powder) formulations that also contained mancozeb, chlorothalonil or copper.

The results of the trials are summarized in the following tables:

Commodity	Crop group	Table
Grapes		32
Lettuce	Leafy vegetables	33
Melons	Fruiting vegetables, Cucurbits	34
Watermelons		35
Onions	Bulb vegetables	36
Potato	Root and tuber vegetables	37
Tomato	Fruiting vegetables, other than Cucurbits	38

Grapes

Table 32 Residues in grapes from residue trials conducted with benalaxyl (WP mancozeb co-formulation) in Brazil, France and Italy.

GRAPE Country (Year) Location (Variety)	Application rate				Residues (mg/kg) Bunch	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no			
France, 1997 Brimont (Pinot Meunier)	0.2	300	0.067	4	0.329 0.456 0.167 0.126 0.086	-0 [a] 0 15 30 42	Ref : 043 Report : 2277 Trial: 9703ISA/V/STA004
France, 1997 Brimont (Pinot Meunier) SC Formulation	0.150	300	0.050	4	0.188 0.346 0.112 0.065 0.055	-0 [a] 0 15 30 42	Ref : 043 Report 2277 Trial: 9703ISA/V/STA004
France, 2000 Vouvray (Chenin)	0.200	300	0.067	4	0.127	40	Ref: 044 Report: 2322 Trial 00 F VI IG P04
France, 2000 Bouzy (Pinot noir)	0.200	200	0.100	4	0.287	39	Ref: 044 Report: 2322 Trial 00 F VI IG P05
France, 2000 Le Mesnil sur Oger (Chardonnay)	0.200	200	0.100	4	0.146	39	Ref: 044 Report: 2322 Trial 00 F VI IG P06
France, 2000 Hermonville (Pinot meunier)	0.200	200	0.100	4	0.140	41	Ref: 044 Report: 2322 Trial 00 F VI IG P07
France, 2000 Vernou sur Brenne (Chenin)	0.200	300	0.067	4	0.189 0.262 0.167 0.153 0.062 0.061	-0 [a] 0 10 15 29 38	Ref: 045 Report: 2326 Trial 00 F VI IG P08
France, 2000 Panzoult (Cabernet franc)	0.200	300	0.067	4	0.144 0.248 0.162 0.122 0.087 0.084	-0 [a] 0 9 15 30 38	Ref: 046 Report: 2327 Trial 00 F VI IG P09
France, 2001 Vernou sur Brenne (Chenin)	0.200	500	0.040	4	0.128 0.176 0.116 0.092 0.048 0.063	-0 [a] 0 11 15 29 40	Ref: 047 Report: 2350 Trial 00 F VI IG P06
Italy, 1999 Salerano sul Lambro (Merlot)	0.200	1000	0.020	4	0.800 0.217 0.139 0.102 0.068	0 15 21 30 42	Ref: 048 Report: 2289 Trial IR2/I/01VI
Italy, 1999 Brentino Belluno (Cabernet Sauvignon)	0.200	1000	0.020	4	0.742 0.150 0.101 0.112 0.064	0 15 21 30 42	Ref: 049 Report: 2290 Trial IR2/I/02VI
Italy, 2000 Torre di Mosto (Verduzzo)	0.200	500	0.040	4	0.074	41	Ref: 050 Report: 2334 Trial IR3/I/05VI

GRAPE Country (Year) Location (Variety)	Application rate				Residues (mg/kg) Bunch	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no			
Italy, 2001 Castenaso (Lambrusco)	0.200	1000	0.020	4	0.071 0.338 0.063 0.055 0.045 0.021	-0 [a] 0 15 20 30 40	Ref: 051 Report: 2351 Trial 0107R
Italy, 2001 Castenaso (Albana)	0.200	1000	0.020	4	0.030	40	Ref: 052 Report: 2353 Trial 0111R
Italy, 2001 Volania di Comacchio (Malvasia)	0.200	1000	0.020	4	0.110	41	Ref: 052 Report: 2353 Trial 0147R
Italy, 2001 Volania di Comacchio (Malvasia)	0.200	1000	0.020	4	0.295 0.684 0.227 0.170 0.150 0.107	-0 [a] 0 15 20 30 41	Ref: 053 Report 2352 Trial 0108R SEE 2353 same last spray date and harvest date
Brazil, 1997 Districto de Guavera (Bordeaux)	0.240	1000	0.024	4	< 0.1,< 0.1 < 0.1,< 0.1 < 0.1,< 0.1	0 7 10	Ref: 054 Report: 040 aS/98
Brazil, 1997 Districto de Guavera (Bordeaux)	0.480	1000	0.048	4	< 0.1,< 0.1 < 0.1,< 0.1 < 0.1,< 0.1	0 7 10	Ref: 054 Report: 040 aS/98
Brazil, 2004 Gentil RS (Isabel)	0.240	1000	0.024	4	< 0.1	7	Ref: 055 Report: R/03/RS575/ PLANTEC
Brazil, 2004 Gentil RS (Isabel)	0.480	1000	0.048	4	< 0.1	7	Ref: 055 Report: R/03/RS575/ PLANTEC

[a] sampling done at time zero, immediately prior to the last application

Lettuce

In 15 trials conducted in Italy and Spain, 2–3 foliar sprays of benalaxyl were applied to outdoor lettuce 2 weeks apart at a target rate of 0.2 or 0.24 kg ai/ha, using water rates of 1000 L/ha. WP formulations used in these trials were either co-formulations of benalaxyl and mancozeb or benalaxyl and copper.

Table 33 Residues in lettuce (outdoor) from residue trials conducted with benalaxyl (WP formulations) in Italy and Spain

LETTUCE Country (Year) Location (Variety)	Form	Application rate				Residues (mg/kg) Leaves	PHI (days)	Reference & Comments
		kg ai/ha	water L/ha	kg ai/hL	no			
Spain, 1995 Sueca (Trocadero)	WP 80	0.200	1000	0.020	2	7.732 0.193 0.119 0.048	0 7 14 21	Ref: 084 Report: 2172 vol. 2 Trial: R 9514/1
Spain, 1995 Cullera (Trocadero)	WP 80	0.200	1000	0.020	2	7.606 0.354 0.064 0.043	0 7 14 21	Ref: 084 Report: 2172 vol. 2 Trial: R 9514/2

LETTUCE Country (Year) Location (Variety)	Form	Application rate				Residues (mg/kg)	PHI (days)	Reference & Comments
		kg ai/ha	water L/ha	kg ai/hL	no	Leaves		
Spain, 1995 Riola (Trocadero)	WP 80	0.200	1000	0.020	2	6.292 0.245 0.039 0.079	0 7 14 21	Ref: 084 Report: 2172 vol. 2 Trial: R 9514/3
Spain, 1996 Sueca (Valladolid)	WP 80	0.200	1000	0.020	2	6.034 0.987 0.154 0.022	0 7 14 21	Ref: 085 Report: 2219 Trial: R 9655/1
Spain, 1996 Cullera (Valladolid)	WP 80	0.200	1000	0.020	2	11.255 2.166 0.425 0.054	0 7 13 21	Ref: 085 Report: 2219 Trial: R 9655/2
Spain, 1996 Malgrat de Mar (Maravilla)	WP 80	0.200	1000	0.020	2	4.685 0.078 0.067 < 0.02	0 7 14 21	Ref: 085 Report: 2219 Trial: R 9655/3
Spain, 1997 Alginet (Trocadero)	WP 80	0.200	1000	0.020	2	7.656 1.382 0.328 0.044	0 7 14 21	Ref: 086 Report: 2241 Trial: R 9770-1
Spain, 1997 Picassent (Romana)	WP 80	0.200	1000	0.020	2	4.168 0.928 0.105 0.059	0 7 14 21	Ref: 086 Report: 2241 Trial: R 9770-2
Italy, 1996 Guidizzolo (Titan)	WP 80	0.240	1000	0.024	3	9.746 0.661 0.105 < 0.02 < 0.02[a]	0 5 11 15 28	Ref: 087 Report: 2209 Trial: BIF/2/2I/L
Italy, 1996 Salerano sul Lambro (Audran)	WP 80	0.240	1000	0.024	3	4.468 1.221 0.527 0.058 0.022	0 4 9 13 27	Ref: 088 Report: 2207 Trial: BIF/2/1I/L
Italy, 1995 Salerano sul Lambro (Regina d'estate)	WP 40	0.200	1000	0.020	3	0.035 6.558 0.617 0.059 < 0.02 < 0.02[a]	-0 0 4 10 14 20	Ref: 089 Report: 2173 Trial: BIF/1/1I/L
Italy, 1995 Lusia (Jeanne)	WP 40	0.200	1000	0.020	3	9.312 1.021 0.034 < 0.02 < 0.02[a]	0 4 11 15 20	Ref: 089 Report: 2173 Trial: BIF/1/2I/L
Italy, 1995 S. Lazzaro di Savena (Jeanne)	WP 40	0.200	1000	0.020	3	0.104 7.040 0.396 0.118 0.089 0.046	-0 0 4 10 14 20	Ref: 089 Report: 2173 Trial: BIF/1/3I/L
Italy, 1996 Guidizzolo (Titan)	WP 40	0.200	1000	0.020	3	7.064 0.491 0.081 < 0.02 < 0.02[a]	0 5 11 15 20	Ref: 090 Report: 2210 Trial: BIF/2/2I/L

LETTUCE Country (Year) Location (Variety)	Form	Application rate				Residues (mg/kg)	PHI (days)	Reference & Comments
		kg ai/ha	water L/ha	kg ai/hL	no	Leaves		
Italy, 1996 Salerano sul Lambro (Audran)	WP 40	0.200	1000	0.020	3	3.548	0	Ref: 091 Report: 2208 Trial: BIF/2/1I/L
						0.854	4	
						0.322	9	
						0.059	13	
						< 0.02	19	

[a] < 0.02 = actual value reported as < LOD (0.01 mg/kg)

WP 80 = benalaxyl & mancozeb co-formulation

WP 40 = benalaxyl & Copper co-formulation

Melons and watermelons

In eight outdoor melon trials and five outdoor watermelon trials conducted in Italy and Spain, two foliar sprays of benalaxyl (WP co-formulated with mancozeb) were applied 2 weeks apart at a target rate of 0.2 kg ai/ha, using water rates of 1000 L/ha. In one melon and one watermelon trial in Italy, 2 applications at a higher rate of 0.24 kg ai/ha was used.

In most trials, the pulp and peel were analysed separately and the whole fruit residues were calculated as the sum of (% peel weight × residues in peel) and (% pulp weight × residues in pulp).

Table 34 Residues in melons (outdoor) from residue trials conducted with benalaxyl (WP mancozeb co-formulations) in Italy and Spain.

MELON Country (Year) Location (Variety)	Application rate				Portion analysed	Residues (mg/kg)	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no				
Spain, 1995 Calasparra (Piel de Sapo)	0.200	1000	0.020	3	Pulp	0.039	0	Ref: 064 Report: 2172 vol. 1 Trial R9513/2
						< 0.02	7	
						< 0.02	14	
						< 0.02 [a]	21	
					Peel	0.212	0	
						0.031	7	
						< 0.02	14	
						< 0.02	21	
					Whole fruit [b]	0.101	0	
						0.020	7	
						< 0.02	14	
						< 0.02	21	
Spain, 1996 Benifayo (Piel de Sapo -Rijz Swan)	0.200	1000	0.020	3	Whole fruit	0.061	0	Ref: 065 Report: 2218 Trial: R9654/3
						0.038	7	
						< 0.02	14	
						< 0.02 [a]	21	
Spain, 1997 Almussafes (Sancho)	0.200	1000	0.020	3	Pulp	0.044	0	Ref: 066 Report: 2243 Trial: R9771-1
						0.046	3	
						0.054	7	
						0.033	14	
					Peel	0.472	0	
						0.092	3	
						0.060	7	
						0.064	14	
					Whole fruit [b]	0.201	0	
						0.065	3	
						0.056	7	

MELON Country (Year) Location (Variety)	Application rate				Portion analysed	Residues (mg/kg)	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no				
						0.044	14	
Spain, 1997 Alginet (Piel de sapo - Bavieca)	0.200	1000	0.020	3	Pulp	0.026 0.027 0.022 0.020	0 3 7 14	Ref: 066 Report: 2243 Trial: R9771-2
					Peel	0.342 0.066 0.029 0.024	0 3 7 14	
					Whole fruit [b]	0.136 0.041 0.025 0.022	0 3 7 14	
Italy, 1996 Isola S. Antonio – Sale (Asgrow)	0.240	1000	0.024	2	Whole fruit	0.062 0.056 0.078 0.057	0 4 10 14	Ref: 067 Report: 2211 Trial: BIF/1/1I/M
Italy, 1998 Badia Polesine (Mambo)	0.200	1000	0.020	3	Pulp	< 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a]	0 3 7 14	Ref: 068 Report: 2268 Trial: BE2/I/01ML
					Peel	0.170 0.160 0.174 0.140	0 3 7 14	
					Whole fruit [b]	0.079 0.076 0.060 0.057	0 3 7 14	
Italy, 1998 San Martino Spino (1227)	0.200	1000	0.020	3	Pulp	< 0.02 < 0.02 < 0.02 < 0.02	0 3 7 14	Ref: 069 Report: 2269 Trial: BE2/I/02ML
					Peel	0.735 0.261 0.305 0.248	0 3 7 14	
					Whole fruit [b]	0.378 0.127 0.154 0.122	0 3 7 14	
Spain, 2003 El Mareny de Barraquetes (Mojete)	0.200	1000	0.020	3	Pulp	0.019 < 0.02 < 0.02	0 3 7	Ref: 070 Report: 2391 Trial: 1D
					Peel	0.272 0.107 0.111	0 3 7	
					Whole fruit [b]	0.114 0.054 0.054	0 3 7	

MELON Country (Year) Location (Variety)	Application rate				Portion analysed	Residues (mg/kg)	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no				
Spain, 2003 Canals (Sancho)	0.200	1000	0.020	3	Pulp	< 0.02	0	Ref: 070 Report: 2391 Trial: 2D
						< 0.02	3	
						< 0.02	7	
					Peel	0.089	0	
						0.070	3	
						0.032	7	
					Whole fruit [b]	0.040	0	
						0.037	3	
						0.022	7	

[a] < 0.02 = actual value reported as < LOD (0.01 mg/kg)

[b] whole fruit residues calculated as the sum of (% peel weight x peel residues) and (% pulp weight x pulp residues)

Table 35 Residues in watermelons (outdoor) from residue trials conducted with benalaxyl (WP mancozeb co-formulations) in Italy and Spain.

WATERMELON Country (Year) Location (Variety)	Application rate				Portion analysed	Residues (mg/kg)	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no				
Spain, 1995 Sueca (Resistent)	0.200	1000	0.020	3	Pulp	< 0.02 [a]	0	Ref: 064 Report: 2172 vol. 1 Trial: R9513/1
						< 0.02 [a]	7	
						< 0.02 [a]	14	
						< 0.02 [a]	21	
					Peel	0.086	0	
						< 0.02	7	
						< 0.02 [a]	14	
						< 0.02 [a]	21	
					Whole fruit [b]	0.030	0	
						< 0.02 [a]	7	
						< 0.02 [a]	14	
						< 0.02 [a]	21	
Spain, 1995 Fortaleny (Resistent)	0.200	1000	0.020	3	Pulp	< 0.02 [a]	0	Ref: 064 Report: 2172 vol. 1 Trial: R9513/3
						< 0.02 [a]	7	
						< 0.02 [a]	14	
						< 0.02 [a]	21	
					Peel	0.096	0	
						< 0.02	7	
						< 0.02 [a]	14	
						< 0.02 [a]	21	
					Whole fruit [b]	0.039	0	
						< 0.02 [a]	7	
						< 0.02 [a]	14	
						< 0.02 [a]	21	
Spain, 1996 Sueca (Resistent)	0.200	1000	0.020	3	Whole fruit	0.045	0	Ref: 071 Report: 2217 Trial: R9654/1
						0.031	7	
						< 0.02 [a]	14	
						< 0.02 [a]	21	
Spain, 1996 Albalat de la Ribera (Resistent)	0.200	1000	0.020	3	Whole fruit	< 0.02	0	Ref: 071 Report: 2217 Trial: R9654/2
						< 0.02	7	
						< 0.02 [a]	14	
						< 0.02 [a]	21	

WATERMELON Country (Year) Location (Variety)	Application rate				Portion analysed	Residues (mg/kg)	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no				
Italy, 1997 Alluvioni Cambiò – Sale (Crimson sweet)	0.240	1000	0.024	2	Whole fruit	0.023 < 0.02 < 0.02 [a] < 0.02 [a]	0 4 10 14	Ref: 072 Report: 2212 Trial: BIF/1/2I/W

[a] < 0.02 = actual value reported as < LOD (0.01 mg/kg)

[b] whole fruit residues calculated as the sum of (% peel weight x peel residues) and (% pulp weight x pulp residues)

Onion

In 12 onion trials conducted in France, Italy, Greece and Spain, 3 foliar sprays of benalaxyl (WP co-formulated with mancozeb) were applied to onions at 2 week intervals at a target rate of 0.16 kg ai/ha or 0.2 kg ai/ha, using water rates of 400–1000 L/ha.

In three reverse decline studies conducted in Brazil, 4 applications of benalaxyl WP (co-formulated with chlorothalonil) were applied at 2 week intervals to separate plots up to 7 and up to 14 days before a single harvest date, at target rates of 0.24 kg ai/ha and 0.48 kg ai/ha.

Table 36 Residues in onion (bulbs) from residue trials conducted with benalaxyl (WP co-formulations) in Italy, Spain and Brazil

ONION, BULB Country (Year) Location (Variety)	Application rate				Residues (mg/kg) Bulbs	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no			
France, 2004 Griesheim-pres-Molsheim (Turbo)	0.160	1000	0.016	3	< 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a]	0 7 14 21 30	Ref: 056 Report: 2392 1D
France, 2003 Dorlisheim (Barito)	0.160	1000	0.016	3	< 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a]	0 7 14 21 30	Ref: 056 Report: 2392 2D
France, 2004 Zellwiller (Jaune paille des vertus)	0.160	1000	0.016	3	< 0.02 [a]	30	Ref: 056 Report: 2392 3H Trial:
France, 2004 Niedernai (Mustang)	0.160	1000	0.016	3	< 0.02 [a]	30	Ref: 056 Report: 2392 4H Trial:
Italy, 2000 Monticelli d'Ongina (Leger)	0.220	500	0.044	3	< 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a]	0 1 3 7 14	Ref: 057 Report: 2336 Trial: IR/I/06CI
Italy, 2000 Salerano sul Lambro (Gigante di Stoccarda)	0.220	500	0.044	3	< 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a]	0 1 3 7 14	Ref: 058 Report: 2337 Trial: IR/I/07CI
Italy, 2001 Contrada Aragona-Centuripe (Nostrana)	0.220	1000	0.022	3	< 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a] < 0.02 [a]	-0 0 14 21 28	Ref: 059 Report: 2355 Trial: 01NA42R

ONION, BULB Country (Year) Location (Variety)	Application rate				Residues (mg/kg)	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no	Bulbs		
Italy, 2002 Mezzolara (Blanco Duro)	0.220	600	0.037	3	< 0.02 [a]	14	Ref: 060 Report: 2378 Trial: SP00177 P1H
Italy, 2002 Poggio Renatico (Density)	0.220	600	0.037	3	< 0.02 [a]	14	Ref: 060 Report: 2378 Trial: SP00177 P2H
Greece, 2002 Eleohoria (Arma)	0.220	600	0.037	3	< 0.02 [a]	14	Ref: 060 Report: 2378 Trial: SP00177 P3H
France, 2002 St. Lurent de la Salanque (Rouge de Toulouge)	0.220	400	0.055	3	< 0.02 [a]	14	Ref: 060 Report: 2378 Trial: SP00177 P4H
Spain, 2002 Purto de Sagunto (Grano de Oro)	0.220	600	0.037	3	< 0.02 [a]	14	Ref: 060 Report: 2378 Trial: SP00177 P5H
Brazil, 2001 Ponta Grossa (Crioula)	0.240	600	0.040	4	< 0.10	7	Ref: 061 Report: 40/02 Trial: HDB00233 Protocol TRP 0006 (Reverse Decline)
	0.240	600	0.040	4	< 0.10	14	
Brazil, 2001 Ponta Grossa (Crioula)	0.480	600	0.080	4	< 0.10	7	Ref: 061 Report: 40/02 Trial: HDB00233 Protocol TRP 0006 (Reverse Decline)
	0.480	600	0.080	4	< 0.10	14	
Brazil, 2001 Pereiras (Tres Marias)	0.240	800	0.030	4	< 0.10	7	Ref: 062 Peport: 41/02 Trial: HDB00234 Protocol TRP 0006 (Reverse decline)
	0.240	800	0.030	4	< 0.10	14	
Brazil, 2001 Pereiras (Tres Marias)	0.480	800	0.060	4	< 0.10	7	Ref: 062 Peport: 41/02 Trial: HDB00234 Protocol TRP 0006 (Reverse decline)
	0.480	800	0.060	4	< 0.10	14	
Brazil, 2001 Santa Cruz do Sul (Roxa)	0.240	600	0.040	4	< 0.10	7	Ref: 063 Report: 42/02 Trial: HDB00235 Protocol TRP 0006 (Reverse decline)
	0.240	600	0.040	4	< 0.10	14	
Brazil, 2001 Santa Cruz do Sul (Roxa)	0.480	600	0.080	4	< 0.10	7	Ref: 063 Report: 42/02 Trial: HDB00235 Protocol TRP 0006 (Reverse decline)
	0.480	600	0.080	4	< 0.10	14	

[a] < 0.02 = actual value reported as < LOD (0.01 mg/kg)

Potato

In six trials conducted in France and Italy and in two trials in Brazil, 4 foliar sprays of benalaxyl (WP co-formulated with mancozeb) were applied to potatoes at 2 week intervals at a target rate of 0.24 kg ai/ha, using water rates of 250 or 500 L/ha. In the Brazilian trials (2003), a higher rate of 0.48 kg ai/ha was also used.

Three trials were also conducted in Brazil using a WP formulation of benalaxyl co-formulated with chlorothalonil, involving 2 applications of 0.24 kg ai/ha and 0.48 kg ai/ha applied at 2 week intervals. Two of these trials were reverse decline studies where 2 sprays were applied at 2 week intervals to separate plots up to 7, 14 and 21 days before a single harvest date.

Table 37 Residues in potato (tubers) from residue trials conducted with benalaxyl (WP co-formulations) in Brazil, France and Italy

POTATO Country (Year) Location (Variety)	Application rate				Residues (mg/kg) Tubers	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no			
France, 2000 Mormelon Le Petit (Caesar)	0.240	250	0.096	4	< 0.02 [a]	15	Ref: 092 Report: 2319 Trial: 00 F PT IG P01
France, 2000 Dampierre sur Moivre (Caesar)	0.240	250	0.096	4	< 0.02 [a]	15	Ref: 092 Report: 2319 Trial: 00 F PT IG P02
France, 2000 Faux-Vesigneul (Capta)	0.240	250	0.096	4	< 0.02 [a]	15	Ref: 092 Report: 2319 Trial: 00 F PT IG P03
Italy, 2000 Budrio (Primula)	0.240	500	0.096	4	< 0.02 [a]	14	Ref: 093 Report: 2329 Trial: IR3/I/01PA
Italy, 2000 Terrazzo (Lisetta)	0.240	500	0.096	4	< 0.02 [a]	14	Ref: 093 Report: 2329 Trial: IR3/I/02PA
Italy, 2000 Castiglione Mantovano (Hermes)	0.240	500	0.096	4	< 0.02 [a]	14	Ref: 093 Report: 2329 Trial: IR3/I/03PA
Brazil, 2003 Gentil RS (Bintje)	0.240	400		4	< 0.10	7	Ref: 097 Report: R/03/RS573/PLANTEC
Brazil, 2003 Gentil RS (Bintje)	0.480	400	0.120	4	< 0.10	7	Ref: 097 Report: R/03/RS573/PLANTEC
Brazil, 2003 Vargem Grande do Sul (Monalisa)	0.240	600	0.033	4	< 0.10	7	Ref: 098 Report: R/03/RS574/PLANTEC
Brazil, 2003 Vargem Grande do Sul (Monalisa)	0.480	600	0.066	4	< 0.10	7	Ref: 098 Report: R/03/RS574/PLANTEC
Brazil, 2001 Pereiras (Jaette Bintje)	0.240	860	0.028	2	< 0.10 < 0.10 < 0.10	7 14 21	Ref: 094 Report: 43/02 Trial: HDB00230
Brazil, 2001 Pereiras (Jaette Bintje)	0.480	860	0.056	2	< 0.10 < 0.10 < 0.10	7 14 21	Ref: 094 Report: 43/02 Trial: HDB00230
Brazil, 2001 Julio de Castilho (Asterix)	0.240	400	0.060	2	< 0.10	7	Ref: 095 Report: 44/02 Trial: HDB00231
	0.240	400	0.060	2	< 0.10	14	Protocol TRP 0004
	0.240	400	0.060	2	< 0.10	21	(Reverse decline)

POTATO Country (Year) Location (Variety)	Application rate				Residues (mg/kg) Tubers	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no			
Brazil, 2001 Julio de Castilho (Asterix)	0.480	400	0.120	2	< 0.10	7	Ref: 095 Report: 44/02 Trial: HDB00231 Protocol TRP 0004 (Reverse decline)
	0.480	400	0.120	2	< 0.10	14	
	0.480	400	0.120	2	< 0.10	21	
Brazil, 2001 Santa Cruz do Sul (Asterix)	0.240	400	0.060	2	< 0.10	7	Ref: 096 Report: 45/02 Trial: HDB00232 Protocol TRP 0004 (Reverse decline)
	0.480	400	0.120	2	< 0.10	7	
	0.240	400	0.060	2	< 0.10	14	
Brazil, 2001 Santa Cruz do Sul (Asterix)	0.480	400	0.120	2	< 0.10	14	Ref: 096 Report: 45/02 Trial: HDB00232 Protocol TRP 0004 (Reverse decline)
	0.240	400	0.060	2	< 0.10	21	
	0.480	400	0.120	2	< 0.10	21	

[a] < 0.02 = actual value reported as < LOD (0.01 mg/kg)

Tomato

In eight trials conducted in France, Italy and Spain and in two trials in Brazil, 4 foliar sprays of benalaxyl (WP co-formulated with mancozeb) were applied to tomatoes at 2 week intervals at a target rate of 0.24 kg ai/ha, using water rates of 400–4000 L/ha. In the Brazilian trials (2003), a higher rate of 0.4 kg ai/ha was also used.

Three trials were also conducted in Brazil (2001) using a WP formulation of benalaxyl co-formulated with chlorothalonil, involving 4 applications of 0.24 kg ai/ha and 0.48 kg ai/ha applied at 2 week intervals. Two of these trials were reverse decline studies where 2 sprays were applied at 2 week intervals to separate plots up to 7 and up to 14 days before harvest.

Table 38 Residues in tomato from residue trials conducted with benalaxyl (WP co-formulations) in Brazil, France, Italy and Spain

TOMATO Country (Year) Location (Variety)	Application rate				Residues (mg/kg) Fruit	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no			
Italy, 1999 Salerano sul Lambro (UC134)	0.240	1000	0.024	4	0.464 0.100 0.097 0.037 0.046	0 3 7 10 15	Ref: 073 Report: 2294 Trial: IR2/I/01PO
Italy, 2000 Contrada Pigno (Ortigia)	0.240	1000	0.024	4	0.036 0.183 0.110 0.082 0.058 0.040	-0 0 3 7 10 14	Ref: 074 Report: 2315 Trial: 00N11R
Italy, 2000 Località Vangelese (Incas)	0.240	4000	0.006	4	0.037 0.173 0.139 0.073 0.028 0.032	-0 0 3 7 10 14	Ref: 075 Report: 2317 Trial: A/0011/R

TOMATO Country (Year) Location (Variety)	Application rate				Residues (mg/kg)	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no	Fruit		
Italy, 2000 Poggio Rusco (1296)	0.240	500	0.048	4	0.270 0.170 0.142 0.057 0.042	0 1 3 7 14	Ref: 076 Report: 2332 Trial: IR3/I/04/PO
Italy, 2001 Contrada Pigno (Marmande)	0.240	1000	0.024	4	0.049	14	Ref: 077 Report: 2354 Trial: 01NA41R
Italy, 2002 Dugliolo (Antalia)	0.240	600	0.040	4	< 0.02	14	Ref: 078 Report: 2379 Trial: 00183P1H
France, 2002 Lavallette (Pavia)	0.240	400	0.060	4	0.023	14	Ref: 078 Report: 2379 Trial: 00183P2H
Spain, 2002 Anna (Rio Grande)	0.240	700	0.034	4	< 0.02	14	Ref: 078 Report: 2379 Trial: 00183P3H
Brazil, 1996 Estado de Sao Paulo (Santo Antonio)	0.200	1000	0.020	4	< 0.10 < 0.10 < 0.10 < 0.10 < 0.10	0 3 7 14 21	Ref: 082 Report: GAL-R-2al:
Brazil, 1996 Estado de Sao Paulo (Santo Antonio)	0.400	1000	0.040	4	< 0.10 < 0.10 < 0.10 < 0.10 < 0.10	0 3 7 14 21	Ref: 082 Report: GAL-R-2al:
Brazil, 2003 Iracemapolis (Carmen)	0.200	1000	0.020	4	< 0.10	7	Ref: 083 Report: R/03/RS576/PLANTEC
Brazil, 2003 Iracemapolis (Carmen)	0.400	1000	0.040	4	< 0.10	7	Ref: 083 Report: R/03/RS576/PLANTEC
Brazil, 2001 Santa Cruz do Sul (Florida)	0.240	1000	0.024	4	< 0.10	0	Ref: 079 Report: 47 T/02 Trial: HDB00237 Protocol TRP 0005 (Reverse decline)
	0.240	1000	0.024	4	< 0.10	3	
	0.240	1000	0.024	4	< 0.10	5	
	0.240	1000	0.024	4	< 0.10	7	
	0.240	1000	0.024	4	< 0.10	14	
Brazil, 2001 Santa Cruz do Sul (Florida)	0.480	1000	0.048	4	< 0.10	0	Ref: 079 Report: 47 T/02 Trial: HDB00237 Protocol TRP 0005 (Reverse decline)
	0.480	1000	0.048	4	< 0.10	3	
	0.480	1000	0.048	4	< 0.10	5	
	0.480	1000	0.048	4	< 0.10	7	
	0.480	1000	0.048	4	< 0.10	14	
Brazil, 2001 Astorga (Debora max)	0.240	1000	0.024	4	< 0.10	0	Ref: 080 Report 49 T/02 Trial: HDB00239 Protocol TRP 0208 (Reverse decline)
	0.240	1000	0.024	4	< 0.10	3	
	0.240	1000	0.024	4	< 0.10	5	
	0.240	1000	0.024	4	< 0.10	7	
	0.240	1000	0.024	4	< 0.10	14	

TOMATO Country (Year) Location (Variety)	Application rate				Residues (mg/kg) Fruit	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no			
Brazil, 2001 Astorga (Debora max)	0.480	1000	0.048	4	< 0.10	0	Ref: 080 Report 49 T/02 Trial: HDB00239 Protocol TRP 0208 (Reverse decline)
	0.480	1000	0.048	4	< 0.10	3	
	0.480	1000	0.048	4	< 0.10	5	
	0.480	1000	0.048	4	< 0.10	7	
	0.480	1000	0.048	4	< 0.10	14	
Brazil, 2001 Engenheiro Coelho Rio Grande Super)	0.240	1000	0.024	4	< 0.10	0	Ref: 081 Report: 50 T/02 Trial: HDB00240 Protocol TRP 0208 (Reverse decline)
	0.240	1000	0.024	4	< 0.10	3	
	0.240	1000	0.024	4	< 0.10	5	
	0.240	1000	0.024	4	< 0.10	7	
	0.240	1000	0.024	4	< 0.10	14	
Brazil, 2001 Engenheiro Coelho Rio Grande Super)	0.480	1000	0.048	4	< 0.10	0	Ref: 081 Report: 50 T/02 Trial: HDB00240 Protocol TRP 0208 (Reverse decline)
	0.480	1000	0.048	4	< 0.10	3	
	0.480	1000	0.048	4	< 0.10	5	
	0.480	1000	0.048	4	< 0.10	7	
	0.480	1000	0.048	4	< 0.10	14	

FATE OF RESIDUES IN STORAGE AND IN PROCESSING

The Meeting received processing studies for grape and tomato. Samples from a residue study performed with Benalaxyl M (Benalaxyl and Mancozeb 8–65% WP) on grapes, were processed into must, pomace, grape juice, and bottled wine. Residue levels are shown in Table 39. Processing was generally found to reduce benalaxyl residue levels, except for pomace, where residues concentrated to give an average processing factor of 3.5×. In wine, residues of benalaxyl were either not detected or are at about the LOQ level.

Table 39 Residues in processed commodities of grapes from residue trials conducted with benalaxyl (WP formulation also containing mancozeb) in France and Italy

GRAPE Country (Year) Location (Variety)	Application rate				Portion analysed	Residues (mg/kg)	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no				
France, 1997 Brimont (Pinot Meunier) WP formulation	0.2	300	0.067	4	Bunch Must Juice Pomace Bottled wine	0.090 < 0.02 < 0.02 [a] 0.294 0.020	43 43 48 49 75	Ref : 043 Report : 2277 Trial: 9703ISA/V/STA004
France, 1997 Brimont (Pinot Meunier) SC formulation	0.150	300	0.050	4	Bunch Must Juice Pomace Bottled wine	0.055 < 0.02 [a] < 0.02 [a] 0.207 < 0.02	43 43 48 49 75	Ref : 043 Report 2277 Trial: 9703ISA/V/STA004
Italy, 1999 Salerano sul Lambro (Merlot)	0.200	1000	0.020	4	Bunch Juice Bottled wine	0.068 < 0.02 [a] < 0.02 [a]	42	Ref: 048 Report: 2289 Trial IR2/I/01VI

GRAPE Country (Year) Location (Variety)	Application rate				Portion analysed	Residues (mg/kg)	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no				
Italy, 1999 Brentino Belluno (Cabernet Sauvignon)	0.200	1000	0.020	4	Bunch Juice Bottled wine	0.064 < 0.02 [a] < 0.02 [a]	42	Ref: 049 Report: 2290 Trial IR2/I/02VI

[a] < 0.02 = actual value reported as < LOD (0.01 mg/kg)

The reported LOQ was 0.02 mg/kg

Table 40 Tomato Processing Study Summary

TOMATO Country (Year) Location (Variety)	Application rate				Portion analysed	Residues (mg/kg) Fruit	PHI (days)	Reference & Comments
	kg ai/ha	water L/ha	kg ai/hL	no				
Italy, 1999 Salerano sul Lambro (UC134)	0.240	1000	0.024	4	Tomato Juice Purée Preserve	0.100 < 0.02 0.021 < 0.02 [a]	3 3 3 3	Ref: 073 Report: 2294 Trial: IR2/I/01PO
					Tomato Juice Purée Preserve	0.046 < 0.02 [a] 0.022 < 0.02 [a]	15 15 15 15	

[a] < 0.02 = actual value reported as <LOD (0.01 mg/kg)

The reported LOQ was 0.02 mg/kg

RESIDUES IN ANIMAL COMMODITIES

Farm animal feeding studies

A bovine feeding study was not provided. Other than for wet grape pomace, which is a feed item only for Australia, there are no cattle feed items resulting from the RACs for which the 2009 Meeting made maximum residue level recommendations. Moreover, as indicated in the FAO Manual [Second Edition] (Section 3.9), a bovine feeding study is not necessary when a ruminant metabolism study with dosing at the equivalent of 10×, where 1× is the anticipated dietary burden, results in levels of the residue of concern below the limit of quantitation (LOQ) in all edible commodities. Accordingly, the Meeting decided that no bovine feeding study was necessary at this time.

A poultry feeding study was not provided. As there were no poultry feed items resulting from the RACs for which the 2009 Meeting made maximum residue level recommendations the Meeting decided no poultry feeding study was necessary.

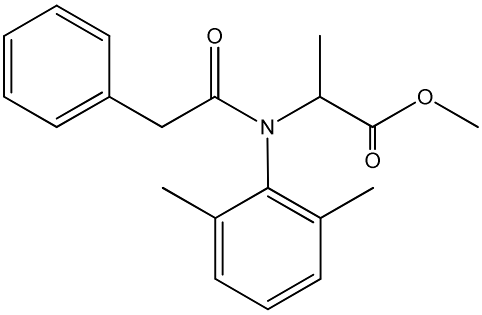
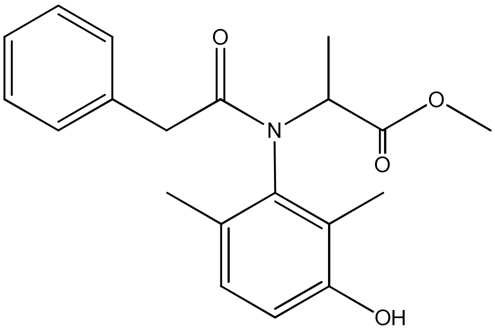
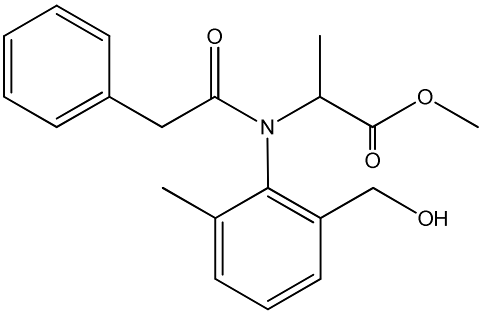
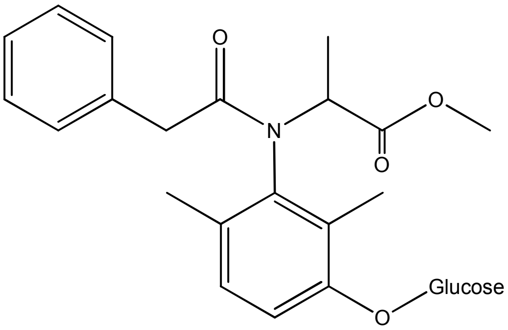
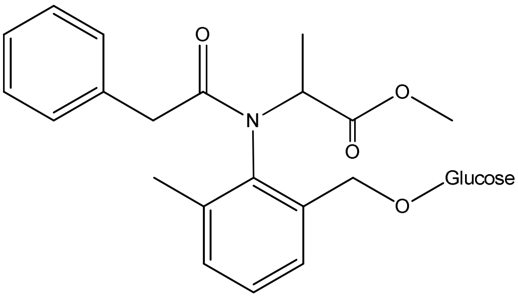
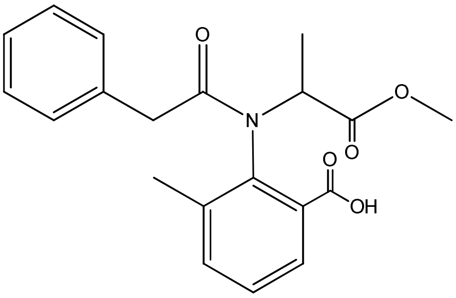
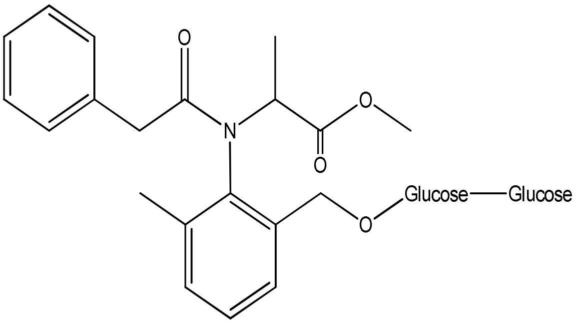
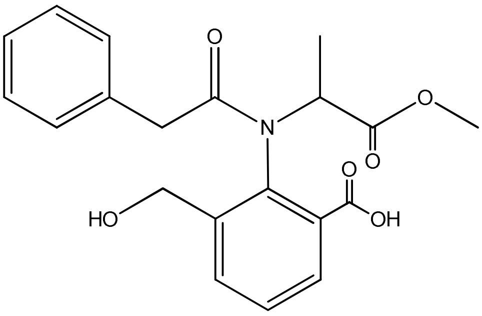
Neither poultry nor bovine feeding studies were provided. The manufacturer stated that, due to the low level of benalaxyl residues in animal feedstuffs, no feeding studies are needed.

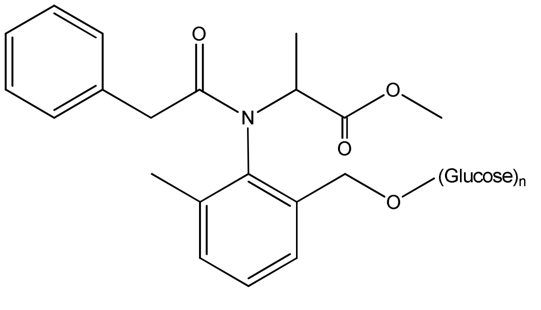
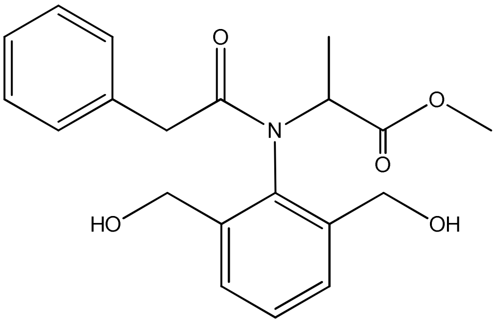
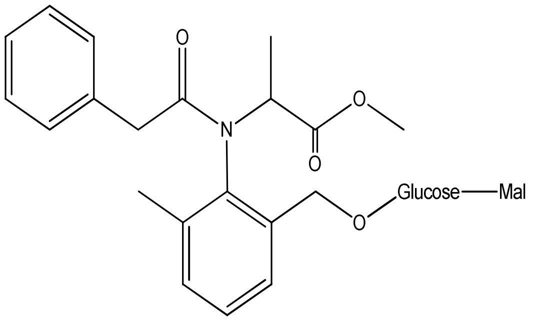
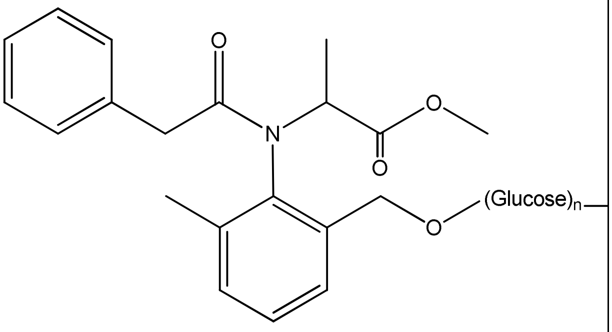
APPRAISAL

Benalaxyl [methyl N-phenylacetyl-N-2,6-xylyl-DL-alaninate] is a broad-spectrum phenylamide fungicide. Residue and analytical aspects of benalaxyl were evaluated by the JMPR in 1986, 1988, 1992, and 1993. It was evaluated for toxicological review by JMPR 2005. The ADI for benalaxyl was established at 0–0.07 mg/kg bw and an ARfD of 0.1 mg/kg bw was established for women of childbearing age. This compound was listed in the Periodic Re-Evaluation Program at the 40th Session of the CCPR for periodic review by the 2009 JMPR.

Residue studies were submitted by the manufacturer to support the use of benalaxyl in or on a variety of fruits and vegetables.

Chemical codes and structures of Benalaxyl and its plant and animal metabolites:

 Benalaxyl [Galben]	 GX5c (Plants)
 GX5a and GX5b (Plants), G8 and G14 (Animals)	 GX1c (Plants)
 GX1a and GX1b (Plants)	 G7a and G7b (Animals)
 GX6 (Plants)	 G6 (Animals)

 GX4 (Plants)	 M4 (Plants and Animals)
 GX11 (Plants)	 GX16 (Plants)

Animal metabolism

The Meeting reviewed studies on the metabolism of ^{14}C-labelled benalaxyl in goats and hens. Two lactating goats received two daily oral administrations of benalaxyl at the equivalent of 40 ppm in the feed for seven consecutive days. The urine and faeces' contained about 80–90% of the administered dose of radioactivity. The maximum levels of radioactive residue in milk and tissues were as follows: milk, 0.011 mg/kg; muscle, 0.017 mg/kg; fat, 0.027 mg/kg; liver 1.1 mg/kg; and kidney 0.37 mg/kg. Minor amounts of benalaxyl (< 2% TRR) were identified in kidney or liver samples. The major metabolites identified in tissues were glucuronide and/or sulphate conjugates of the hydroxylated metabolites G8 and G14. However, poor extractability and analysis difficulties hampered metabolite identification, particularly in liver samples, resulting in 20–30% TRR being unidentified but characterized as polar species.

Ten laying hens were dosed once daily for fourteen days with capsules containing ^{14}C-labelled benalaxyl at a dose of approximately 60 mg/kg diet/day. The TRR levels were as follows: eggs, 0.35 mg/kg, fat, 0.04 mg/kg; kidney, 0.72 mg/kg; liver, 1.4 mg/kg; and muscle, 0.05 mg/kg. The residue profile was qualitatively similar to that of the goat. Benalaxyl was not found in any of the hen tissues except blood (9%). The major metabolite identified was the hydroxymethylcarboxy metabolite G6 at 21% TRR in egg yolk. As with the goat metabolism study, large portions of the TRR were characterized as a sum of polar metabolites each comprising less than 10% TRR, with 10–15% TRR unidentified.

The 2005 JMPR Toxicological Evaluation provides a description of the metabolic profile of benalaxyl in rats that is qualitatively similar to that discussed above for goats and hens.

Based on the results of the goat and hen metabolism studies, a metabolic profile for benalaxyl was proposed. Benalaxyl is oxidised giving the G8 and G14 hydroxymethyl derivatives. The G8 and G14 compounds are further oxidised to form the G7A and G7B carboxy derivatives. The G6 hydroxymethylcarboxy metabolite is a further oxidation product. Conjugation appears to occur with all the compounds. Enzymatic hydrolysis increased the levels of extractable ^{14}C-residue in the tissues and egg yolk. Thus, it is likely that oxidation followed by conjugation is the main route of benalaxyl metabolism in animals.

Plant metabolism

The studies on plant metabolism show that [^{14}C]benalaxyl penetrates into grape, tomato, and potato plants. In grapes, more than 75% of radioactivity applied was found in the fruit 8 days after application while in tomato 40% of radioactivity was inside the fruit 28 days after treatment. Benalaxyl sprayed on potato plant leaves or present in soil, due to dripping after spraying, doesn't transfer to tuber since no significant radioactivity was found in tubers (< 0.005 mg/kg).

The rate of degradation depends on the plant species. In grapes, more than 50% of existing radioactivity corresponds to the active ingredient itself, 24 days after application; in tomato fruit, more than 15% was found as benalaxyl 35 days after treatment; in potato leaves, the parent compound percentage was more than 25%, 10 days after treatment.

The metabolites identified in grapes are GX1, GX5a, GX5b, GX5c and GX6; only GX1 and GX6 were present in significant levels (25% and 10%, respectively). In wine, besides some of those metabolites, minor levels of metabolites GX4, GX7 and GX8 were found. In tomato, several metabolites were found in low concentrations, except for GX11 which is significant (> 10% TRR).

The most important component of residue is the parent compound. However, metabolites GX1 and GX6 in grapes, and GX11 in tomatoes comprise more than 10% TRR. These metabolites result from oxidation and linkage of the parent compound to one (GX1) or more (GX6) molecules of glucose or (GX11) molecules of glucose plus malonic acid. Although these glucoside metabolites were not identified in the rat metabolism study, these plant metabolites are more polar and likely less toxic than the parent compound.

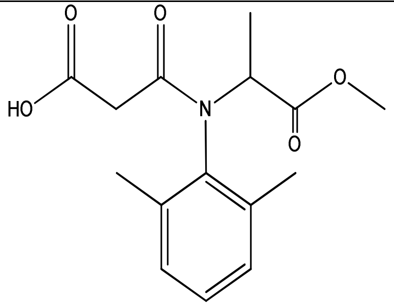
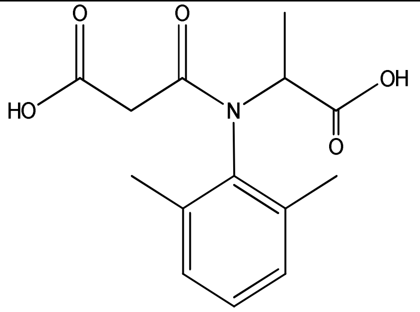
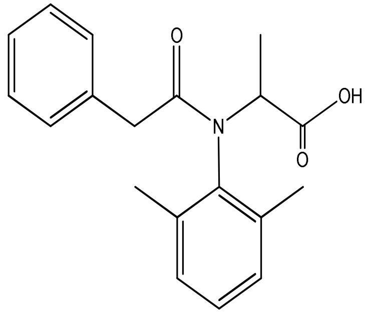
Environmental fate

Soil

The metabolism of [^{14}C-α position of the ester moiety] benalaxyl in aerobic conditions was investigated in previously sieved silt loam soil. Benalaxyl degraded very slowly in the first 28 days after treatment suggesting a lag phase followed by a steady degradation until the end of the incubation period (133 days after treatment). By this time the radioactivity associated with benalaxyl represented only 11.7% AR.

The DT$_{50}$ of benalaxyl taking into account the lag period was estimated to be 77 days. Excluding the initial lag phase of 28 days acclimation/adaptation period during which very little benalaxyl degradation occurred, a shorter DT$_{50}$ of approximately 42 days was estimated.

Chemical Codes and Structures of Benalaxyl Soil Metabolites:

		
Compound A: methyl-N-(2,6-xylyl)-N-malonyl alaninate	Compound B: N-(2,6-xylyl)-N-malonyl alanine	Benalaxyl acid: N-(2,6-xylyl)-N-(phenylacetyl) alanine

Two main degradation products were identified in soil extracts.

Compound A, identified as methyl-N-(2,6-xylyl)-N-malonyl alaninate and Compound B, identified as N-(2,6-xylyl)-N-malonyl alanine with maximum soil concentrations after treatment at 133 days (31% AR) and 98 days (34.1% AR), respectively.

Benalaxyl acid, identified as phenylacetyl-N-2,6-xylyl-DL-alanine was found with maximum soil concentration at 28 days (4.9% AR). In the first period (1–28 day) this is the only metabolite present in soil then from 56 to 133 day the other two metabolites (compound A and compound B) are detectable.

The results of a study of benalaxyl degradation rates in four different soil types (loam/sandy loam, loam, clay loam, and sandy loam) under identical incubation conditions demonstrated DT_{50} values ranging from 77–100 days. The same experiment with one-tenth the initial concentration of benalaxyl gave DT_{50} values of 36–85 days. These results demonstrate that benalaxyl is stable in most soils and show the range of half-life variability in four different soil types.

The degradation rate of benalaxyl in soil essentially depends on the presence of micro-organisms. The concentration and activity of these agents can vary significantly in different soils and account for the range of half-lives determined in the study cited above. The DT_{50} value in sterilized soil was reported as greater than 300 days. Evidence of microbial adaptation was also reported in this study.

Photolysis

Labelled [^{14}C]benalaxyl (≥ 98% radiochemical purity; 100 KBq/mg specific activity) was irradiated under natural sunlight conditions in a distilled sterilized buffer solution at pH 7 and test concentration of 10 mg ai/L for up to 64 days. After 64 days, 60% AR was still present as benalaxyl. At least 15 different compounds were recorded but none of them represented individually more than 5.0% of the applied radioactivity and therefore were not identified. No degradation of benalaxyl was observed under dark conditions. The study was conducted during June–August, 1984 in Milan, Italy.

In a separate experiment, the degree of photolytic degradation and the quantum yield of benalaxyl were determined by irradiation with xenon light at 306 + 12 nm at 20 °C. The absorption coefficients of benalaxyl in the relevant wavelength range around 300 nm were very low (approximately 5–10 mol/L^{-1} cm^{-1}). Just 2% benalaxyl degradation was found after 5 days and 3% degradation after 10 days. Degradation products could not be detected. The quantum yields of the photodegradation as estimated from the 5 days and 10 days irradiations were both 0.01. Thus, benalaxyl may be considered a photolytically stable compound.

Rotational Crops

Rotational crop studies using radiolabelled benalaxyl are available showing very low levels of residues in the following crops (lettuce, tomato, carrot, and wheat) even after application at highly exaggerated rates (approximately 10×). Based on the behaviour of benalaxyl in soil and the findings in the radio-labelled studies, it is unlikely that residues above the limit of quantitation would occur in succeeding crops.

Methods of analysis

The Meeting received description and validation data for a single-residue analytical method for benalaxyl in samples of plant and animal origin. The method is based on extraction with acetone, followed by liquid-liquid extraction using water and dichloromethane and an additional clean-up on an alumina column. The determination of benalaxyl residues is performed using GC-NPD. The method was validated for grapes, lettuce, bovine milk, bovine meat and poultry eggs with a LOQ of 0.02 mg/kg. The recoveries for plant and animal matrices were in the range of 81–102% and 73–

110%, respectively, with RSDs < 10%. The method was used in the supervised trials on plant commodities evaluated by this Meeting (grapes, onions, melons, tomatoes, lettuce, and potatoes) with concurrent recoveries within the range of 80–120% and RSD < 10%.

The Meeting noted that there are several multiresidue methods available (e.g., the German DFG S19 or the QuEChERS methods) that are used in routine monitoring laboratories for the analysis of benalaxyl residues (using GC-MS or GC-NPD for determination).

Adequate multi- and single-residue methods exist for both gathering data in supervised trials and other studies and for monitoring and enforcing benalaxyl MRLs in samples of plant and animal origin.

Stability of pesticide residues in stored analytical samples

The Meeting received information on the stability of benalaxyl in freezer-stored samples of grapes, grape must and pomace, potatoes and tomatoes. The samples were fortified at different concentration levels and stored at –20° C for up to 3 years. The concurrent recoveries were in the range of 98–100%, with RSDs of 4.0–6.4%. The residues remaining after 3 years of storage were in the range of 95–106%, demonstrating very good freezer-storage stability of benalaxyl residues in the tested commodities during the period of 3 years, which well covers the storage intervals in the supervised trials evaluated by this Meeting.

Stability of benalaxyl residues in frozen livestock commodity samples was not demonstrated, but only livestock metabolism studies were conducted.

Definition of the residue

The plant metabolism studies indicate that significant portions of benalaxyl are oxidized and then converted to the corresponding glucoside in plant matrices. However, due to the low absolute levels of metabolites expected in crops at the label use rates and presumed lower toxicity of the polar conjugates formed, the Meeting concluded that the residue definition for plant commodities for purposes of enforcement is benalaxyl. The Meeting also concluded that for purposes of dietary intake considerations, the residue definition is also benalaxyl alone.

The ruminant and poultry metabolism studies showed an initial oxidation step as observed in plants. However, animal metabolism proceeds with further oxidation reactions to form carboxylic acids rather than the glucosides generated in plants. Noting the low levels of benalaxyl residues expected in animal tissues, the Meeting concluded that the residue definition for animal commodities for purposes of enforcement and dietary intake considerations is benalaxyl.

The octanol-water partition coefficient of benalaxyl ($\log K_{OW} = 3.5$) implied that benalaxyl may be fat-soluble. However, the results of the goat metabolism study were inconclusive about the fat solubility issue since such low levels of benalaxyl were found. The Meeting agreed that insufficient information was available to reach a conclusion regarding the fat solubility of benalaxyl.

Results of supervised trials on crops

The NAFTA calculator was used as a tool in the estimation of the maximum residue level from the selected residue data set obtained from trials conducted according to GAP. As a first step, the Meeting reviewed all relevant factors related to each data set in arriving at a best estimate of the maximum residue level using expert judgement. Then, the NAFTA calculator was employed. If the statistical calculation spreadsheet suggested a different value from that recommended by the JMPR, a brief explanation of the deviation was supplied. Some common factors that may lead to rejection of the statistical estimate include when the number of data points is < 15 or when there are a large number of values < LOQ.

Grape

The Meeting received results from supervised trials with benalaxyl used on grapes in France, Italy, and Brazil.

The GAP in Italy specifies 0.20 kg ai/ha, four applications, and a 20 day PHI. There were four trials in Italy at the GAP with a PHI of 20–21 days and five trials in France conducted at the Italian GAP rate with four applications and a PHI of 15 days. Based on the decline results obtained in the same French trials, the benalaxyl residues at the GAP PHI of 20 days are expected to be within ± 25% of the residues obtained at a PHI of 15 days. The benalaxyl residues from trials in Italy and France, ranked order, were (n = 9): 0.055, 0.092, 0.10, 0.11, 0.12, 0.14, 0.15, and 0.17 (2) mg/kg.

The GAP in Brazil specifies 0.24 kg ai/ha, four applications, and a 7 day PHI. Two trials in Brazil were conducted at the GAP. There were also two additional trials at a double rate. Benalaxyl residues were < 0.1 mg/kg in all four trials.

Based on the trials in France and Italy, the Meeting estimated a maximum residue level for benalaxyl in grapes of 0.3 mg/kg to replace the previous recommendation of 0.2 mg/kg, an STMR of 0.12 mg/kg, and an HR of 0.17 mg/kg.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 0.30 mg/kg, which was in agreement with the Meeting’s estimation.

Onion, bulb

The Meeting received results from supervised trials with benalaxyl used on onions in Brazil, France, Italy, Greece, and Spain.

The GAP in Brazil for onions specifies 0.24 kg ai/ha, four applications, and a 7 day PHI. Three trials in Brazil were conducted at the GAP. There were also three additional trials conducted at a double rate. Benalaxyl residues were < 0.1 mg/kg in all six trials.

In Europe, the GAP of Cyprus, Spain, Italy, and France specify 0.20 kg ai/ha, 3 applications, and a PHI of 14, 15, 20 and 28 days, respectively. Ten trials in Greece, France and Italy were conducted at the GAP rate of Cyprus with a PHI of 14 days. Benalaxyl residues were < 0.02 (10) mg/kg. The Meeting noted that the residues were < LOQ of 0.02 mg/kg at PHIs from 0 to 30 days, concluding that benalaxyl residues are unlikely to occur in onions.

The Meeting estimated a maximum residue level for benalaxyl in onion, bulb of 0.02(*) mg/kg to replace the previous recommendation of 0.2 mg/kg, an STMR of 0 mg/kg and an HR of 0 mg/kg.

Cucumber

No residue data were available for cucumber. The Meeting withdrew the previous benalaxyl maximum residue level recommendation of 0.05 mg/kg for cucumber.

Melons, except watermelon

The Meeting received results from supervised trials with benalaxyl used on melons in Italy and Spain. The GAP of Spain for melon specifies 0.20 kg ai/ha, 3 applications, and a 7 day PHI.

Benalaxyl residues in whole fruit, in ranked order, were (n = 9): 0.02 (2), 0.03, 0.04, 0.05, 0.06 (2), 0.08, and 0.15 mg/kg. For melon pulp (n = 7), the ranked order of residues was: < 0.02 (4), 0.02, and 0.05 mg/kg.

The Meeting estimated a maximum residue level of 0.3 mg/kg for melons, except watermelon to replace the previous recommendation of 0.1 mg/kg, an STMR of 0.02 mg/kg and an HR of 0.05 mg/kg.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 0.25 mg/kg, which when rounded up, was in agreement with the Meeting's estimation.

Watermelon

The Meeting received results from supervised trials with benalaxyl used on watermelon in Italy and Spain. The GAP of Spain for watermelon specifies 0.20 kg ai/ha, 3 applications, and a 7 day PHI.

Benalaxyl residues in whole fruit, in ranked order, were (n = 5): < 0.02 (4) and 0.03 mg/kg. In two trials from Spain, benalaxyl residues in watermelon pulp were < 0.02 (2) mg/kg.

The Meeting estimated a maximum residue level of 0.1 mg/kg for watermelon, an STMR of 0.02 mg/kg and an HR of 0.02 mg/kg.

Peppers

No residue data were available for peppers. The Meeting withdrew the previous benalaxyl maximum residue level recommendations of 0.05 mg/kg for peppers, sweet and 0.5 mg/kg for chilli peppers, dry.

Tomato

The Meeting received results from supervised trials with benalaxyl used on tomato in Brazil, France, Italy and Spain. The GAPs of Spain, Italy and France for tomato specify 0.24 kg ai/ha, 4 applications, and a PHI 3, 7, and 14 days.

Four trials in Italy were conducted according to the GAP of Spain with a PHI of 3 days. Benalaxyl residues, in ranked order, were (n = 4): 0.10, 0.11, and 0.14 (2) mg/kg. The Meeting agreed that four tomato trials were insufficient for a maximum residue level estimate.

Eight trials in France, Italy and Spain were conducted according to the GAP of France with a PHI of 14 days. Benalaxyl residues, in ranked order, were (n = 8): < 0.02 (2), 0.02, 0.03, 0.04 (2), and 0.05 (2) mg/kg.

The GAP of Brazil for tomato specifies 0.24 kg ai/ha, 4 applications, and a 7 day PHI. Five trials in Brazil were conducted at the GAP. There were also four additional trials conducted at a double rate. Benalaxyl residues were < 0.1 mg/kg in all nine trials.

Based on the trials in France, Italy, and Spain according to the French GAP, the Meeting estimated a maximum residue level of 0.2 mg/kg for tomato to replace the previous recommendation of 0.5 mg/kg, an STMR of 0.035 mg/kg and an HR of 0.05 mg/kg.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 0.15 mg/kg (making use of Maximum Likelihood Estimate [MLE] procedures to fit data points below the LOQ to a lognormal distribution), which when rounded up was in agreement with the Meeting's estimation.

Lettuce, Head

The Meeting received results from supervised trials with benalaxyl used on head lettuce in Italy and Spain. The GAP of Italy and Spain specify 0.20 kg ai/ha, 3 applications, and a PHI 15 days.

The trials in Spain (n = 8) were conducted with 2 applications but, based on the data from Italian trials, the benalaxyl residues determined prior to the last application were insignificant in comparison with the residues determined on day 0 of the last application. Therefore, the Meeting considered the Spanish trials together with the trials in Italy (n = 7), which were conducted at the GAP of Italy with 3 applications.

The benalaxyl residues in head lettuce, in ranked order, were (n = 15): < 0.02 (4), 0.06 (3), 0.07, 0.08, 0.09, 0.11, 0.12, 0.15, 0.33, and 0.43 mg/kg.

The Meeting estimated a maximum residue level for benalaxyl in lettuce, head of 1 mg/kg, an STMR of 0.07 mg/kg, and an HR of 0.43 mg/kg.

The maximum residue level estimate derived from the use of the NAFTA statistical calculator was 1.0 mg/kg (making use of MLE procedures), which was in agreement with the Meeting's estimation.

Potato

The Meeting received results from supervised trials with benalaxyl used on potato in Brazil, France, and Italy.

The GAP of Brazil for potato specifies 0.24 kg ai/ha, 2 applications, and a 7 day PHI. Five trials in Brazil were conducted at the GAP. There were also five additional trials conducted at a double rate. Benalaxyl residues were < 0.1 mg/kg in all 10 trials.

The GAPs of France and Italy for potato specify 0.24 kg ai/ha, 4 applications, and a 7 day PHI. Six trials in France and Italy were conducted at the GAP rate, with benalaxyl residues being < 0.02 (6) mg/kg.

Based on the results of the potato metabolism study, which showed no transfer of radioactivity to the tubers, the Meeting agreed that no benalaxyl residues are expected in potatoes.

The Meeting estimated a maximum residue level for benalaxyl in potato of 0.02(*) mg/kg to confirm its previous recommendation, an STMR of 0 mg/kg and an HR of 0 mg/kg.

Hops, dry

No residue data were available for dry hops. The Meeting withdrew the previous benalaxyl maximum residue level recommendation of 0.2 mg/kg for hops, dry.

Fate of residues during processing

The Meeting received processing studies for grape and tomato. The residue definition recommended for plant commodities will suffice for processed plant commodities (parent only).

The processing (or transfer) factors derived from the processing studies and the resulting recommendations for STMR-P values are summarized in the table below. The factors are the ratio of the total residue in the processed commodity divided by the total residue in the raw agricultural commodity (RAC).

Processing (Transfer) Factors from the Processing of Raw Agricultural Commodities (RACs) with Field-Incurred Residues from Foliar Treatment with Benalaxyl

RAC	RAC STMR	Processed Commodity	Processing Factor[a]	Processed Commodity STMR-P
Grapes	0.12	Juice	0.11, 0.18, 0.15, 0.16 Median: 0.155	0.019
		Wet Pomace	3.3, 3.8 Mean: 3.5	0.42
		Bottled Wine	0.22, 0.36, 0.15, 0.16, Median:0.19	0.03
Tomato	0.035	Juice	0.22, 0.22 Mean: 0.22	0.0077
		Puree	0.21, 0.48 Mean: 0.344	0.012
		Preserve	0.10, 0.22 Mean: 0.16	0.0056

[a] Each value represents a separate study. The processing factor is the ratio of the total residue in the processed item divided by the total residue in the RAC.

Based on the STMR-P value of 0.42 mg/kg and dry-weight content of 15% for grape pomace, wet, the Meeting estimated an STMR-P value of 2.8 mg/kg and a maximum residue level of 3 mg/kg for benalaxyl in grape pomace, dry.

Estimated maximum and mean dietary burdens of farm animals

Dietary burden calculations for beef cattle and dairy cattle are provided below. The calculations were made according to the animal diets from Canada-USA, EU, and Australia in the Table of OECD Feedstuffs Derived from Field Crop (Annex 6 of the 2006 JMPR Report).

Grape pomace, dry is the only potential cattle feed item.

Animal dietary burden, benalaxyl residue, ppm of dry matter diet				
		US-Canada	EU	Australia
Beef/Dairy cattle	Max	0	0	0.56
	Mean	0	0	0.56

Animal commodity maximum residue levels

A bovine feeding study was not provided. However, there are no cattle feed items resulting from the RACs for which the 2009 Meeting made maximum residue level recommendations, except for wet grape pomace, which is a feed item only for Australia. Moreover, as indicated in the *FAO Manual* [Second Edition] (Section 3.9), a bovine feeding study is not necessary when a ruminant metabolism study with dosing at the equivalent of 10×, where 1× is the anticipated dietary burden, results in levels of the residue of concern below the limit of quantitation (LOQ) in all edible commodities. Accordingly, the Meeting determined that no bovine feeding study is necessary at this time.

The Meeting estimated maximum residue levels of 0.02(*) mg/kg and STMR and HR values of 0 mg/kg for benalaxyl in meat from mammals (other than marine mammals), edible offal (mammalian), and milks.

A poultry feeding study was not provided. However, as there are no poultry feed items resulting from the RACs for which the 2009 Meeting made maximum residue level recommendations, there was no need to recommend maximum residue levels for poultry commodities.

RECOMMENDATIONS

On the basis of the data from supervised trials the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits and for IEDI and IESTI assessments.

Definition of the residue (for compliance with MRL and estimation of dietary intake) for plant and animal commodities: *benalaxyl*.

Commodity		MRL, mg/kg		STMR or STMR-P, mg/kg	HR, mg/kg
CCN	Name	New	Previous		
VC 0424	Cucumber	W [a]	0.05		
DH 1100	Hops, dry	W [a]	0.2		
HS 0444	Pepper, chilli, dry	W [a]	0.5		
VO 0445	Peppers, sweet	W [a]	0.05		
MO 0105	Edible offal (mammalian)	0.02*		0	0

Commodity		MRL, mg/kg		STMR or	HR, mg/kg
CCN	Name	New	Previous	STMR-P, mg/kg	
MM 0095	Meat from mammals (other than marine mammals)	0.02*		0	0
ML 0106	Milks	0.02*		0	
FB 0269	Grapes	0.3	0.2	0.12	0.17
AB 0269	Grape pomace, dry	3		2.8	
VL 0482	Lettuce, head	1		0.07	0.43
VC 0046	Melons, except watermelon	0.3	0.1	0.02	0.05
VA 0385	Onion, bulb	0.02*	0.2	0	0
VR 0589	Potato	0.02*	0.02*	0	0
VO 0448	Tomato	0.2	0.5	0.035	0.05
VC 0432	Watermelon	0.1		0.02	0.02
JF 0269	Grape juice			0.019	
JF 0448	Tomato juice			0.0077	
	Tomato preserve			0.0056	
	Tomato puree			0.012	
	Wine			0.023	

[a] W: the recommendation is withdrawn as no data were submitted in support of this crop.

* : at or about the limit of quantitation.

DIETARY RISK ASSESSMENT

Long-term intake

The evaluation of benalaxyl has resulted in recommendations for MRLs and STMRs for raw and processed commodities. These commodities were included at the appropriate levels in the dietary intake calculations. The International Estimated Daily Intakes (IEDI) for the 13 GEMS/Food Consumption Cluster Diets, based on estimated STMRs were in the range 0–1% of the maximum ADI of 0.07 mg/kg bw The results are shown in Annex 3 of the 2009 JMPR Report.

The Meeting concluded that the long-term intake of residues of benalaxyl from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-Term Intake (IESTI) for benalaxyl was calculated for the food commodities (and their processing fractions) for which maximum residue levels and HRs were estimated and for which consumption data were available. The results are shown in Annex 4 of the 2009 JMPR Report. For benalaxyl, the IESTI varied from 0–4% of the ARfD (0.1 mg/kg bw) for women of childbearing age using the intake figures for the general population.

The Meeting concluded that the short-term intake of residues of benalaxyl from uses that have been considered by the JMPR is unlikely to present a public health concern.

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