Abstract

According to Article 12 of Regulation (EC) No 396/2005, EFSA has reviewed the maximum residue levels (MRLs) currently established at European level for the pesticide active substance benalaxyl-M. To assess the occurrence of benalaxyl-M residues in plants, processed commodities, rotational crops and livestock, EFSA considered the conclusions derived in the framework of Commission Regulation (EU) No 188/2011, the European authorisations for benalaxyl-M reported by Member States (including the supporting residues data) and also the authorised uses of benalaxyl reviewed by EFSA in the past (as they may generate common residues with benalaxyl-M) and the MRLs established for benalaxyl by the Codex Alimentarius Commission. Based on the assessment of the available data, MRL proposals were derived and a consumer risk assessment was carried out. Although no apparent risk to consumers was identified, some information required by the regulatory framework was missing. Hence, the consumer risk assessment is considered indicative only and all MRL proposals derived by EFSA still require further consideration by risk managers.

Keywords: benalaxyl-M, MRL review, benalaxyl, Regulation (EC) No 396/2005, consumer risk assessment, fungicide

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Summary

Benalaxyl-M was approved on 1 May 2014 by means of Commission Implementing Regulation (EU) No 1175/2013 under Regulation (EC) No 1107/2009 as amended by Commission Implementing Regulations (EU) No 540/2011 and 541/2011.

As the active substance was approved after the entry into force of Regulation (EC) No 396/2005 on 2 September 2008, the European Food Safety Authority (EFSA) is required to provide a reasoned opinion on the review of the existing maximum residue levels (MRLs) for that active substance in compliance with Article 12(1) of the aforementioned regulation.

As the basis for the MRL review, on 15 September 2017 EFSA initiated the collection of data for this active substance. In a first step, Member States were invited to submit by 13 October 2017 their national Good Agricultural Practices (GAPs) in a standardised way, in the format of specific GAP forms, allowing the designated rapporteur Member State Portugal to identify the critical GAPs in the format of a specific GAP overview file. Subsequently, Member States were requested to provide residue data supporting the critical GAPs, within a period of 1 month, by 2 January 2018. On the basis of all the data submitted by Member States and by the EU Reference Laboratories for Pesticides Residues (EURL), EFSA asked the rapporteur Member State (RMS) to complete the Pesticide Residues Overview File (PROFile) and to prepare a supporting evaluation report. The PROFile and evaluation report, together with an updated GAP overview file, were provided by the RMS to EFSA on 4 April 2018. Subsequently, EFSA performed the completeness check of these documents with the RMS. The outcome of this exercise including the clarifications provided by the RMS, if any, was compiled in the completeness check report.

Based on the information provided by the RMS, Member States and the EURL, and taking into account the conclusions derived by EFSA in the framework of Commission Regulation (EU) No 188/2011 and the MRLs established for benalaxyl by the Codex Alimentarius Commission, EFSA prepared in March–April 2019 a draft reasoned opinion, which was circulated to Member States for consultation via a written procedure. Comments received by 15 May 2019 were considered during the finalisation of this reasoned opinion. The following conclusions are derived.

The metabolism of benalaxyl-M in plant was investigated in primary and rotational crops. It is noted that the available metabolites have several shortcomings. Therefore, representative studies fully characterising the nature of residues of benalaxyl-M in all crop groups are still required. Studies investigating the nature of residues of benalaxyl-M or racemic benalaxyl are not available and not required.

A specific residue definition for rotational crops is not deemed necessary since residues of benalaxyl-M in the soil above the limit of quantification (LOQ) are not expected. Nevertheless, the outcome of the renewal assessment of benalaxyl-M may need to be considered following its finalisation.

Based on the available data, the plant residue definition for monitoring can be tentatively proposed as ‘benalaxyl-M’ noting that it is compatible with the current definition for enforcement, namely, ‘benalaxyl including other mixtures of constituent isomers including benalaxyl-M (sum of isomers)’ which can still be considered.

Benalaxyl-M may not be the only toxicologically relevant compound as indicated during the renewal assessment of benalaxyl and following its finalisation new information may needs to be considered. Meanwhile, the plant residue definition for risk assessment previously derived in the peer review is tentatively maintained: ‘benalaxyl-M’. Considering that benalaxyl-M and benalaxyl are of similar toxicity, this residue definition is also applicable in the frame of a comprehensive risk assessment comprising benalaxyl. It is to be noted that this might need to be reconsidered in the future based on the outcome of the ongoing renewal of benalaxyl.

Fully validated analytical methods are available for the enforcement of benalaxyl-M in high water and high acid commodities at the LOQ of 0.02 mg/kg. According to the EURLs, the for benalaxyl a LOQ of 0.01 mg/kg is achievable by using the QuEChERS method in routine analyses.

The storage stability of racemic benalaxyl and benalaxyl-M was demonstrated in high water and acid commodities (grapes, wine, potatoes and tomatoes) for 3 years at –20°C.

Available residue trials data were considered appropriate to derive tentative MRL proposals as well as risk assessment values for all commodities under evaluation. Considering the general data gap identified for the metabolism studies covering fruit, leafy, root and tuber crops, all the MRL proposals are considered as tentative.

Benalaxyl-M is authorised for use on crops that might be fed to livestock. Livestock dietary burden calculations were therefore performed for different groups of livestock according to the OECD guidance. Since the calculated dietary burdens for all groups of livestock were found to be below the...
trigger value of 0.1 mg/kg dry matter (DM), further investigation of residues as well as the setting of MRLs in commodities of animal origin is unnecessary noting that the metabolism of benalaxyl which is considered comparable to the one in benalaxyl-M residues in livestock was investigated in lactating goats and laying hens at dose rate covering the maximum dietary burdens calculated in this review.

An analytical method for the enforcement of benalaxyl-M at the LOQ of 0.01 mg/kg in fat, kidney and liver and at the LOQ of 0.02 mg/kg in meat, eggs and milk is available. According to the EURLs, the screening detection limit (SDL) of 0.0025 mg/kg for benalaxyl is achievable by using the QuEChERS method in routine analyses (EURL, 2018).

Chronic consumer exposure resulting from the authorised uses reported in the framework of this review was calculated using revision 2 of the EFSA Pesticide Residues Intake Model (PRIMo). For those commodities where data were insufficient to derive a MRL, EFSA considered the existing EU MRL for an indicative calculation. The highest chronic exposure represented 1.1% of the acceptable daily intake (ADI) (WHO Cluster diet B).

Apart from the MRLs evaluated in the framework of this review, internationally recommended CXLs have also been established for benalaxyl. Furthermore, uses for benalaxyl were reviewed by EFSA previously and since these may generate residues of benalaxyl-M, they were considered in this review.

Therefore, in a second scenario, the overall exposure to benalaxyl resulting from the authorised uses of benalaxyl and benalaxyl-M in EU and outside EU was assessed. For that purpose, all available MRL values from benalaxyl and benalaxyl-M reviews and CXLs were compared and the highest of these three sources was taken for the consumer exposure estimate. It is assumed that benalaxyl and benalaxyl-M are not used simultaneously according to the authorised uses. The highest chronic exposure for this second scenario represented 2.2% of the ADI (WHO Cluster diet B).
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Background

Regulation (EC) No 396/2005 (hereinafter referred to as 'the Regulation') establishes the rules governing the setting and the review of pesticide maximum residue levels (MRLs) at European level. Article 12(1) of that Regulation stipulates that the European Food Safety Authority (EFSA) shall provide within 12 months from the date of the inclusion or non-inclusion of an active substance in Annex I to Directive 91/414/EEC a reasoned opinion on the review of the existing MRLs for that active substance.

As benalaxyl-M was approved on 1 May 2014 by means of Commission Implementing Regulation (EU) No 1175/2013 under Regulation (EC) No 1107/2009 as amended by Commission Implementing Regulations (EU) No 540/2011 and 541/2011, EFSA initiated the review of all existing MRLs for that active substance.

By way of background information, in the framework of Commission Regulation (EU) No 188/2011 Benalaxyl-M was evaluated by Portugal, designated as rapporteur Member State (RMS). Subsequently, a peer review on the initial evaluation of the RMS was conducted by EFSA, leading to the conclusions as set out in the EFSA Conclusion (EFSA, 2013a).

According to the legal provisions, EFSA shall base its reasoned opinion in particular on the relevant assessment report prepared under Directive 91/414/EEC repealed by Regulation (EC) No 1107/2009. It should be noted, however, that, in the framework of Regulation (EC) No 1107/2009, only a few representative uses are evaluated, whereas MRLs set out in Regulation (EC) No 396/2005 should accommodate all uses authorised within the European Union (EU), and uses authorised in third countries that have a significant impact on international trade. The information included in the assessment report prepared under Regulation (EC) No 1107/2009 is therefore insufficient for the assessment of all existing MRLs for a given active substance.

To gain an overview of the pesticide residues data that have been considered for the setting of the existing MRLs, EFSA developed the Pesticide Residues Overview File (PROFile). The PROFile is an inventory of all pesticide residues data relevant to the risk assessment and MRL setting for a given active substance. This includes data on:

- the nature and magnitude of residues in primary crops;
- the nature and magnitude of residues in processed commodities;
- the nature and magnitude of residues in rotational crops;
- the nature and magnitude of residues in livestock commodities;
- the analytical methods for enforcement of the proposed MRLs.

As the basis for the MRL review, on 15 September 2017 EFSA initiated the collection of data for this active substance. In a first step, Member States were invited to submit by 13 October 2017 their Good Agricultural Practices (GAPs) that are authorised nationally, in a standardised way, in the format of specific GAP forms. In the framework of this consultation, 13 Member States provided feedback on their national authorisations of benalaxyl-M. Based on the GAP data submitted, the designated RMS Portugal was asked to identify the critical GAPs to be further considered in the assessment, in the format of a specific GAP overview file. Subsequently, in a second step, Member States were requested to provide residue data supporting the critical GAPs by 2 January 2018.

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1 Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1–16.
2 Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1–32. Repealed by Regulation (EC) No 1107/2009.
3 Commission Implementing Regulation (EU) No 1175/2013 of 20 November 2013 approving the active substance benalaxyl-M, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. OJ L 312, 21.11.2013, p. 18–22.
4 Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.
5 Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p. 1–186.
6 Commission Implementing Regulation (EU) No 541/2011 of 1 June 2011 amending Implementing Regulation (EU) No 540/2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p. 187–188.
7 Commission Regulation (EU) No 188/2011 of 25 February 2011 laying down detailed rules for the implementation of Council Directive 91/414/EEC as regards the procedure for the assessment of active substances which were not on the market 2 years after the date of notification of that Directive. OJ L 53, 26.2.2011, p. 51–55.
On the basis of all the data submitted by Member States and the EU Reference Laboratories for Pesticides Residues (EURL), EFSA asked Portugal to complete the PROFile and to prepare a supporting evaluation report. The PROFile and the supporting evaluation report, together with an updated GAP overview file, were submitted to EFSA on 4 April 2018. Subsequently, EFSA performed the completeness check of these documents with the RMS. The outcome of this exercise including the clarifications provided by the RMS, if any, was compiled in the completeness check report.

Considering all the available information, and taking into account the MRLs established for benalaxyl by the Codex Alimentarius Commission (CAC) (i.e. codex maximum residue limit; CXLs), EFSA prepared in March-April 2019 a draft reasoned opinion, which was circulated to Member States for commenting via a written procedure. All comments received by 15 May 2019 were considered by EFSA during the finalisation of the reasoned opinion.

The evaluation report submitted by the RMS (Portugal, 2018), taking into account also the information provided by Member States during the collection of data (Austria, 2017), and the EURL report on analytical methods (EURL, 2018) are considered as main supporting documents to this reasoned opinion and, thus, made publicly available.

In addition, further supporting documents to this reasoned opinion are the completeness check report (EFSA, 2019a) and the Member States consultation report (EFSA, 2019b). These reports are developed to address all issues raised in the course of the review, from the initial completeness check to the reasoned opinion. Furthermore, the exposure calculations for all crops reported in the framework of this review performed using the EFSA Pesticide Residues Intake Model (PRIMo) and the PROFile as well as the GAP overview file listing all authorised uses are key supporting documents and made publicly available as background documents to this reasoned opinion. A screenshot of the report sheet of the PRIMo is presented in Appendix C.

It is underlined that the peer review for the renewal of benalaxyl in accordance with Regulation (EC) No 1107/2009 is currently ongoing and therefore the conclusions reported in this reasoned opinion might need to be reconsidered in the light of the outcome of the peer review for the renewal.

Terms of Reference

According to Article 12 of Regulation (EC) No 396/2005, EFSA shall provide a reasoned opinion on:

- the inclusion of the active substance in Annex IV to the Regulation, when appropriate;
- the necessity of setting new MRLs for the active substance or deleting/modifying existing MRLs set out in Annex II or III of the Regulation;
- the inclusion of the recommended MRLs in Annex II or III to the Regulation;
- the setting of specific processing factors as referred to in Article 20(2) of the Regulation.

The active substance and its use pattern

Benalaxyl-M is the ISO common name for methyl \(N\)-(phenylacetyl)-\(N\)-(2,6-xylyl)-\(\alpha\)-alaninate (IUPAC). The chemical structure of the active substance and its main metabolites are reported in Appendix F. Benalaxyl-M is the purified \(R\)-enantiomer of benalaxyl, a racemic mixture of two enantiomers. The EU MRLs for benalaxyl-M are established in Annexes II and IIIB of Regulation (EC) No 396/2005, as amended by Commission Regulations (EC) No 839/2008\(^8\) and No 149/2008\(^9\). It is noted that MRLs specifically for benalaxyl-M are not defined since the MRLs are set for the unresolved mixture ‘Benalaxyl including other mixtures of constituent isomers including benalaxyl-M (sum of isomers)’. CXLs for benalaxyl, were also established by the CAC. It is be noted that CXLs specifically for benalaxyl-M are not established. An overview of the MRL changes that occurred since the entry into force of the Regulation mentioned above is provided below (Table 1).

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\(^{8}\) Commission Regulation (EC) No 839/2008 of 31 July 2008 amending Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards Annexes II, III and IV on maximum residue levels of pesticides in or on certain products. OJ L 234, 30.8.2008, p. 1-216.

\(^{9}\) Commission Regulation (EC) No 149/2008 of 29 January 2008 amending Regulation (EC) No 396/2005 of the European Parliament and of the Council by establishing Annexes II, III and IV setting maximum residue levels for products covered by Annex I thereto. OJ L 58, 1.3.2008, p. 1-398.
For the purpose of this MRL review, all the uses of benalaxyl-M currently authorised within the EU as submitted by the Member States during the GAP collection, have been reported by the RMS in the GAP overview file. The critical GAPs identified in the GAP overview file were then summarised in the PROFile and considered in the assessment. The details of the authorised critical GAPs for benalaxyl-M are given in Appendix A. The RMS did not report any use authorised in third countries that might have a significant impact on international trade. It is noted that the critical uses of benalaxyl have been collected and previously reviewed (EFSA, 2013b). Considering that benalaxyl-M and benalaxyl may generate common residues, the uses on benalaxyl were also considered in the present review.

**Assessment**

EFSA has based its assessment on the following documents:

- the PROFile submitted by the RMS;
- the evaluation report accompanying the PROFile (Portugal, 2018);
- the draft assessment report (DAR) on benalaxyl-M and its addenda prepared under Council Directive 91/414/EEC and Commission Regulation (EU) No 188/2011 (Portugal, 2003, 2007, 2013);
- the conclusion on the peer review of the pesticide risk assessment of the active substance benalaxyl-M (EFSA, 2013a);
- the Joint Meeting on Pesticide residues (JMPR) Evaluation report (FAO, 2009);
- the previous reasoned opinion on the review of the existing MRLs on benalaxyl (EFSA, 2013b).

The assessment is performed in accordance with the legal provisions of the uniform principles for evaluation and authorisation of plant protection products as set out in Commission Regulation (EU) No 546/2011 and the currently applicable guidance documents relevant for the consumer risk assessment of pesticide residues (European Commission, 1997a-g, 2000, 2010a,b, 2017; OECD, 2011, 2013).

More detailed information on the available data and on the conclusions derived by EFSA can be retrieved from the list of end points reported in Appendix B.

### 1. Residues in plants

#### 1.1. Nature of residues and methods of analysis in plants

##### 1.1.1. Nature of residues in primary crops

The available metabolism studies were carried out either comparatively with $^{14}$C-labelled benalaxyl-M (the pure R-enantiomer of benalaxyl) and $^{14}$C-labelled benalaxyl (fruiting vegetables (wine leaves and tomato leaves),) and root and tuber vegetables (potato leaves)) or with $^{14}$C-labelled benalaxyl only, namely on fruiting vegetables (grape and tomato), on leafy vegetables (tobacco) and on root and tuber vegetables (potato). Studies investigating leaves and not analysing the edible part of plant commodities...
were considered as informative only and were not considered representative for edible fruits and vegetables. All studies were considered during the peer review of benalaxyl-M (EFSA, 2013a).

In wine leaves, after foliar applications of benalaxyl-M, the major components identified were benalaxyl-M, representing 1 day after treatment (DAT) 95.64% total radioactive residues (TRR) and 14 DAT 31.57% TRR and metabolite GX1 (monoglucobenalaxyl) which was present 1 DAT with 1.47% TRR and 14 DAT with 54.88% TRR. Other metabolites were not significant. The study was performed in parallel on another set of plants treated with benalaxyl. Benalaxyl was reported to have an identical metabolic pathway as benalaxyl-M with the parent being present with 43.23% TRR and metabolite GX1 representing 51.72% TRR 14 DAT. Residues in grapes were not investigated in both studies.

In tomato leaves, after foliar applications benalaxyl-M, the major components identified were benalaxyl-M, representing one DAT 28.02% and 14 DAT 19.21% TRR and metabolite GX11 (malonyl conjugate of GX1) which was not present 1 DAT and augmented to 36.35% TRR 14 DAT. Other metabolites were not significant. The study was performed in parallel on another set of tomato plants with benalaxyl, which was present 1 DAT with 20.11% TRR and 14 DAT with 12.4% TRR whereby GX11 represented 55.32% TRR. Residues in tomatoes were not investigated in both studies.

In another study presented during the peer review, the metabolism benalaxyl-M and benalaxyl, respectively on potato plants was investigated. In potato leaves, benalaxyl-M augmented to 28.44% TRR (benalaxyl to 20.61% TRR) 14 DAT with GX11 being the major metabolite with 36.50% TRR and 32.51% TRR, respectively. Metabolite GX12 (malonyl derivate of GX6 (di-gluco-benalaxyl) noting that the stereochemistry of the conjugation is not known) represented 13.06% TRR for benalaxyl treatment and 7.36% TRR for the benalaxyl-M treated leaves 14 DAT.

In the studies on wine and tomato leaves, it was confirmed by high-performance liquid chromatography (HPLC) measurement that the ratio of the R- and S-enantiomers of benalaxyl did not change and that benalaxyl-M was always made up of the R-enantiomer and racemisation did not occur. While benalaxyl was reported to have a similar pathway, it was absorbed and metabolised faster than benalaxyl-M. This stereo-selective degradation was also indicated in the peer reviewed literature and leads to an enrichment of benalaxyl-M (EFSA, 2013a).

The metabolism of benalaxyl was studied in tomato fruits and highest concentrations of benalaxyl (10.68% TRR, 0.063 mg eq/kg) were found insight tomatoes 14 DAT. Metabolite GX11 increased to 14.09% TRR (0.036 mg eq/kg) 21 DAT.

The metabolism of benalaxyl was studied in ripe grapes where highest concentrations of benalaxyl (97.74-93.30% TRR, 3.46-3.48 mg eq/kg) were found 3 h to 3 DAT. Metabolites GX1 increased to 17.65% TRR (0.36 mg eq/kg) 15 DAT and GX6 to 10.81% TRR (0.28 mg eq/kg) 24 DAT, respectively.

In potato plants treated with benalaxyl, the major component identified 26 DAT in the leaves was the parent, representing 16.37% TRR (0.55 mg eq/kg) while metabolite GX11 was present at 20.53% TRR (0.69 mg eq/kg), metabolite GX13 (structure not identified) was present at 21.43% TRR (0.72 mg eq/kg), while additionally identified metabolites GX1, were below 10% TRR (Portugal, 2007). In this study, residue levels of benalaxyl and metabolites in the roots/tuber could not be identified since residues were below the LOQ and between 0.002 and 0.005 mg eq/kg at all days after treatment. It was concluded that the parent does not transfer to tubers following foliar application on leaves or due to dripping in soil (Portugal, 2007).

An additional study of benalaxyl in potato leaves, showed that in the leaves benalaxyl increased to 26.47% TRR (2.91 mg eq/kg) 16 DAT. Metabolites GX11 and GX12 increased to 21.70% TRR (2.39 mg eq/kg) and 19.51% TRR (2.15 mg eq/kg), respectively (Portugal, 2007).

An earlier study on tobacco leaves showed that in the leaves benalaxyl increased from 11 to 12 mg eq/kg (76-81.4%TRR) 21 DAT. It was reported that the polar metabolite GX11 tends to degrade to GX1 (0.15 mg/k; 21 DAT) and GX16 (0.86 mg/kg; 21 DAT) (malonyl derivate of GX4) degrades to GX4 (n-glucobenalaxyl, structure not unambiguously identified (0.18 mg/kg; 21 DAT)) depending on release of malonic acid bound to glucose molecule (Portugal, 2003). Notably, the study duration does not cover the preharvest interval (PHI) of 28 days of the critical GAP (cGAP) for lettuce in this review and deconjugation of longer to shorter metabolites is reported.

During the peer review, it was concluded that the metabolic pathway of benalaxyl-M and racemic benalaxyl was similar in fruiting, leafy and root and tuber vegetables. After application, benalaxyl-M is progressively incorporated into plant material by formation of glucosides. Metabolites resulted from hydroxylation of the parent and subsequent conjugation with one or more molecules of glucose, or additionally with malonic acid (EFSA, 2013a).
It is further noted that, in the framework of the renewal assessment of benalaxyl, which is ongoing, an additional metabolism study on tomatoes is under assessment which was not assessed in this Art. 12 review (Romania, 2018).

It is noted that the available studies are not fully representative for the cGAPs (except for melons and watermelons) of this review because of a too short PHI (lettuces and garlic or an underdosed application rate (potato, tomato, aubergines) or both (wine and table grapes, onions, shallots and leek) and can therefore only be considered on a tentative basis. Therefore, metabolism studies in three primary crop groups are still required (data gap).

1.1.2. Nature of residues in rotational crops

Benalaxyl-M is authorised on crops that may be grown in rotation. The field DT₉₀ reported in the soil degradation studies evaluated in the framework of the peer review was up to 326 days (EFSA, 2013a).

In the MRL review of benalaxyl, the metabolism of benalaxyl in rotational crops was studied in tomato, lettuce, carrot, wheat and tobacco with ¹⁴C-labelled benalaxyl (Portugal, 2003; EFSA, 2013b). The radiolabelled active substance was applied on a bare soil once at an application rate of 2.25 kg a.s./ha and crops were sown or planted around 30, 110 to 120 and 295–370 DAT. Studies are summarised in Table B.1.1.1.

TRRs for all plant-back intervals ranged between 0.02 and 0.11 mg eq/kg in mature lettuce, 0.01 and 0.10 mg eq/kg in tomato, 0.02 to 0.06 mg eq/kg in the root parts of carrot, up to 0.16 mg eq/kg in wheat grain and up to 0.24 mg eq/kg in wheat straw (Portugal, 2003; EFSA, 2013b).

Despite the fact, that the TRR levels were significant in most of the samples, no further characterisation or identification of the residues was conducted. Consequently, it was not possible to conclude on the comparability of the metabolic patterns in rotational and primary crops (EFSA, 2013b). This conclusion is supported in this review. It is further noted that the available studies were performed with racemic benalaxyl only and are considered representative for benalaxyl-M.

It is noted, however, that in the ongoing renewal assessment of benalaxyl, new data were made available and the metabolite benalaxyl acid (M9) was considered relevant because of its high persistence in soil (Romania, 2018). The possible uptake of M9 in rotational crops is however expected to be assessed during the ongoing renewal where an additional confined rotational crops study has been made available.

1.1.3. Nature of residues in processed commodities

There were no studies investigating the nature of residues of benalaxyl-M or racemic benalaxyl in processed commodities available for this review. During the peer review of benalaxyl-M, the absence of data on the nature of residues in processed commodities was noted and considered not necessary based on the requirements at the time (EFSA, 2013a).

Since in the current review, the estimated total theoretical maximum daily intake is below 10% of the ADI standard hydrolysis studies to investigate the nature of residues in processed commodities are not required.

This may need to be reconsidered in the future if new primary metabolism studies become available, and/or the dietary consumer risk assessment needs to be updated.

1.1.4. Methods of analysis in plants

During the peer-review, for enforcement a validated hyphenated analytical method based on HPLC with a chiral column coupled to mass spectrometric (MS) detection was validated in grape bunches and wine, with a LOQ of 0.02 mg/kg and was proposed. This primary method is supported by an ILV (EFSA, 2013a). Another hyphenated analytical method based on HPLC with a chiral column coupled to MS/MS detection for all commodities however open for ILV and a confirmatory method, with a LOQ of 0.01 mg/kg was evaluated during the peer review (EFSA, 2013a).

During the completeness check, the EURLs provided a QuEChERS multiresidue analytical method using gas chromatography with tandem mass spectrometry (GC-MS/MS with a LOQ of 0.01 mg/kg for routine analyses validated with benalaxyl in high water and high acid commodities (EURL, 2018). The proposed method for routine analysis without a chiral column cannot differentiate between benalaxyl and benalaxyl-M. However, based on the experience of the EURLs with other enantiomers, the two enantiomers of benalaxyl are expected to show an equivalent analytical behaviour and therefore the validation data for benalaxyl and considered transferable to benalaxyl-M (EURLs, 2018).
1.1.5. Stability of residues in plants

The storage stability of racemic benalaxyl was evaluated on high water and acid commodities (grapes, wine, potatoes and tomatoes) stored in the dark at $-20^\circ \text{C}$ over 3 years of storage during the peer review (EFSA, 2013a). During this period no appreciable degradation occurred. The racemic benalaxyl demonstrated to remain stable for up to 3 years. It was assessed that the $R$-isomer (benalaxyl-M) does not degrade in the same storage conditions.

Based on the above studies, it is concluded that the $R$-enantiomer benalaxyl-M is also stable under these storage conditions.

1.1.6. Proposed residue definitions

The metabolic pathway was indicatively similar in all crop groups investigated (Section 1.1.1). After application, benalaxyl-M is expected to progressively be incorporated into plant material by formation of glucosides. Metabolites resulted from hydroxylation of the parent and subsequent conjugation with one or more molecules of glucose, or additionally with malonic acid. These glucoside and malonic acid glucoside conjugates of the parent of hydroxylated parent were found at significant proportions.

Based on the available data, the plant residue definition for monitoring can be tentatively proposed as ‘benalaxyl-M’ noting that it is compatible with the current definition for enforcement, namely, benalaxyl including other mixtures of constituent isomers including benalaxyl-M (sum of isomers) which can still be considered. It is noted that this conclusion might need to be reconsidered after the peer review for the renewal of benalaxyl, when all available metabolism studies and toxicological information on the metabolites will be re-assessed.

In the currently ongoing renewal assessment of benalaxyl, it was noted that benalaxyl and benalaxyl-M may not be the only toxicologically relevant compounds (Romania, 2018). However, since a toxicological assessment is currently ongoing within the renewal assessment of benalaxyl, it is not further pursued in this Art. 12 review.

Meanwhile, the plant residue definition for risk assessment previously derived in the peer review (EFSA, 2013a) is tentatively maintained: ‘benalaxyl-M’. Considering that benalaxyl-M and benalaxyl are of similar toxicity, this residue definition is also applicable in the frame of a comprehensive risk assessment comprising benalaxyl (see also Section 3.2).

An analytical method for the enforcement of the proposed residue definition at the LOQ of 0.02 mg/kg in high acid and high-water matrices is available (EFSA, 2013a).

Based on the available information on the nature of residues in rotational crops, it cannot be concluded whether the metabolism in rotational crops is equivalent to the metabolism in primary crops. However, considering the expected magnitude of residue uptake in edible parts of plant commodities as outlined in Section 1.2.2 specific residue definitions for rotational crops are not considered necessary.

Processing studies of benalaxyl-M which investigated the nature and magnitude of residues are not available and are at present not required. However, this may need to be re-considered in the future if additional data becomes available (see Section 1.1.2).

1.2. Magnitude of residues in plants

1.2.1. Magnitude of residues in primary crops

To assess the magnitude of benalaxyl-M residues resulting from the reported GAPs, EFSA considered all residue trials reported by the RMS in its evaluation report (Portugal, 2018) as well as the residue trials evaluated in the framework of the peer review and review of the existing MRLs for benalaxyl (EFSA, 2013a,b). All residue trial samples considered in this framework were stored in compliance with the conditions for which storage stability of residues was demonstrated. Decline of residues during storage of the trial samples is therefore not expected.

The number of residue trials and extrapolations were evaluated in accordance with the European guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs (European Commission, 2017).

The residue trials considered are not fully supporting the authorisations on table and wine grapes and lettuces. Therefore, although tentative MRL and risk assessment values could be derived for these crops, the following data gaps were identified:
- **Table and wine grapes:** Four trials on table or wine grapes compliant with the northern outdoor GAP are required. In addition, eight trials compliant with the southern outdoor GAP on table grapes and four trials compliant with the southern outdoor GAP on wine grapes are required.

- **Lettuces:** Only eight overdosed trials on lettuces are available to support the southern outdoor GAP. Although tentative MRL and risk assessment values can be derived, eight trials on lettuces (open leaf variety) compliant with the southern outdoor GAP are still required.

For all other crops, available residue trials are appropriate to derive tentative MRL and risk assessment values, taking note of the following considerations:

- **Tomatoes and aubergines:** The number of residue trials supporting the southern outdoor GAP is not compliant with the data requirements for this crop since one additional GAP-compliant trial is missing. However, the reduced number of residue trials is considered acceptable in this case because the indoor GAP, which is fully supported, is expected to lead to higher residues levels. Further residue trials are therefore not required.

- **Potatoes:** The number of residue trials supporting the northern and southern outdoor GAPs is not compliant with the data requirements for this crop. However, the reduced number of residue trials is considered acceptable in this case because all results were below the LOQ and a no residues situation is expected. Further residue trials are therefore not required.

- **Onions, garlic and shallots:** Only three GAP-compliant trials on onions are available to support the northern outdoor GAP and two GAP-compliant trials on onions to support the southern outdoor GAP. However, the reduced number of residue trials is considered acceptable in this case because all residues were below LOQ and no residue situation is expected. Further residue trials are therefore not required.

- **Leeks:** The residue trials supporting the northern and southern outdoor GAPs are not fully compliant with the data requirements for this crop. However, this is considered acceptable in this case because all results were below the LOQ and a no residues situation is expected. Further residue trials are therefore not required.

### 1.2.2. Magnitude of residues in rotational crops

There were no studies investigating the magnitude of residues in rotational crops available for this review. The available rotational crop studies were described and discussed previously (Section 1.1.2).

Considering the cGAP on tomatoes reported in this review (three applications at BBCH 17-85 at a rate of 100 g a.s./ha), assuming a soil density of 1.5 g/L, soil depth of 5 cm, crop interception of 50% and considering a DT₅₀ in soil of 98 days, the plateau concentration derived in soil, taking into account accumulation over the years, is 0.0041 mg/kg after 10 years (EFSA, 2013a). The maximum plateau concentration considering accumulation following seasonal applications will be 0.20 mg/kg. With a cultivation mixing depth of 20 cm the concentration is 0.05 mg/kg.

Information on the soil used was provided, however, information on soil cultivation practice and mixing depth was not. Nevertheless, considering that 2.25 kg benalaxyl/ha (7.3N) (equivalent to 1.125 kg benalaxyl-M/ha (3.7N)) were applied directly to soil and assuming the behaviour of the enantiomer benalaxyl-M is equivalent in soil to that of benalaxyl, it is concluded that the plateau concentration expected after use of benalaxyl-M at the critical GAP rate is covered by these studies.

Based on the rotational confined crop study and the fact that benalaxyl was applied to a bare soil (interception of active substance by the plants is expected in practice), it was concluded previously during the Art. 12 review of benalaxyl that residues above the LOQ would not be expected (EFSA, 2013b). This is considered valid for this review and benalaxyl-M residue levels in rotational commodities are not expected to exceed 0.01 mg/kg, provided benalaxyl-M is applied in compliance with the GAPs reported in Appendix A.

Since metabolite M9 was reported as highly persistent in the draft renewal assessment report of benalaxyl (Romania, 2018) its plateau concentration needs to be considered (Section 1.2.1). However, noting that the peer review of the renewal assessment is not yet finalised, further considerations were not included in this Art. 12 review.

### 1.2.3. Magnitude of residues in processed commodities

The effect of industrial processing was assessed on studies conducted on grapes and tomatoes (Portugal, 2003, 2018; EFSA, 2013a,b). An overview of all available processing studies is available in Appendix B.1.2.3. In the absence of studies investigating the nature of the residue (Section 1.1.3)
robust processing factors (fully supported by data) could not be derived for any of the investigated processed commodities. Further processing studies are not required as they are not expected to affect the outcome of the risk assessment. However, if more robust processing factors were to be required by risk managers, for enforcement purposes, additional processing studies would be needed.

1.2.4. Proposed MRLs

The available data are considered adequate to derive tentative MRL proposals as well as risk assessment values for all commodities under evaluation from the GAPs authorised for benalaxyl-M. Considering the general data gap identified for fully representative metabolism studies for all crop groups under assessment, MRLs for all commodities are considered tentative.

2. Residues in livestock

Benalaxyl-M and benalaxyl are authorised for use on potatoes that might be fed to livestock. Livestock dietary burden calculations were therefore performed for different groups of livestock according to OECD guidance (OECD, 2013), which has now also been agreed upon at European level. The input values for all relevant commodities are summarised in Appendix D.1. The input values derived from benalaxyl-M are based on a LOQ of 0.02 mg/kg whereby those of benalaxyl were considering 0.01 mg/kg (EFSA, 2013b). Therefore, the input values for benalaxyl-M cover the animal dietary burden which would result from benalaxyl uses reviewed earlier (EFSA, 2013b).

Since the calculated dietary burdens for all groups of livestock were found to be below the trigger value of 0.1 mg/kg dry matter (DM), further investigation of residues as well as the setting of MRLs in commodities of animal origin is unnecessary.

The metabolism of 14C-labelled benalaxyl (however not specifically of the R-enantiomer benalaxyl-M) residues in livestock was investigated in lactating goats and laying hens (Appendix B.2.1.1) at dose rate covering the maximum dietary burdens calculated in this review (Portugal, 2003, 2007, 2018). These studies were assessed in the framework of the review of existing MRLs for benalaxyl (EFSA, 2013b).

Notably, the metabolism in rats of benalaxyl-M did not differ significantly from the metabolism of benalaxyl in goats and hen. It was therefore concluded during the peer review that the metabolism of farm animals which was investigated with benalaxyl is representative also for benalaxyl-M (Portugal, 2007). This conclusion is supported during this review and studies performed with benalaxyl are considered appropriate to support benalaxyl-M metabolism.

The study performed on lactating goats indicates a significant transfer of residues to that transfer of residues in milk were relatively low (up to 0.011 mg/kg), fat (up to 0.03 mg/kg) and muscle (up to 0.022 mg/kg) when compared to the TRR levels in liver (up to 1.14 mg/kg) and kidney (up to 0.37 mg/kg). For liver, the extraction efficiency was poor (65% TRR extracted). After enzymatic hydrolysis, 55% of TRR remained unidentiﬁed. The parent compound was not detected. The most abundant single compound was a hydroxymethyl derivative of benalaxyl (6.2% TRR; 0.04 mg eq/kg). For kidney, the extraction was more efﬁcient (ca. 100%) however 58% TRR remained unidentiﬁed. Hydroxymethyl derivatives of benalaxyl were the most abundant single identiﬁed compounds (21.3% and 14.9% TRR; 0.07 mg eq/kg and 0.05 mg eq/kg) (EFSA, 2013b).

In the hen metabolism study, 81% of the administrated dose was found in excreta. The TRR levels was relatively low in egg white (up to 0.05 mg/kg), fat (up to 0.04 mg/kg) and muscle (up to 0.05 mg/kg) when compared to the TRR levels in liver (up to 1.4 mg/kg), kidney (up to 0.72 mg/kg) and egg yolk (up to 0.30 mg/kg). Liver and egg yolk were extracted and analysed to identify metabolites. Hydroxy-carboxy-benalaxyl was the most abundant compound in egg yolk (20.5% TRR; 0.06 mg eq/kg). No compound was identiﬁed in liver. In all samples, parent compound was found at low level or even not found at all (EFSA, 2013b).

Since the metabolism in hen is equal to the metabolism in goats, which is similar in rats, a general metabolic pathway can be proposed. Benalaxyl is oxidised, giving hydroxymethyl derivatives such as G8. These can be further oxidised to form carboxy derivate of benalaxyl. Conjugation can occur with all compounds.

The storage stability of benalaxyl including benalaxyl-M was not investigated (Portugal, 2003, 2007, 2018) and it is not required.

The parent compound was not a suitable marker however hydroxymethyl and carboxy metabolites can potentially considered as markers in livestock commodities, however a high level of TRR remained unidentified in the metabolism studies and further clarifications on the identity/characteristics of the
radioactive residue would still be required. Therefore, based on the currently available information residue definitions for enforcement or risk assessment cannot be proposed.

An analytical method using HPLC-MS with a chiral column was fully validated for the determination of benalaxyl-M in fat, kidney and liver with a LOQ of 0.01 mg/kg and in meat, eggs and milk a LOQ of 0.02 mg/kg (EFSA, 2013a). However, an analytical method for the enforcement of the proposed residue tentative residue definition is not available. Although a fully validated analytical method for enforcement in animal commodities is not available, the EURL informed EFSA that a screening detection limit (SLD) of 0.0025 mg/kg for monitoring of benalaxyl in milk, meat, egg and honey is achievable with a liquid chromatography quadrupole time-of-flight mass spectrometry (LC-MS-q-ToF) based QuEChERS method (EURLs, 2018).

The calculated dietary burden, considering authorised uses of this review, was shown to be below the trigger value and there is currently no need to derive a residue definition, or to establish MRLs, for commodities of animal origin.

3. **Consumer risk assessment**

In the framework of this review, only the uses of benalaxyl-M reported by the RMS in Appendix A were considered. However, residues of benalaxyl-M may also be generated from the use of benalaxyl (a racemic mixture of two enantiomers containing benalaxyl-M with ca. 50%).

Benalaxyl uses were previously also assessed by EFSA (2013b) in the framework of the MRL review of benalaxyl and this assessment also included the CXLs of benalaxyl previously derived by JMPR (FAO, 2009) which need to be considered by European risk managers when establishing MRLs for benalaxyl.

In the present reasoned opinion, the consumer exposure was firstly calculated specifically for the authorised uses of benalaxyl-M evaluated in this review. In a second scenario the uses of benalaxyl and the existing CXLs were evaluated. This second calculation corresponds to an update of the previous EFSA opinion (EFSA, 2013b) with consideration of additional intake resulting from the uses of benalaxyl-M.

3.1. **Consumer risk assessment only considering the authorised uses of benalaxyl-M**

Chronic and acute exposure calculations for all crops reported in the framework of this review were performed using revision 2 of the EFSA PRIMo (EFSA, 2007). Input values for the exposure calculations were derived in compliance with the decision tree reported in Appendix E. Hence, for those commodities where a (tentative) MRL could be derived by EFSA in the framework of this review, input values were derived according to the internationally agreed methodologies (FAO, 2009). All input values included in the exposure calculations are summarised in Appendix D.2. Acute exposure calculations were not carried out because an acute reference dose (ARfD) was not deemed necessary for this active substance (EFSA, 2013a).

The exposure values calculated were compared with the toxicological reference value for benalaxyl-M, derived by EFSA (2013a). The highest chronic exposure was calculated for WHO Cluster diet B, representing 1.1% of the acceptable daily intake (ADI). Although major uncertainties remain due to the data gaps identified in the previous sections, this indicative exposure calculation did not indicate a risk to consumer’s health.

Based on the above calculations, EFSA concludes that the existing authorised uses for benalaxyl-M are not expected to be of concern for the European consumers.

3.2. **Consumer risk assessment with consideration of benalaxyl residues resulting from EU uses of benalaxyl and benalaxyl-M, plus existing CXLs for benalaxyl**

In the previous EFSA review of existing MRLs for benalaxyl indicative exposure calculations were performed, resulting in the highest chronic exposure for WHO cluster diet B, representing 2% of the ADI (EFSA, 2013b). This calculation is now updated in the present review considering the additional data linked to benalaxyl-M uses and CXLs.

In order to assess the overall exposure to benalaxyl resulting from the authorised uses of benalaxyl and benalaxyl-M in EU and outside EU, all available MRL values from benalaxyl and benalaxyl-M reviews and CXLs were compared and the highest of these three sources taken for the consumer.
exposure estimate. It is assumed that benalaxyl and benalaxyl-M are not used simultaneously according to the authorised uses.

For each plant commodity, the input values for consumer exposure estimate is based on the highest residue level observed following the use of either benalaxyl-M or benalaxyl and the existing CXL for benalaxyl. An overview of this comparison is reported in the table below.

Table 2: Overview table on selection of highest MRLs for the consumer risk assessment in this review

| Code number | Commodity               | Calculated MRL (mg/kg) from benalaxyl uses (EFSA, 2013b) | Existing CXL (mg/kg) for benalaxyl | Calculated MRL (mg/kg) from benalaxyl-M uses | Highest selected MRL for dietary consumer estimate |
|-------------|-------------------------|---------------------------------------------------------|-----------------------------------|---------------------------------------------|--------------------------------------------------|
| 0151010     | Table grapes            | 0.6                                                     | 0.3                               | 0.7                                         | 0.7                                              |
| 0151020     | Wine grapes             | 0.1                                                     | 0.3                               | 0.15                                        | 0.3                                              |
| 0211000     | Potatoes                | 0.02                                                    | 0.02*                             | 0.02*                                       | 0.02*                                            |
| 0220010     | Garlic                  | –                                                       | –                                 | 0.02*                                       | 0.02*                                            |
| 0220020     | Onions                  | 0.01*                                                   | 0.02*                             | 0.02*                                       | 0.02*                                            |
| 0220030     | Shallots                | 0.01*                                                   | –                                 | 0.02*                                       | 0.02*                                            |
| 0231010     | Tomatoes                | 0.5                                                     | 0.2                               | 0.3                                         | 0.5                                              |
| 0231020     | Peppers                 | 0.2                                                     | –                                 | –                                           | 0.2                                              |
| 0231030     | Aubergines/eggplants    | 0.5                                                     | –                                 | 0.3                                         | 0.5                                              |
| 0233010     | Melons                  | 0.1                                                     | 0.3                               | 0.15                                        | 0.3                                              |
| 0233030     | Watermelons             | 0.1                                                     | 0.1                               | 0.15                                        | 0.15                                             |
| 0251020     | Lettuces                | 0.5                                                     | 1                                 | 3                                           | 3                                                |
| 0270060     | Leeks                   | –                                                       | –                                 | 0.02*                                       | 0.02*                                            |
| 0401060     | Rape seeds              | 0.05*                                                   | –                                 | –                                           | 0.05*                                            |

MRL: maximum residue level; CXL: codex maximum residue limit.
In bold: the highest selected MRL from the three sources, which was taken for the consumer exposure estimate.
+
*: Indicates that the MRL is set at the limit of quantification.

As shown in Table 2 above, most of the input values used in the risk assessment are based on tentative MRL proposals (and their associated risk assessment values) derived from the use of benalaxyl-M. For potatoes, tomatoes and aubergines however, the originally proposed MRL values for benalaxyl were higher and were used instead. Furthermore, MRL proposals for peppers and rape seeds linked to benalaxyl uses were also considered in the consumer risk assessment noting that no authorised uses were reported during this review for benalaxyl-M. It is noted that all CXLs established for benalaxyl are covered by either the MRLs derived for benalaxyl and benalaxyl-M with exception of wine grapes and melons where the CXL values were used.

An overview of the input values used for this exposure calculation is also provided in Appendix D.3.

Chronic and acute exposure calculations were also performed using revision 2 of the EFSA PRIMo and the exposure values calculated were compared with the toxicological reference value derived for benalaxyl (equal to benalaxyl-M). The highest chronic exposure was calculated for WHO Cluster diet B, representing 2.3% of the ADI. Based on these calculations, EFSA concludes that the overall dietary exposure to benalaxyl, resulting from the uses of benalaxyl or benalaxyl-M, is not of concern for European consumers, noting however that the consumer risk assessment is indicative only.

Conclusions

The metabolism of benalaxyl-M in plant was investigated in primary and rotational crops. It is noted that the available metabolites have several shortcomings. Therefore, representative studies fully characterising the nature of residues of benalaxyl-M for all crop groups are still required. Studies investigating the nature of residues of benalaxyl-M or racemic benalaxyl in processed commodities are not available and are not required.

A specific residue definition for rotational crops is not deemed necessary since residues of benalaxyl-M in the soil above the LOQ are not expected. Nevertheless, the outcome of the renewal assessment of benalaxyl may needs to be considered following its finalisation.
Based on the available data, the **plant residue definition for monitoring** can be tentatively proposed as 'benalaxyl-M' noting that it is compatible with the current definition for enforcement, namely, benalaxyl including other mixtures of constituent isomers including benalaxyl-M (sum of isomers) can still be considered.

Benalaxyl-M may not be the only toxicologically relevant compound, as indicated during the renewal assessment of benalaxyl and following its finalisation new information may needs to be considered. Meanwhile, the **plant residue definition for risk assessment** previously derived in the peer review is tentatively maintained: 'benalaxyl-M’. Considering that benalaxyl-M and benalaxyl are of similar toxicity, this residue definition is also applicable in the frame of a comprehensive risk assessment comprising benalaxyl. It is noted that this might need to be reconsidered in the future based on the outcome of the ongoing renewal of benalaxyl.

Fully validated analytical methods are available for the enforcement of benalaxyl-M in high water and high acid commodities at the LOQ of 0.02 mg/kg. According to the EURLs the for benalaxyl a LOQ of 0.01 mg/kg is achievable by using the QuEChERS method in routine analyses.

The storage stability of racemic benalaxyl and benalaxyl-M was demonstrated in high water and acid commodities (grapes, wine, potatoes and tomatoes) for 3 years at –20°C.

Available residue trials data were considered appropriate to derive tentative MRL proposals as well as risk assessment values for all commodities under evaluation. Considering the general data gap identified for the metabolism studies covering fruit, leafy, root and tuber crops, all the MRL proposals are considered as tentative.

Benalaxyl-M is authorised for use on crops that might be fed to livestock. Livestock dietary burden calculations were therefore performed for different groups of livestock according to OECD guidance. Since the calculated dietary burdens for all groups of livestock were found to be below the trigger value of 0.1 mg/kg DM, further investigation of residues as well as the setting of MRLs in commodities of animal origin is unnecessary noting that the metabolism of benalaxyl which is considered comparable to the one in benalaxyl M residues in livestock was investigated in lactating goats and laying hens at dose rate covering the maximum dietary burdens calculated in this review.

An analytical method for the enforcement of benalaxyl-M at the LOQ of 0.01 mg/kg in fat, kidney and liver and at the LOQ of 0.02 mg/kg in meat, eggs and milk is available. According to the EURLs, the SDL of 0.0025 mg/kg for benalaxyl is achievable by using the QuEChERS method in routine analyses (EURL, 2018).

Chronic consumer exposure resulting from the authorised uses reported in the framework of this review was calculated using revision 2 of the EFSA PRIMo. For those commodities where data were insufficient to derive an MRL, EFSA considered the existing EU MRL for an indicative calculation. The highest chronic exposure represented 1.1% of the ADI (WHO Cluster diet B).

Apart from the MRLs evaluated in the framework of this review, internationally recommended CXLs have also been established for benalaxyl. Furthermore, uses for benalaxyl were reviewed by EFSA previously and since these may generate residues of benalaxyl-M, they were considered in this review.

Therefore, in a second scenario, the overall exposure to benalaxyl resulting from the authorised uses of benalaxyl and benalaxyl-M in EU and outside EU was assessed. For that purpose, all available MRL values from benalaxyl and benalaxyl-M reviews and CXLs were compared and the highest of these three sources was taken for the consumer exposure estimate. It is assumed that benalaxyl and benalaxyl-M are not used simultaneously according to the authorised uses. The highest chronic exposure for this second scenario represented 2.2% of the ADI (WHO Cluster diet B).

**Recommendations**

MRL recommendations were derived in compliance with the decision tree reported in Appendix E of the reasoned opinion (see Table 3).

In this assessment, the MRLs derived for benalaxyl-M based on authorised uses of benalaxyl-M were integrated into the current list of MRLs defined for benalaxyl (sum of isomers) which was derived in the earlier review of uses of benalaxyl (EFSA, 2013b). The proposed MRLs are derived for benalaxyl (sum of isomers) and are covering both uses of benalaxyl and benalaxyl-M, assuming both active substances would not be used simultaneously according to the authorised uses. It is noted that risk managers have also the possibility to set a specific list of MRLs for benalaxyl-M derived from the only uses assessed in the present review. Chiral analytical methods for enforcement purpose were reported for this specific isomer. However, EFSA was not in position to assess the levels of benalaxyl-M which
would result from the authorised uses of benalaxyl. Therefore, such a list of MRLs was not reported in the present recommendations.

It has further to be noticed that the recommendation of this assessment might need to be reconsidered in the future based on the outcome of the renewal of benalaxyl.

All MRL values listed as ‘Recommended’ in the table are sufficiently supported by data and are therefore proposed for inclusion in Annex II to the Regulation. The remaining MRL values listed in the table are not recommended for inclusion in Annex II because they require further consideration by risk managers (see Table 3 footnotes for details). Particularly, some tentative MRLs and existing EU MRLs need to be confirmed by the following data:

- representative metabolism studies in all three primary crop groups, preferably tomato fruits, potato tuber and lettuces (this data gap is applicable to all MRLs except for wine grapes, aubergines, melons and watermelons;
- additional residue trials on table grapes and lettuces supporting the critical GAPs of benalaxyl-M;
- additional residues trials on peppers and rape seeds supporting the critical GAPs of benalaxyl (see EFSA, 2013b).

It is highlighted, however, that one MRL results from a CXL, whereas other GAPs reported by the RMS were not fully supported by data. EFSA therefore identified the following data gap which is not expected to impact on the validity of the MRLs derived but which might have an impact on national authorisations:

- additional residue trials on wine grapes supporting the critical GAPs of benalaxyl-M.

### Table 3: Summary table

| Code number | Commodity            | Existing EU MRL (mg/kg) for benalaxyl | Existing CXL (mg/kg) for benalaxyl | Outcome for benalaxyl-M MRL (mg/kg) | Outcome of the review for benalaxyl-M considering the previous review of benalaxyl+ | MRL (mg/kg) | Comment                      |
|-------------|----------------------|---------------------------------------|------------------------------------|-------------------------------------|------------------------------------------------------------------------------------|------------|-------------------------------|
| 0151010     | Table grapes         | 0.3                                   | 0.3                                | 0.7                                 | 0.7 Further consideration needed (benalaxyl-M)(a)                                  |            |                               |
| 0151020     | Wine grapes          | 0.3                                   | 0.3                                | 0.15                                | 0.3 Recommended (CXL benalaxyl)(b)                                                |            |                               |
| 0211000     | Potatoes             | 0.05*                                 | 0.02*                              | 0.02*                               | 0.02* Further consideration needed (benalaxyl-M)(c)                                 |            |                               |
| 0220010     | Garlic               | 0.05*                                 | –                                  | 0.02*                               | 0.02* Further consideration needed (benalaxyl-M)(c)                                 |            |                               |
| 0220020     | Onions               | 0.2                                   | 0.02*                              | 0.02*                               | 0.02* Further consideration needed (benalaxyl-M)(c)                                 |            |                               |
| 0220030     | Shallots             | 0.05*                                 | –                                  | 0.02*                               | 0.02* Further consideration needed (benalaxyl-M)(c)                                 |            |                               |
| 0231010     | Tomatoes             | 0.5                                   | 0.2                                | 0.3                                 | 0.5 Recommended (benalaxyl)(d)                                                    |            |                               |
| 0231020     | Peppers              | 0.2                                   | –                                  | –                                   | 0.2 Further consideration needed (EU MRL benalaxyl)(e)                               |            |                               |
| 0231030     | Aubergines/eggplants | 0.5                                   | –                                  | 0.3                                 | 0.5 Recommended (benalaxyl)(f)                                                    |            |                               |
| 0233010     | Melons               | 0.3                                   | 0.3                                | 0.15                                | 0.3 Recommended (CXL benalaxyl)(g)                                                |            |                               |
| 0233030     | Watermelons          | 0.1                                   | 0.1                                | 0.15                                | 0.15 Recommended (benalaxyl-M)(h)                                                 |            |                               |
| 0251020     | Lettuces             | 1                                     | 1                                  | 3                                   | 3 Further consideration needed (benalaxyl-M)(h)                                    |            |                               |
| 0270060     | Leeks                | 0.05*                                 | –                                  | 0.02*                               | 0.02* Further consideration needed (benalaxyl-M)(h)                                 |            |                               |
| 0401060     | Rape seeds           | 0.05*                                 | –                                  | –                                   | 0.05* Further consideration needed (EU MRL benalaxyl)(h)                            |            |                               |

* Enforcement residue definition: Benalaxyl including other mixtures of constituent isomers including benalaxyl-M (sum of isomers)
**Review of the existing MRLs for benalaxyl-M**

| Code number | Commodity | Existing EU MRL (mg/kg) for benalaxyl | Existing CXL (mg/kg) for benalaxyl | Outcome for benalaxyl-M MRL (mg/kg) | Outcome of the review for benalaxyl-M considering the previous review of benalaxyl* |
|-------------|-----------|--------------------------------------|-----------------------------------|-------------------------------------|---------------------------------------------------------------------------------|
| –           | Other commodities of plant and/or animal origin | See Reg. 520/2011 | – | – | Further consideration needed\(^{(i)}\) |

MRL: maximum residue level; CXL: codex maximum residue limit.

\(^{+}\): EFSA (2013b).

\(^{*}\): Indicates that the MRL is set at the limit of quantification.

\(^{(F)}\): The residue definition is fat soluble.

\(^{(a)}\): Tentative MRL is derived from a GAP evaluated at EU level, which is not fully supported by data but for which no risk to consumers was identified (assuming the existing residue definition); existing CXL is covered by the tentative MRL (combination F-III in Appendix E).

\(^{(b)}\): MRL is derived from the existing CXL, which is supported by data and for which no risk to consumers is identified; GAP evaluated at EU level, which is not fully supported by data, leads to a lower tentative MRL (combination F-VII in Appendix E).

\(^{(c)}\): Tentative MRL is derived from a GAP evaluated at EU level, which is not fully supported by data but for which no risk to consumers was identified (assuming the existing residue definition); no CXL is available (combination F-I in Appendix E).

\(^{(d)}\): MRL is derived from the existing CXL, which is supported by data and for which no risk to consumers is identified; GAP evaluated at EU level, which is also fully supported by data, leads to a lower MRL (combination H-VII in Appendix E).

\(^{(e)}\): GAP evaluated at EU level is not supported by data but no risk to consumers was identified for the existing EU MRL (also assuming the existing residue definition); no CXL is available (combination D-I in Appendix E).

\(^{(f)}\): MRL is derived from a GAP evaluated at EU level, which is fully supported by data and for which no risk to consumers is identified; no CXL is available (combination H-I in Appendix E).

\(^{(g)}\): MRL is derived from the existing CXL, which is supported by data and for which no risk to consumers is identified; GAP evaluated at EU level, which is also fully supported by data, leads to a lower MRL (combination H-VII in Appendix E).

\(^{(h)}\): MRL is derived from a GAP evaluated at EU level, which is fully supported by data and for which no risk to consumers is identified; existing CXL is covered by the recommended MRL (combination H-III in Appendix E).

\(^{(i)}\): There are no relevant authorisations or import tolerances reported at EU level; no CXL is available. Either a specific LOQ or the default MRL of 0.01 mg/kg may be considered (combination A-I in Appendix E).

**References**

Austria, 2017. Evaluation report prepared under Article 12.1 of Regulation (EC) No 396/2005. Additional data to be considered for the review of the existing MRLs for benalaxyl-M, December 2017. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2007. Reasoned opinion on the potential chronic and acute risk to consumers’ health arising from proposed temporary EU MRLs. EFSA Journal 2007;5(3):32r; 1141 pp. https://doi.org/10.2903/j.efsa.2007.32r

EFSA (European Food Safety Authority), 2013a. Conclusion on the peer review of the pesticide risk assessment of the active substance benalaxyl-M. EFSA Journal 2013;11(4):3148, 58 pp. https://doi.org/10.2903/j.efsa.2013.3148

EFSA (European Food Safety Authority), 2013b. Reasoned opinion on the review of the existing maximum residue levels (MRLs) for benalaxyl according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(10):3405, 43 pp. https://doi.org/10.2903/j.efsa.2013.3405

EFSA (European Food Safety Authority), 2019a. Completeness check report on the review of the existing MRLs of benalaxyl-M prepared by EFSA in the framework of Article 12 of Regulation (EC) No 396/2005; 12 April 2019. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2019b. Member States consultation report on the review of the existing MRLs of benalaxyl-M prepared by EFSA in the framework of Article 12 of Regulation (EC) No 396/2005, 24 April-15 May 2019. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2010. Scientific and technical support for preparing a EU position in the 42nd Session of the Codex Committee on Pesticide Residues (CCPR). EFSA Journal 2010;8(11):1560, 51 pp. https://doi.org/10.2903/j.efsa.2010.1560

EURL (European Union Reference Laboratories for Pesticide Residues), 2018. Evaluation report prepared under Article 12 of Regulation (EC) No 396/2005. Analytical methods validated by the EURLs and overall capability of official laboratories to be considered for the review of the existing MRLs for benalaxyl-M. March 2018. Available online: www.efsa.europa.eu

European Commission, 1997a. Appendix A. Metabolism and distribution in plants. 7028/IV/95-rev., 22 July 1996.
European Commission, 1997b. Appendix B. General recommendations for the design, preparation and realization of residue trials. Annex 2. Classification of (minor) crops not listed in the Appendix of Council Directive 90/642/EEC. 7029/VI/95-rev. 6, 22 July 1997.
European Commission, 1997c. Appendix C. Testing of plant protection products in rotational crops. 7524/VI/95-rev. 2, 22 July 1997.
European Commission, 1997d. Appendix E. Processing studies. 7035/VI/95-rev. 5, 22 July 1997.
European Commission, 1997e. Appendix F. Metabolism and distribution in domestic animals. 7030/VI/95-rev. 3, 22 July 1997.
European Commission, 1997f. Appendix H. Storage stability of residue samples. 7032/VI/95-rev. 5, 22 July 1997.
European Commission, 1997g. Appendix I. Calculation of maximum residue level and safety intervals. 7039/VI/95, 22 July 1997. As amended by the document: classes to be used for the setting of EU pesticide maximum residue levels (MRLs). SANCO 10634/2010, finalised in the Standing Committee on the Food Chain and Animal Health at its meeting of 23–24 March 2010.
European Commission, 2000. Residue analytical methods. For pre-registration data requirement for Annex II (part A, section 4) and Annex III (part A, section 5 of Directive 91/414. SANCO/3029/99-rev. 4.
European Commission, 2010a. Classes to be used for the setting of EU pesticide Maximum Residue Levels (MRLs). SANCO 10634/2010-rev. 0, Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting of 23–24 March 2010.
European Commission, 2010b. Residue analytical methods. For post-registration control. SANCO/825/00-rev. 8.1, 16 November 2010.
European Commission, 2017. Appendix D. Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs. 7525/VI/95-rev.10.3, June 2017.
FAO (Food and Agriculture Organization of the United Nations), 2009. Benalaxyl. In: Pesticide residues in food – 2009. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 196.
OECD (Organisation for Economic Co-operation and Development), 2011. OECD MRL calculator: spreadsheet for single data set and spreadsheet for multiple data set, 2 March 2011. In: Pesticide Publications/Publications on Pesticide Residues. Available online: http://www.oecd.org
OECD (Organisation for Economic Co-operation and Development), 2013. Guidance document on residues in livestock. In: Series on Pesticides No 73. ENV/JM/MONO(2013)8, 4 September 2013.
Portugal, 2003. Draft assessment report on the active substance benalaxyl-M prepared by the rapporteur Member State Portugal in the framework of Council Directive 91/414/EEC, July 2003. Available online: www.efsa.europa.eu
Portugal, 2007. Final addendum to the draft assessment report on the active substance benalaxyl-M, compiled by EFSA, June 2007. Available online: www.efsa.europa.eu
Portugal, 2013. Final addendum to the draft assessment report on the active substance benalaxyl-M, compiled by EFSA, February 2013. Available online: www.efsa.europa.eu
Portugal, 2018. Evaluation report prepared under Article 12.1 of Regulation (EC) No 396/2005. Review of the existing MRLs for benalaxyl-M, April 2018. Revised in July (rev. 1) and September 2018 (rev. 2). Available online: www.efsa.europa.eu
Romania, 2018. Revised renewal Assessment Report (RAR) on benalaxyl prepared by the rapporteur Member State Romania in the framework of Regulation (EC) No 1107/2009, January 2018. Available online: www.efsa.europa.eu

Abbreviations

a.i. active ingredient
a.s. active substance
ADI acceptable daily intake
ARFD acute reference dose
BBCH growth stages of mono- and dicotyledonous plants
bw body weight
CAC Codex Alimentarius Commission
CF conversion factor for enforcement residue definition to risk assessment residue definition
cGAP critical GAP
CS capsule suspension
CV coefficient of variation (relative standard deviation)
CXL codex maximum residue limit
DAR draft assessment report
DAT days after treatment
DB dietary burden
DM dry matter
DT$_{90}$ period required for 90% dissipation (define method of estimation)
eq residue expressed as a.s. equivalent
EURLs European Union Reference Laboratories for Pesticide Residues (former CRLs)
FAO Food and Agriculture Organization of the United Nations
GAP Good Agricultural Practice
GC-MS gas chromatography with mass spectrometry
GC-MS/MS gas chromatography with tandem mass spectrometry
HPLC-MS high-performance liquid chromatography with mass spectrometry
HPLC-MS/MS high-performance liquid chromatography with tandem mass spectrometry
HR highest residue
IEDI international estimated daily intake
InChiKey International Chemical Identifier Key
ILV independent laboratory validation
ISO International Organisation for Standardization
IUPAC International Union of Pure and Applied Chemistry
JMPR Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
LC-MS/MS liquid chromatography with tandem mass spectrometry
LOQ limit of quantification
Mo monitoring
MRL maximum residue level
MS Member States
NEDI national estimated daily intake
NEU northern European Union
NTMDI national theoretical maximum daily intake
OECD Organisation for Economic Co-operation and Development
PBI plant-back interval
PF processing factor
PHI preharvest interval
PRIMo (EFSA) Pesticide Residues Intake Model
PROFile (EFSA) Pesticide Residues Overview File
QqQ triple-quadrupole mass spectrometer
q-ToF quadrupole time-of-flight
QuEChERS Quick, Easy, Cheap, Effective, Rugged, and Safe (analytical method)
RA risk assessment
RAC raw agricultural commodity
RD residue definition
RMS rapporteur Member State
SANCO Directorate-General for Health and Consumers
SDL screening detection limit
SEU southern European Union
SMILES simplified molecular-input line-entry system
STMR supervised trials median residue
TAR total applied radioactivity
TMDI theoretical maximum daily intake
TRR total radioactive residue
WG water-dispersible granule
WHO World Health Organization
WP wettable powder
Appendix A – Summary of authorised uses considered for the review of MRLs

A.1. Authorised outdoor uses in northern EU

| Crop and/or situation | MS or country | F G I (a) | Pests or group of pests controlled | Preparation | Application | Application rate per treatment | PHI (days)(d) | Remarks |
|-----------------------|--------------|-----------|-----------------------------------|-------------|------------|-------------------------------|---------------|---------|
|                       |              |           |                                   | Type(b)     | Conc. a.s. | Method kind                   | a.s./hl min-max | Water L/ha min-max | Rate and unit |               |
|                       |              |           |                                   | Range of growth stages & season(c) | Number min- max | Interval between application (min) |               |               |               |
| Table grapes          | HU           | F         | Plasmopara viticola, Pseudopezicula tracheiphila | WP          | 40 g/kg   | Foliar treatment - broadcast spraying | 77            | 1–3          | 10           | –          | 100 g a.i./ha | 42          |
| Wine grapes           | FR, HU, RO  | F         | Plasmopora viticola               | WP          | 40 g/kg   | Foliar treatment - broadcast spraying | 13–81         | 1–3          | 10           | –          | 100 g a.i./ha | 42          |
| Potatoes              | NL, RO, PL  | F         | Phytophthora infestans            | WP          | 40 g/kg   | Foliar treatment - broadcast spraying | 21–85         | 1–3          | 7            | –          | 100 g a.i./ha | 7           |
| Onions                | FR, HU, RO, PO | F     | Peronospora destructor, downy mildew | WP          | 40 g/kg   | Foliar treatment - broadcast spraying | 20–48         | 1–3          | 7            | –          | 100 g a.i./ha | 28          |
| Shallots              | FR           | F         | Downy mildew                      | WP          | 40 g/kg   | Foliar treatment - general (see also comment field) | –             | 3            | –            | –          | 100 g a.i./ha | 28          |
| Tomatoes              | RO, PO       | F         | Phytophthora infestans             | WP          | 40 g/kg   | Foliar treatment - broadcast spraying | 17–85         | 1–3          | 7            | –          | 100 g a.i./ha | 3           |
| Leeks                 | BE           | F         | Mildew                            | WP          | 40 g/kg   | Foliar treatment - broadcast spraying | –             | 1–3          | 21           | –          | 100 g a.i./ha | 56 PHI: 60 days |

MS: Member State; a.s.: active substance; WP: wettable powder; a.i.: active ingredient.
(a): Outdoor or field use (F), greenhouse application (G) or indoor application (I).
(b): CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide.
(c): Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including, where relevant, information on season at time of application.
(d): PHI: minimum preharvest interval.
### A.2. Authorised outdoor uses in southern EU

| Crop and/or situation | MS or country | G or F or T | Pests or group of pests controlled | Preparation | Application | Application rate per treatment | PHI (days) | Remarks |
|-----------------------|---------------|-------------|------------------------------------|-------------|-------------|--------------------------------|-----------|---------|
| Table grapes          | FR            | F           | _                                  | WG          | Foliar treatment – spraying | _ | 3 | 10 | 100 g a.i./ha | 42 |
| Wine grapes           | IT, HR        | F           | Plasmopara viticola                | WG          | Foliar treatment – spraying | 13–81 | 3 | 10 | 75 g a.i./ha | 28 |
| Potatoes              | ES            | F           | Mildew                            | WP          | Foliar treatment – spraying | 17–85 | 3 | 10 | 100 g a.i./ha | 7 |
| Garlic                | PT            | F           | Peronospora destructor            | WP          | Foliar treatment – spraying | 15–46 | 2 | 7 | 100 g a.i./ha | 28 |
| Onions                | ES            | F           | Mildew                            | WP          | Foliar treatment – spraying | _ | 3 | 10 | 100 g a.i./ha | 28 |
| Shallots              | PT            | F           | Peronospora destructor            | WP          | Foliar treatment – spraying | 15–46 | 2 | 7 | 100 g a.i./ha | 28 |
| Tomatoes              | ES            | F           | Mildew                            | WP          | Foliar treatment – spraying | _ | 3 | 10 | 100 g a.i./ha | 3 |
| Aubergines            | ES            | F           | Mildew                            | WP          | Foliar treatment – spraying | _ | 3 | 10 | 100 g a.i./ha | 3 |
| Melons                | HR, ES        | F           | Pseudoperonospora cubensis, mildew | WG          | Foliar treatment – spraying | _ | 2 | 7 | 100 g a.i./ha | 7 |
| Watermelons           | ES            | F           | Mildew                            | WP          | Foliar treatment – spraying | _ | 2 | 10 | 100 g a.i./ha | 7 |
| Lettuces              | ES            | F           | Mildew                            | WP          | Foliar treatment – spraying | _ | 1 | – | 100 g a.i./ha | 28 |
| Leeks                 | HR            | F           | Phytophthora porri, Peronospora destructor | WP          | Foliar treatment – spraying | _ | 2 | 10 | 100 g a.i./ha | 56 |

**MS**: Member State; **a.s.**: active substance; **WG**: water-dispersible granule; **WP**: wettable powder; **a.i.**: active ingredient. 
(a): Outdoor or field use (F), greenhouse application (G) or indoor application (T). 
(b): CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide. 
(c): Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including, where relevant, information on season at time of application. 
(d): PHI: minimum preharvest interval.
### A.3. Authorised indoor uses in EU

| Crop and/or situation | MS or country | F or G or I(a) | Pests or Group of pests controlled | Preparation | Application | Application rate per treatment | PHI (days)(d) | Remarks |
|-----------------------|---------------|---------------|-----------------------------------|-------------|-------------|-------------------------------|--------------|---------|
| Tomatoes              | ES            | I             | Mildew                            | WP 40 g/kg | Foliar treatment – spraying | 17–89 3 10 100 g a.i./ha     | 3            | Automated equipment > 1 m height |
| Aubergines            | ES            | I             | Mildew                            | WP 40 g/kg | Foliar treatment – spraying | 17–89 3 10 100 g a.i./ha     | 3            | Automated equipment > 1 m height |

**MS:** Member State; **a.s.**: active substance; **WP**: wettable powder; **a.i.**: active ingredient.

(a): Outdoor or field use (F), greenhouse application (G) or indoor application (I).

(b): CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide.

(c): Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including, where relevant, information on season at time of application.

(d): PHI: minimum preharvest interval.
Appendix B – List of end points

B.1. Residues in plants

B.1.1. Nature of residues and methods of analysis in plants

B.1.1.1. Metabolism studies, methods of analysis and residue definitions in plants

| Primary crops (available studies) | Crop groups | Crop(s) | Application (s) | Sampling (DAT) | Comment/source |
|----------------------------------|-------------|---------|----------------|----------------|----------------|
| Fruit crops                      | Grape       | 1 × 240 g a.s./ha | 0.1, 3, 8, 15, 24 | Foliar treatment with $^{14}$C-benalaxyl (R- and S-enantiomers; ca 50%) of vines with grapes at the stage of darkening. Ripe grapes harvested and analysed 3 h and 3, 8, 15, 24 DAT (Portugal, 2007) |
|                                  |             | 5 × 300 g a.s./ha | Not reported   | Foliar treatment with $^{14}$C-benalaxyl labelled in the alpha position of the ester moiety; characterisation of metabolite GX1 in wine leaves (Portugal, 2007) |
|                                  |             | 4 × 240 g a.s./ha | 22            | Foliar treatment with $^{14}$C-benalaxyl, identification of metabolite GX6 and a glucose conjugate of GX1 in vine leaves (Portugal, 2007) |
|                                  |             | Not reported   | 16            | Foliar treatment with $^{14}$C-benalaxyl; quantification of metabolite GX1 and parent in vine leaves (Portugal, 2007) |
|                                  |             | 1 × 120 g a.s./ha | 0.08, 1, 3, 7, 14 | Foliar treatment with $^{14}$C-benalaxyl-M and analyses of residues in leaves only (Portugal, 2007) |
|                                  |             | 1 × 240 g a.s./ha | 0.08, 1, 3, 7, 14 | Foliar treatment with $^{14}$C-benalaxyl and analyses of residues in leaves only (Portugal, 2007) |
|                                  | Tomato      | 1 × 250 g a.s./ha | 1, 7, 14, 28, 35 | Foliar treatment of plants when most fruits where at the darkening stage with $^{14}$C-benalaxyl; metabolism in tomato fruits (Portugal, 2007) |
|                                  |             | 1 × 120 g a.s./ha | 0.08, 1, 3, 7, 14 | Foliar treatment with $^{14}$C-benalaxyl-M; metabolism investigated in tomato leaves only (Portugal, 2007) |
|                                  |             | 1 × 240 g a.s./ha | 0.08, 1, 3, 7, 14 | Foliar treatment with $^{14}$C-benalaxyl; metabolism investigated in tomato leaves only (Portugal, 2007) |
| Root crops                       | Potato      | 1 × 250 g a.s./ha | 5, 12, 19, 26 | Foliar treatment with $^{14}$C-benalaxyl; metabolism in potato leaves and tuber (Portugal, 2007) |
|                                  |             | 1 × 300 g a.s./ha | 0.8, 1, 2, 3, 4, 7, 10, 16 | Foliar treatment with $^{14}$C-benalaxyl; metabolism in potato leaves (Portugal, 2007) |
|                                  |             | 1 × 120 g a.s./ha | 0.08, 1, 3, 7, 14 | Foliar treatment with $^{14}$C-benalaxyl-M; metabolism in potato leaves (Portugal, 2007) |
|                                  |             | 1 × 240 g a.s./ha | 0.08, 1, 3, 7, 14 | Foliar treatment with $^{14}$C-benalaxyl; metabolism in potato leaves (Portugal, 2007) |
| Leafy crops                      | Tobacco     | 2 × 250 g a.s./ha | 7, 14, 21 | Foliar treatment with $^{14}$C-benalaxyl; metabolism in tobacco leaves (Portugal, 2003) |
| Rotational crops (available studies) | Crop groups | Crop(s) | Application(s) | PBI (DAT) | Comment/source |
|-----------------------------------|-------------|---------|----------------|----------|----------------|
| Fruits and fruiting vegetables    | Tomato      | 1 × 2.25 kg/ha | 33, 111, 363 | Soil treatment with 14C-labelled benalaxyl; harvest interval 84–98 days after transplanting (Portugal, 2003; EFSA, 2013b) |
| Root/tuber crops                  | Carrot      | 1 × 2.25 kg/ha | 32, 110, 370 | Soil treatment with 14C-labelled benalaxyl; harvest interval 70–80 and 112–115 days after sowing (Portugal, 2003; EFSA, 2013b) |
| Leafy crops                       | Lettuce     | 1 × 2.25 kg/ha | 31, 117, 360 | Soil treated with 14C-labelled benalaxyl; harvest interval 15–20 (Portugal, 2003; EFSA, 2013b) |
| Cereal (small grain)              | Wheat       | 1 × 2.25 kg/ha | 30, 120, 295 | Soil treated with 14C-labelled benalaxyl; harvest interval 272 DAT (Portugal, 2003; EFSA, 2013b) |

| Processed commodities (hydrolysis study) | Conditions | Stable? | Comment/source |
|------------------------------------------|------------|---------|----------------|
|                                          | Pasteurisation (20 min, 90 °C, pH 4) | Inconclusive | No studies available (Portugal, 2018) |
|                                          | Baking, brewing and boiling (60 min, 100 °C, pH 5) | Inconclusive | No studies available (Portugal, 2018) |
|                                          | Sterilisation (20 min, 120 °C, pH 6) | Inconclusive | No studies available (Portugal, 2018) |
|                                          | Other processing conditions | Yes | Wine processing following foliar treatment with 14C-labelled benalaxyl (1 × 24 g a.s./ha; 24 DAT ripe grapes were harvested and crushed to must which was used for wine making (Portugal, 2007) |
Can a general residue definition be proposed for primary crops?
Yes However only tentatively because of shortcomings of the metabolism studies since these do not cover all cGAPs of this review

Rotational crop and primary crop metabolism similar?
Inconclusive A characterisation or identification of residues was not performed.

Residue pattern in processed commodities similar to residue pattern in raw commodities?
Inconclusive No study available and not required. It is to be noted that this may need to be reconsidered in the future when additional information is available

Plant residue definition for monitoring (RD-Mo)

Plant residue definition for risk assessment (RD-RA)

Methods of analysis for monitoring of residues (analytical technique, matrix groups, LOQs)

| Matrix Type                      | Analytical Technique                  | LOQ (mg/kg) | Validation Data |
|----------------------------------|---------------------------------------|-------------|-----------------|
| Matrices with high water and high acid content: | | | |
| Benalaxyl-M with a chiral method: | | 0.02 | | |
| Multiresidue method with chiral column for specific determination of benalaxyl Validated in grapes, potatoes and wine (Portugal, 2007). ILV available (EFSA, 2013a). | | | |
| HPLC–MS/MS with chiral column (LOQ=0.01 mg/kg for all type of commodities; confirmatory method and ILV missing (EFSA, 2013a)). | | | |
| Benalaxyl (racemic, sum of enantiomers) | | | |
| QuEChERS multiresidue method using GC-MS/MS with LOQ of 0.01 mg/kg (citrate buffered according to EN 15662:2008 but excluding the dSPE step. Validation data for benalaxyl in lettuce and orange (EURLs, 2018). | | | |
| Dry matrices: | | | |
| GC–QqQ-MS/MS (LOQ = 0.01 mg/kg, QuEChERS-method according to EN 15662:2008 excluding sSPE step). Validated data for benalaxyl in wheat, barley, rice and rye (EURLs, 2018). | | | |
| Matrices with high oil content: | | | |
| LC–MS/MS (LOQ=0.01 mg/kg; BVL L 13.04-5:2013-08 (QuOil method); validation data for benalaxyl in almonds (EURLs, 2018). | | | |

a.s.: active substance; DAT: days after treatment; PBI: plant-back interval; HPLC–MS: high-performance liquid chromatography with mass spectrometry; HPLC–MS/MS: high-performance liquid chromatography with tandem mass spectrometry; LC–MS/MS: liquid chromatography with tandem mass spectrometry; GC–MS/MS: Gas chromatography with tandem mass spectrometry; LOQ: limit of quantification; ILV: independent laboratory validation; QuEChERS: Quick, Easy, Cheap, Effective, Rugged, and Safe.
B.1.1.2. Stability of residues in plants

| Plant products (available studies) | Category     | Commodity | T (°C) | Stability period | Compounds covered | Comment/source                                      |
|-----------------------------------|--------------|-----------|--------|------------------|-------------------|---------------------------------------------------|
|                                   | High water content | Potatoes | –20    | 72 Months        | Benalaxyl and benalaxyl-M | EFSA (2013a), Portugal (2018) |
|                                   | Tomatoes     |           | –20    | 72 Months        | Benalaxyl and benalaxyl-M | EFSA (2013a), Portugal (2018) |
|                                   | High acid content | Grapes    | –20    | 72 Months        | Benalaxyl and benalaxyl-M | EFSA (2013a), Portugal (2018) |
|                                   | Processed products | Wine     | –20    | 72 Months        | Benalaxyl and benalaxyl-M | EFSA (2013a), Portugal (2018) |

B.1.2. Magnitude of residues in plants

B.1.2.1. Summary of residues data from the supervised residue trials – Primary crops

| Commodity   | Region/ indoor(a) | Residue levels observed in the supervised residue trials (mg/kg) | Comments/source | Calculated MRL (mg/kg) | HR(b) (mg/kg) | STMR(c) (mg/kg) |
|-------------|-------------------|-----------------------------------------------------------------|-----------------|------------------------|--------------|-----------------|
| Table grapes | NEU               | GAP-compliant trials: < 0.02; 0.022; 0.03; 0.047<br>Overdosed trials with 4 x 0.12 kg as/ha: 0.027; 0.028; 0.033; 0.046; 0.055; 0.070; 0.071; 0.090<br>Overdosed trials with 4 x 0.012 kg as/ha: 0.027; 0.028; 0.033; 0.046; 0.055; 0.070; 0.071; 0.090 | Trials on wine grapes (Austria, 2017; Portugal, 2018). Extrapolated to table grapes MRL_{OECD} = 0.14 | 0.15(d),(e3),(f) (tentative) | 0.09 | 0.04 |
|             | SEU               | Overdosed trials with 4 x 0.004 kg as/ha: < 0.02; < 0.02; < 0.02; 0.38<br>Overdosed trial with 4 x 0.075 kg as/ha: < 0.02<br>Overdosed trials with 4 x 0.2 kg as/ha: < 0.02; 0.044 | Trials on wine grapes (Portugal, 2018) extrapolated to table grapes MRL_{OECD} = 0.61 | 0.7(d),(e3),(f) (tentative) | 0.38 | 0.02 |
| Wine grapes  | NEU               | GAP-compliant trials: < 0.02; 0.022; 0.03; 0.047<br>Overdosed trials with 4 x 0.12 kg as/ha: 0.027; 0.028; 0.033; 0.046; 0.055; 0.070; 0.071; 0.090<br>Overdosed trials with 2 x 0.2 kg as/ha: 0.023; 0.066 | Trials on wine grapes (Austria, 2017; Portugal, 2018). Extrapolated to table grapes MRL_{OECD} = 0.14 | 0.15(d),(e3),(f) (tentative) | 0.09 | 0.04 |
|             | SEU               | GAP-compliant trials: 0.026; 0.047; 0.056; 0.063<br>Overdosed trials with 2 x 0.2 kg as/ha: 0.023; 0.066 | Trials on wine grapes (Portugal, 2018) MRL_{OECD} = 0.14 | 0.15(d),(e3),(f) (tentative) | 0.07 | 0.05 |
| Commodity          | Region/indoor(a) | Residue levels observed in the supervised residue trials (mg/kg)                                                                 | Comments/source                                                                                                                                                                                                 | Calculated MRL (mg/kg) | HR(b) (mg/kg) | STMR(c) (mg/kg) |
|-------------------|------------------|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|--------------|-----------------|
| Potatoes          | NEU              | < 0.02; < 0.02                                                                                                                                                                 | GAP-compliant trials on potatoes (Portugal, 2018) Additional 15 trials on potatoes available performed with benalaxyl with residues below LOQ (EFSA, 2013b). Available trials deemed sufficient to concluded on a no residues situation | 0.02*(e2) (tentative) | < 0.02       | < 0.02          |
|                   | SEU              | < 0.02; < 0.02; < 0.02                                                                                                                                                          | GAP-compliant trials on potatoes (Portugal, 2018) deemed sufficient to concluded on a no residues situation                                                                                                    | 0.02*(e2) (tentative) | < 0.02       | < 0.02          |
| Onions, shallots  | NEU              | GAP-compliant trials: < 0.02; < 0.02; < 0.02 Trials with a shorter PHI (first 18 and last two 3 days): < 0.02; < 0.01                                                                 | Trials on onions (Portugal, 2018). Extrapolated to shallots deemed sufficient to concluded on a no residues situation. Extrapolated to shallots MRL<sub>OECD</sub> = 0.02 | 0.02*(e3) (tentative) | < 0.02       | < 0.02          |
|                   | SEU              | GAP-compliant trials: < 0.02; < 0.02 Trials with a shorter PHI (3–14 days): < 0.02; < 0.02; < 0.01                                                                                   | Trials on onions (Portugal, 2018) deemed sufficient to concluded on a no residues situation. Extrapolated to shallots MRL<sub>OECD</sub> = 0.02                                                                 | 0.02*(e3) (tentative) | 0.02         | 0.02            |
| Garlic            | SEU              | Overdosed trials with 3 x 0.1 kg as/ha: < 0.02; < 0.02 Trials with a shorter PHI (3–14 days): < 0.02; < 0.02; < 0.01                                                                 | Trials on onions (Portugal, 2018) deemed sufficient to concluded on a no residues situation. Extrapolated to garlic MRL<sub>OECD</sub> = 0.02                                                              | 0.02*(e1) (tentative) | 0.02         | 0.02            |
| Tomatoes, aubergines | NEU          | 0.017; 0.018; 0.021; 0.028; 0.029; 0.041; 0.052; 0.114                                                                                                                            | GAP-compliant trials on tomatoes (Portugal, 2018). No authorisation on aubergines NEU MRL<sub>OECD</sub> = 0.17                                                                                             | 0.2*(e2) (tentative)  | 0.11         | 0.03            |
|                   | SEU              | GAP-compliant trials: 0.035; 0.050; 0.053; 0.075; 0.076; 0.115; 0.122 Overdosed trials with 4 x 0.1 kg as/ha: 0.028; 0.049; 0.056; 0.062 | Trials on tomatoes (Portugal, 2018). Tentatively extrapolated to aubergines MRL<sub>OECD</sub> = 0.2                                                                                                        | 0.2*(d),(e2),(f) (tentative) | 0.12         | 0.06            |
| Commodity          | Region/indoor(a) | Residue levels observed in the supervised residue trials (mg/kg)                                                                 | Comments/source                                                                 | Calculated MRL (mg/kg) | HR(b) (mg/kg) | STMR(c) (mg/kg) |
|--------------------|------------------|---------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|------------------------|------------|----------------|
| EU                 |                  | GAP-compliant trials on tomatoes (Portugal, 2018). Tentatively extrapolated to aubergines. MRL_{OECD} = 0.22                       |                                                                                  | 0.3(e2) (tentative) | 0.12       | 0.07           |
| Melons, water melons | SEU              | GAP-compliant trials on melons (Portugal, 2018); extrapolation to watermelons is applicable. MRL_{OECD} = 0.12                    |                                                                                  | 0.15       | 0.07       | 0.03           |
| Lettuces           | SEU              | Overdosed trials with 3 × 0.12 kg as/ha, PHI = 7 days: Open-leaf variety: < 0.01; 0.022; 0.815; 1.542                             | Trials on lettuce (Portugal, 2018) MRL_{OECD} = 2.58                             | 3(e1),(f) (tentative) | 1.54       | 0.03           |
| Leeks              | NEU              | Overdosed trials on leek (4 × 100 g as/ha; PHI-42 days): < 0.02; < 0.02; < 0.02                                              | Trials on leek and spring onions (Portugal, 2018; EFSA, 2019b)                   | 0.02*(e3) (tentative) | –          | –              |
|                    | SEU              | Overdosed trials (3 × 100 g a.s./ha; PHI = 28 days): < 0.02; < 0.02; < 0.02                                                | Trials on spring onions (Portugal, 2018). Extrapolation to leek possible       | 0.02*(d),(e3), (f) (tentative) | 0.02       | 0.02           |

GAP: Good Agricultural Practice; OECD: Organisation for Economic Co-operation and Development; MRL: maximum residue level; Mo: residue levels expressed according to the monitoring residue definition; RA: residue levels expressed according to risk assessment residue definition. *: Indicates that the MRL is proposed at the limit of quantification.

(a): NEU: Outdoor trials conducted in northern Europe, SEU: Outdoor trials conducted in southern Europe, Indoor: indoor EU trials or Country code: if non-EU trials.
(b): Highest residue. The highest residue for risk assessment (RA) refers to the whole commodity and not to the edible portion.
(c): Supervised trials median residue. The median residue for risk assessment (RA) refers to the whole commodity and not to the edible portion.
(d): Tentative MRL because of reduced number of GAP-compliant trials.
(e): Tentative MRL because of data gaps on metabolism studies (section 1.1.1) ((1) too short PHI; (2) underdosed compared to application rate of cGAP; (3) PHI too short and underdosed).
(f): Tentative MRL because of trials to a more critical GAP compared with the reported GAP.

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B.1.2.2. Residues in rotational crops

Overall summary

| Residues in rotational and succeeding crops expected based on confined rotational crop study? | No |
| Residues in rotational and succeeding crops expected based on field rotational crop study? | No for the current residue definition |

Rotational crop studies using radiolabelled benalaxyl are available showing very low levels of residues in the following crops (lettuce, tomato, carrot and wheat) after application of an overdosed rate. 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 quantification would occur in succeeding crops without radiolabelling.

No for the current residue definition

No rotational field crop study available. This may need to be reconsidered pending the ongoing renewal assessment of benalaxyl.

B.1.2.3. Processing factors

| Processed commodity | Number of valid studies(a) | Processing Factor (PF) | Comment/source |
|---------------------|---------------------------|------------------------|----------------|
|                     |                           | Individual values | Median PF(b) | |
| Grape, must         | 6                         | 0.035; 0.052; 0.082; 0.085; 0.14; 0.45 | 0.08 | Residue levels in grape must were lower or equal to 0.01 mg/kg (Portugal, 2003) |
| Grape, juice        | 4                         | 0.036; 0.063; 0.11; 0.33 | 0.09 | Residue levels in grape juice were lower or equal to 0.01 mg/kg (Portugal, 2003, 2018) |
| Grape, pomace       | 1                         | 3.9 | 3.9 | Tentative(c); Portugal (2018) |
| Grape, wine         | 6                         | 0.035; 0.036; 0.052; 0.063; 0.082; 0.085 | 0.06 | Portugal (2003) |
| Tomato, juice       | 3                         | 0.27; 0.36; 0.41 | 0.36 | Residue levels in tomato juice were not detectable, lower or equal to 0.02 mg/kg (Portugal, 2018) |
| Tomato, puree       | 3                         | 0.24; 0.37; 0.71 | 0.37 | Residue levels in tomato puree were not detectable, lower or equal to 0.02 mg/kg (Portugal, 2018) |
| Tomato, ketchup     | 2                         | 0.24; 0.27 | 0.26 | Tentative(c); Portugal (2018) |
| Tomato, paste       | 2                         | 0.49; 0.55 | 0.52 | Tentative(c); Portugal (2018) |
| Tomato, canned      | 3                         | 0.20; 0.22; 0.36 | 0.22 | Residue levels in canned tomato were not detectable, lower or equal to 0.02 mg/kg (Portugal, 2018) |

PF: Processing factor (= Residue level in processed commodity expressed according to RD-Mo/Residue level in raw commodity expressed according to RD-Mo); CFp: Conversion factor for risk assessment in processed commodity (= Residue level in processed commodity expressed according to RD-RA/Residue level in processed commodity expressed according to RD-Mo).

(a): Studies with residues in the RAC at or close to the LOQ were disregarded (unless concentration may occur)
(b): Median of the individual conversion factors for each processing residues trial.
(c): A tentative PF is derived based on a limited dataset.

B.2. Residues in livestock

| Relevant groups (subgroups) | Dietary burden expressed in mg/kg bw per day | Most critical subgroup(5) | Most critical commodity(y)(6) | Trigger exceeded (Y/N) | Comments |
|-----------------------------|---------------------------------------------|---------------------------|-------------------------------|------------------------|----------|
|                             | Median | Maximum | Median | Maximum |                         |          |          |
| Cattle (all)                | 0.0031 | 0.0031  | 0.10  | 0.10    | Cattle (dairy) | Potato, process waste | No       | –        |
### Relevant groups (subgroups)

| Relevant groups (subgroups) | Dietary burden expressed in mg/kg bw per day | Most critical subgroup | Most critical commodity | Trigger exceeded (Y/N) | Comments |
|-----------------------------|---------------------------------------------|------------------------|-------------------------|------------------------|----------|
|                            | Median | Maximum | Median | Maximum |                      |                       |                       |
| Cattle (dairy only)        | 0.0031 | 0.0031  | 0.08   | 0.08   | Cattle (dairy)        | Potato, process waste | No                     |
| Sheep (all)                | 0.0032 | 0.0032  | 0.10   | 0.10   | Sheep (ram, ewe)      | Potato, process waste | No                     |
| Sheep (ewe only)           | 0.0032 | 0.0032  | 0.10   | 0.10   | Sheep (ewe)           | Potato, process waste | No                     |
| Swine (all)                | 0.0019 | 0.0019  | 0.08   | 0.08   | Swine (breeding)      | Potato, process waste | No                     |
| Poultry (all)              | 0.0014 | 0.0014  | 0.02   | 0.02   | Poultry (turkey)      | Potato, culls         | No                     |
| Poultry (layer only)       | 0.0009 | 0.0009  | 0.01   | 0.01   | Poultry (layer)       | Potato, culls         | No                     |
| Fish                       | –      | –       | –      | –      | –                     | –                     | Not calculated         |

(a): When one group of livestock includes several subgroups (e.g. poultry 'all' including broiler, layer and turkey), the result of the most critical subgroup is identified from the maximum dietary burdens expressed as 'mg/kg bw per day'.

(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as 'mg/kg bw per day'.

### B.2.1. Nature of residues and methods of analysis in livestock

#### B.2.1.1. Metabolism studies, methods of analysis and residue definitions in livestock

| Livestock (available studies) | Animal         | Dose (mg/kg bw/d) | Duration (days) | Comment/source                                      |
|-------------------------------|----------------|-------------------|-----------------|-----------------------------------------------------|
|                               | Laying hen     | 5.5               | 12              | Laying hen were dosed once a day (average weight 1.3 kg; ¹⁴C-benalaxyl; 59.6 mg/kg diet per day) (Portugal, 2003, 2007) |
|                               | Lactating ruminants (goats) | 1.35; 2.42 | 7               | Two goats (34.5 and 53.6 kg) dosed twice daily with 36.1 mg/kg ¹⁴C-benalaxyl (Portugal, 2003, 2007) |
Time needed to reach a plateau concentration in milk and eggs (days)

| Product  | Milk: > 7 days | Was not observed for the duration of the study. |
|----------|----------------|-----------------------------------------------|
| Eggs: 9 days | Hen metabolism study.          |

Metabolism in rat and ruminant similar

| Similarity | Yes | The rat metabolism of benalaxyl-M and benalaxyl was similar and it can be therefore assumed that the metabolism studies of benalaxyl in goats and hen is also valid for benalaxyl-M |

Can a general residue definition be proposed for animals?

| Definition required | Not required | Based on the goat and laying hen metabolism studies from benalaxyl, a common pathway for benalaxyl-M has depicted. However, in the studies a high level of TRR remains unidentified |

Animal residue definition for monitoring (RD-Mo)

| Definition required | Not required |

Animal residue definition for risk assessment (RD-RA)

| Definition required | Not required |

Fat soluble residues

| Soluble requirement | No | In the goat and hen metabolism studies, no accumulation in fat is reported. (EFSA, 2013b) |

Methods of analysis for monitoring of residues (analytical technique, matrix groups, LOQs)

| Analytical technique | Benalaxyl-M with chiral method: Fat, kidney, liver: | HPLC–MS with chiral column (LOQ=0.01 mg/kg) Confirmatory method and ILV available (EFSA, 2013a) |
|---------------------|-------------------------------------------------|----------------------------------------------------------------------------------|
|                     | Meat, eggs, milks: | HPLC–MS with chiral column (LOQ=0.02 mg/kg) Confirmatory method and ILV available (EFSA, 2013a) |
|                     | Benalaxyl with non-chiral method: Milk, meat, eggs and honey: | LC-MS-q-ToF (QuEChERS AO, Screening Detection Limit (SDL) of 0.0025 mg/kg validated for benalaxyl in milk, honey, meat (red and white meat, low and high fat fish) and eggs (EURLs, 2018) |

HPLC–MS: high-performance liquid chromatography with mass spectrometry; GC–MS/MS: gas chromatography with tandem mass spectrometry; ILV: independent laboratory validation.

**B.2.1.2. Stability of residues in livestock**

| Animal products (available studies) | Animal | Commodity | T (°C) | Stability period Value | Stability period Unit | Compounds covered | Comment/source |
|------------------------------------|--------|-----------|--------|----------------------|----------------------|------------------|----------------|
|                                    | No storage stability studies are available and not required | | | | | | |

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### B.2.2. Magnitude of residues in livestock

#### B.2.2.1. Summary of the residue data from livestock feeding studies

| Animal commodity      | Residues at the closest feeding level (mg/kg) | Estimated value at 1N | MRL proposal (mg/kg) | CF<sup>(c)</sup> |
|-----------------------|-----------------------------------------------|------------------------|----------------------|-------------------|
|                       | Mean                                         | Highest                | STMR<sub>Mo</sub><sup>(a)</sup> (mg/kg) | HR<sub>Mo</sub><sup>(b)</sup> (mg/kg) |
| Cattle (all)          | –                                             | –                      | –                    | –                 |
| Cattle (dairy only)   | –                                             | –                      | –                    | –                 |
| Sheep (all)           | –                                             | –                      | –                    | –                 |
| Sheep (ewe only)      | –                                             | –                      | –                    | –                 |
| Swine (all)           | –                                             | –                      | –                    | –                 |
| Poultry (all)         | –                                             | –                      | –                    | –                 |
| Poultry (layer only)  | –                                             | –                      | –                    | –                 |

n.a.: not applicable; n.r.: not reported.

*: Indicates that the MRL is proposed at the limit of quantification.

(a): Median residues expressed according to the residue definition for monitoring, recalculated at the 1N rate for the median dietary burden.

(b): Highest residues expressed according to the residue definition for monitoring, recalculated at the 1N rate for the maximum dietary burden.

(c): Conversion factor to recalculate residues according to the residue definition for monitoring to the residue definition for risk assessment.

### B.3. Consumer risk assessment

#### B.3.1. Consumer risk assessment only considering the authorised uses of benalaxyl-M

| ARfD                  | Not allocated (EFSA, 2013a) |
|-----------------------|-----------------------------|

Not relevant since no ARfD has been considered necessary (EFSA, 2013a).

| ADI                   | 0.04 mg/kg bw per day (EFSA, 2013a) |
|-----------------------|-------------------------------------|
| TMDI according to EFSA PRIMo | Not assessed in this review |
| NTMDI                 | Not assessed in this review         |
| Highest IEDI, according to EFSA PRIMo (rev.2) | 1.1% ADI (WHO Cluster diet B) |
| NEDI (% ADI)          | Not assessed in this review         |
| Assumptions made for the calculations | The calculation is based on the median residue levels derived for raw agricultural commodities. The contributions of commodities where no GAP on benalaxyl-M was reported in the framework of the MRL review were not included in the calculation (see Section 3.1) |

ADI: acceptable daily intake; bw: body weight; NEDI: national estimated daily intake; PRIMo: (EFSA) Pesticide Residues Intake Model; WHO: World Health Organization; TMDI: theoretical maximum daily intake; NTMDI: national theoretical maximum daily intake; GAP: Good Agricultural Practice; MRL: maximum residue level.
Consumer exposure assessment through drinking water resulting from groundwater metabolite(s) according to SANCO/221/2000 rev.10 Final (25/02/2003)

| Metabolite(s) | Not assessed in this review |
|---------------|----------------------------|
| ADI (mg/kg bw per day) | Not assessed in this review |
| Intake of groundwater metabolites (% ADI) | Not assessed in this review |

B.3.2. Consumer risk assessment with consideration of benalaxyl residues resulting from the EU uses of benalaxyl and benalaxyl-M, plus existing CXLs for benalaxyl

ARfD

Not allocated (EFSA, 2013a)

Not relevant since no ARfD has been considered necessary.

| ADI         | Not assessed in this review |
|-------------|----------------------------|
| TMDI according to EFSA PRIMo | Not assessed in this review |
| NTMDI, according to (to be specified) | Not assessed in this review |
| Highest IEDI, according to EFSA PRIMo (rev.2.1) | 2.2% ADI (WHO Cluster diet B) |

Assumptions made for the calculations

The calculation is based on the median residue levels derived for raw agricultural commodities based on a comparison between MRL proposals derived in this review (from benalaxyl-M uses) and in the previous review of benalaxyl. Furthermore, CXL values were considered where these were not covered by proposed EU MRLs for benalaxyl. It is further assumed that benalaxyl and benalaxyl-M are not used simultaneously according to the authorised uses (see Section 3.1). The contributions of commodities where no GAP on benalaxyl-M or benalaxyl and for which no CXL are defined were not included in the calculation.

ADI: acceptable daily intake; bw: body weight; NEDI: national estimated daily intake; PRIMo: (EFSA) Pesticide Residues Intake Model; WHO: World Health Organization; TMDI: theoretical maximum daily intake; NTMDI: national theoretical maximum daily intake; GAP: Good Agricultural Practice; MRL: maximum residue level; CXL: codex maximum residue limit.

B.4. Proposed MRLs

| Code number | Commodity     | Existing EU MRL (mg/kg) for benalaxyl | Existing CXL (mg/kg) for benalaxyl | Outcome for benalaxyl-M MRL (mg/kg) | Outcome of the review for benalaxyl-M considering the previous review of benalaxyl
|-------------|---------------|--------------------------------------|------------------------------------|-----------------------------------|----------------------------------------------------------------------------------|
|             |               |                                      |                                    |                                   |                                                                                 |
|             |               |                                      |                                    |                                   | MRL (mg/kg) | Comment                                                                 |
| 0151010     | Table grapes  | 0.3                                   | 0.3                                | 0.7                               | 0.7 | Further consideration needed (benalaxyl-M) |
| 0151020     | Wine grapes   | 0.3                                   | 0.3                                | 0.15                              | 0.3 | Recommended (CXL benalaxyl) |

Enforcement residue definition: Benalaxyl including other mixtures of constituent isomers including benalaxyl-M (sum of isomers)
## Review of the existing MRLs for benalaxyl-M

| Code number | Commodity     | Existing EU MRL (mg/kg) for benalaxyl | Existing CXL (mg/kg) for benalaxyl | Outcome for benalaxyl-M MRL (mg/kg) | Outcome of the review for benalaxyl-M considering the previous review of benalaxyl+ | MRL (mg/kg) | Comment |
|-------------|---------------|---------------------------------------|-----------------------------------|------------------------------------|---------------------------------------------------------------------------------|------------|---------|
| 0211000     | Potatoes      | 0.05*                                 | 0.02*                             | 0.02*                              | Further consideration needed (benalaxyl-M)                                        | 0.02*      |         |
| 0220010     | Garlic        | 0.05*                                 | –                                 | 0.02*                              | Further consideration needed (benalaxyl-M)                                        | 0.02*      |         |
| 0220020     | Onions        | 0.2                                   | 0.02*                             | 0.02*                              | Further consideration needed (benalaxyl-M)                                        | 0.02*      |         |
| 0220030     | Shallots      | 0.05*                                 | –                                 | 0.02*                              | Further consideration needed (benalaxyl-M)                                        | 0.02*      |         |
| 0231010     | Tomatoes      | 0.5                                   | 0.2                               | 0.3                                | Recommended (benalaxyl)                                                            | 0.5        |         |
| 0231020     | Peppers       | 0.2                                   | –                                 | –                                 | Further consideration needed (EU MRL benalaxyl)                                    | 0.2        |         |
| 0231030     | Aubergines/eggplants | 0.5                         | –                                 | 0.3                                | Recommended (benalaxyl)                                                            | 0.5        |         |
| 0233010     | Melons        | 0.3                                   | 0.3                               | 0.15                               | Recommended (CXL benalaxyl)                                                       | 0.3        |         |
| 0233030     | Watermelons   | 0.1                                   | 0.1                               | 0.15                               | Recommended (benalaxyl-M)                                                         | 0.15       |         |
| 0251020     | Lettuces      | 1                                     | 1                                 | 3                                 | Further consideration needed (benalaxyl-M)                                        | 3          |         |
| 0270060     | Leeks         | 0.05*                                 | –                                 | 0.02*                              | Further consideration needed (benalaxyl-M)                                        | 0.02*      |         |
| 0401060     | Rape seeds    | 0.05*                                 | –                                 | –                                 | Further consideration needed (EU MRL benalaxyl)                                    | 0.05*      |         |
| –           | Other commodities of plant and/or animal origin | See Reg. 520/2011 | –                                 | –                                 | Further consideration needed                                                      | –          |         |

MRL: maximum residue level; CXL: codex maximum residue limit.

+: EFSA (2013b).

*: Indicates that the MRL is set at the limit of quantification.

(F): The residue definition is fat soluble.

(a): Tentative MRL is derived from a GAP evaluated at EU level, which is not fully supported by data but for which no risk to consumers was identified (assuming the existing residue definition); existing CXL is covered by the tentative MRL (combination F-III in Appendix E).

(b): MRL is derived from the existing CXL, which is supported by data and for which no risk to consumers is identified; GAP evaluated at EU level, which is not fully supported by data, leads to a lower tentative MRL (combination F-VII in Appendix E).

(c): Tentative MRL is derived from a GAP evaluated at EU level, which is not fully supported by data but for which no risk to consumers was identified (assuming the existing residue definition); GAP evaluated at EU level, which is also fully supported by data, leads to a lower tentative MRL (combination F-V in Appendix E).

(d): GAP evaluated at EU level is not supported by data but no risk to consumers was identified for the existing EU MRL (also assuming the existing residue definition); no CXL is available (combination D-I in Appendix E).

(e): MRL is derived from a GAP evaluated at EU level, which is fully supported by data and for which no risk to consumers is identified; no CXL is available (combination H-I in Appendix E).

(g): MRL is derived from the existing CXL, which is supported by data and for which no risk to consumers is identified; GAP evaluated at EU level, which is also fully supported by data, leads to a lower MRL (combination H-VII in Appendix E).

(h): MRL is derived from a GAP evaluated at EU level, which is fully supported by data and for which no risk to consumers is identified; existing CXL is covered by the recommended MRL (combination H-III in Appendix E).

(i): There are no relevant authorisations or import tolerances reported at EU level; no CXL is available. Either a specific LOQ or the default MRL of 0.01 mg/kg may be considered (combination A-I in Appendix E).
## Appendix C – Pesticide Residue Intake Model (PRIMo)

### PRIMo(EU)

| Benalaxyl-M | Status of the active substance: | Included Code no. |
|-------------|--------------------------------|-------------------|
|             | LOQ (mg/kg bw): 0.01 Proposed LOQ |                   |

| Toxicological end points | ADI (mg/kg bw per day): 0.04 | ARfD (mg/kg bw): n.a. |
|--------------------------|-------------------------------|-----------------------|
| Source of ADI: | EFSA | Source of ARfD: | EFSA |
| Year of evaluation: | 2013 | Year of evaluation: | 2013 |

| Code no. | Year of evaluation: 2013 | Source of ADI: EFSA | Source of ARfD: EFSA | Year of evaluation: 2013 | LOQ (mg/kg bw): 0.01 Proposed LOQ |
|-----------|--------------------------|---------------------|----------------------|--------------------------|-----------------------------------|
| Code no.  | Year of evaluation: 2013 | Source of ADI: EFSA | Source of ARfD: EFSA | Year of evaluation: 2013 | LOQ (mg/kg bw): 0.01 Proposed LOQ |

| Toxicological end points | ADI (mg/kg bw per day): 0.04 | ARfD (mg/kg bw): n.a. |
|--------------------------|-------------------------------|-----------------------|
| Source of ADI: | EFSA | Source of ARfD: | EFSA |
| Year of evaluation: | 2013 | Year of evaluation: | 2013 |

### Chronic risk assessment – refined calculations

| Commodity/ | TMDI | minimum – maximum |
|------------|------|-------------------|
| group of commodities | TMDI (range) in % of ADI | 0 | 1 |

| No of diets exceeding ADI: | --- |
|---------------------------|-----|

| Commodity/ | TMDI | minimum – maximum |
|------------|------|-------------------|
| group of commodities | TMDI (range) in % of ADI | 0 | 1 |

| Commodity/ | TMDI | minimum – maximum |
|------------|------|-------------------|
| group of commodities | TMDI (range) in % of ADI | 0 | 1 |

### Conclusion:

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI. A long-term intake of residues of benalaxyl-M is unlikely to present a public health concern.
### Acute risk assessment/children – refined calculations

The acute risk assessment is based on the ARfD. For each commodity, the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS, an average European unit weight was used for the IESTI calculation.

In the IESTI 1 calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002); for lettuce, a variability factor of 5 was used. In the IESTI 2 calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce, the calculation was performed with a variability factor of 3.

Threshold MRL is the calculated residue level which would lead to an exposure equivalent to 100% of the ARfD.

#### Highest % of ARfD/ADI processed commodities

| Commodity | pTMRL/Threshold MRL (mg/kg) |
|-----------|-----------------------------|
| Lettuce   | 5                           |

No exceedance of the ARfD/ADI was identified for any unprocessed commodity.

| Processed commodities | pTMRL/Threshold MRL (mg/kg) |
|-----------------------|-----------------------------|
| Lettuce               | 5                           |

#### No of critical MRLs (IESTI 1)

| No of commodities for which ARfD/ADI is exceeded (IESTI 1): |
|--------------------------------------------------------------|
| None                                                         |

#### No of critical MRLs (IESTI 2)

| No of commodities for which ARfD/ADI is exceeded (IESTI 2): |
|--------------------------------------------------------------|
| None                                                         |

### Conclusion:

For benalaxyl-M, IESTI 1 and IESTI 2 were calculated for food commodities for which pTMRLs were submitted and for which consumption data are available. No exceedance of the ARfD/ADI was identified for any unprocessed commodity.

For processed commodities, no exceedance of the ARfD/ADI was identified. Acute exposure calculations were not carried out because an acute reference dose (ARfD) was not deemed necessary for this active substance (EFSA, 2013a).
Benalaxyl-M

Status of the active substance: Included

Proposed LOQ: 0.02

ADI (mg/kg bw per day): 0.04

Source of ADI: EFSA

Year of evaluation: 2013

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.

A long-term intake of residues of benalaxyl-M is unlikely to present a public health concern.
The acute risk assessment is based on the ARfD.

For each commodity, the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS, an average European unit weight was used for the IESTI calculation.

In the IESTI 1 calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002); for lettuce, a variability factor of 5 was used.

In the IESTI 2 calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce, the calculation was performed with a variability factor of 3.

Threshold MRL is the calculated residue level which would lead to an exposure equivalent to 100% of the ARfD.

### Acute risk assessment/children – refined calculations

| No of commodities for which ARfD/ADI is exceeded (IESTI 1): | No of commodities for which ARfD/ADI is exceeded (IESTI 2): | No of commodities for which ARfD/ADI is exceeded (IESTI 1): | No of commodities for which ARfD/ADI is exceeded (IESTI 2): |
|---|---|---|---|
| IESTI 1 | IESTI 2 | IESTI 1 | IESTI 2 |
| Highest % of ARfD/ADI Commodities | pTMRL/ threshold MRL (mg/kg) | Highest % of ARfD/ADI Commodities | pTMRL/ threshold MRL (mg/kg) | Highest % of ARfD/ADI Commodities | pTMRL/ threshold MRL (mg/kg) | Highest % of ARfD/ADI Commodities | pTMRL/ threshold MRL (mg/kg) |

No of critical MRLs (IESTI 1) --- No of critical MRLs (IESTI 2) ---

### Acute risk assessment/adults/general population – refined calculations

| No of commodities for which ARfD/ADI is exceeded: | No of commodities for which ARfD/ADI is exceeded: |
|---|---|
| IESTI 1 | IESTI 2 |
| Highest % of ARfD/ADI Processed commodities | pTMRL/ threshold MRL (mg/kg) | Highest % of ARfD/ADI Processed commodities | pTMRL/ threshold MRL (mg/kg) |

No exceedance of the ARfD/ADI was identified for any unprocessed commodity.

**pTMRL: provisional temporary MRL.**

**Conclusion:**

For benalaxyl-M, IESTI 1 and IESTI 2 were calculated for food commodities for which pTMRLs were submitted and for which consumption data are available. No exceedance of the ARfD/ADI was identified for any unprocessed commodity.

For processed commodities, no exceedance of the ARfD/ADI was identified.

Acute exposure calculations were not carried out because an acute reference dose (ARfD) was not deemed necessary for this active substance (EFSA, 2013a).
Appendix D – Input values for the exposure calculations

D.1. Livestock dietary burden calculations

| Risk assessment residue definition: benalaxyl-M | Median dietary burden | Maximum dietary burden |
|-----------------------------------------------|-----------------------|------------------------|
|                                               | Input value (mg/kg)   | Comment                |
| Feed commodity                                |                       |                        |
| Potato, culls                                 | 0.02*                 | STMR                   |
| Potato, process waste                         | 0.02*                 | STMR(a)                |
| Potato, dried pulp                            | 0.02*                 | STMR(a)                |
|                                               | 0.02*                 | HR                     |
|                                               | 0.02*                 | STMR(a)                |

STMR: supervised trials median residue; HR: highest residue; PF: processing factor.
*: Indicates that the input value is proposed at the limit of quantification.
(a): For potato, process waste and potato dried pulp no default processing factor was applied because benalaxyl-M is applied early in the growing season and residues are expected to be below the LOQ. Concentration of residues in these commodities is therefore not expected.

D.2. Consumer risk assessment only considering the authorised uses of benalaxyl-M

| Commodity                  | Chronic risk assessment |
|----------------------------|-------------------------|
|                            | Input value (mg/kg)     | Comment                |
| Risk assessment residue definition: benalaxyl-M |                       |
| Table grapes               | 0.04                    | STMR (benalaxyl-M)     |
| Wine grapes                | 0.052                   | STMR (benalaxyl-M)     |
| Potatoes                   | 0.02*                   | STMR (benalaxyl-M)     |
| Garlic                     | 0.02*                   | STMR (benalaxyl-M)     |
| Onions                     | 0.02*                   | STMR (benalaxyl-M)     |
| Shallots                   | 0.02*                   | STMR (benalaxyl-M)     |
| Tomatoes                   | 0.068                   | STMR (benalaxyl-M)     |
| Aubergines/eggplants       | 0.068                   | STMR (benalaxyl-M)     |
| Melons                     | 0.026                   | STMR (benalaxyl-M)     |
| Watermelons                | 0.026                   | STMR (benalaxyl-M)     |
| Lettuces                   | 0.033                   | STMR (benalaxyl-M)     |
| Leeks                      | 0.02*                   | STMR (benalaxyl-M)     |

STMR: supervised trials median residue.
*: Indicates that the input value is proposed at the limit of quantification.

D.3. Consumer risk assessment with consideration of benalaxyl residues resulting from EU uses of benalaxyl and benalaxyl-M, plus existing CXLs for benalaxyl

| Commodity                  | Chronic risk assessment |
|----------------------------|-------------------------|
|                            | Input value (mg/kg)     | Comment                |
| Risk assessment residue definition: benalaxyl |                       |
| Table grapes               | 0.04                    | STMR (benalaxyl-M)     |
| Wine grapes                | 0.12                    | STMR (CXL) (benalaxyl) |
| Potatoes                   | 0.01*                   | STMR (benalaxyl-M)     |
| Garlic                     | 0.02*                   | STMR (benalaxyl-M)     |
| Onions                     | 0.02*                   | STMR (benalaxyl-M)     |
| Shallots                   | 0.02*                   | STMR (benalaxyl-M)     |
| Tomatoes                   | 0.14                    | STMR (benalaxyl-M)     |
| Peppers                    | 0.20                    | EU MRL (benalaxyl)     |
### Chronic risk assessment

| Commodity             | Input value (mg/kg) | Comment                           |
|-----------------------|--------------------|-----------------------------------|
| Aubergines/eggplants  | 0.14               | STMR (benalaxyl; EFSA, 2013b)     |
| Melons                | 0.045              | STMR (CXL) (benalaxyl)            |
| Watermelons           | 0.026              | STMR (benalaxyl-M)                |
| Lettuces              | 0.033              | STMR (benalaxyl-M)                |
| Leeks                 | 0.02*              | STMR (benalaxyl-M)                |
| Rape seeds            | 0.05*              | EU MRL (benalaxyl; EFSA, 2013b)   |

STMR: supervised trials median residue; CXL: codex maximum residue limit; MRL: maximum residue level.

*: Indicates that the input value is proposed at the limit of quantification.
Appendix E – Decision tree for deriving MRL recommendations
### Appendix F – Used compound codes

| Code/trivial name<sup>(a)</sup> | IUPAC name/SMILES notation/InChIKey<sup>(b)</sup> | Structural formula<sup>(c)</sup> |
|-------------------------------|-----------------------------------------------|---------------------------------|
| Benalaxyl-M                  | methyl N-(phenylacetyl)-N-(2,6-xylyl)-D-alaninate  
C[C@@H](N(C(=O)C1cccc1)c1c(C)cccc1C(-)=O)OC  
CJPQIRJHZUAQNP-MRXNPFEDSA-N  | ![Structural formula for Benalaxyl-M](image) |
| benalaxyl                    | methyl N-(phenylacetyl)-N-(2,6-xylyl)-DL-alaninate  
CC1=C(N(C(C(C=CC=CC=CC2=O)C(O)=O)C(C)=CC=CC1)CJPQIRJHZUAQNP-UHFFFAOYSA-N  | ![Structural formula for benalaxyl](image) |
| GX5a/GX5b isomers (2-hydroxy methyl benalaxyl) | methyl N-(2-(hydroxymethyl)-6-methylphenyl)-N-(2-phenylacetyl)alaninate  
CC(C(O)=O)NC1=CC=CC=CC=CC=C2=O  
QHSRUJWLHQLNFRUHFFFAOYSA-N  | ![Structural formula for GX5a/GX5b isomers](image) |
| GX1 (monoglucobenalaxyl)    | methyl N-(2-D-glucopyranosyl-oxy-methyl-6-methyl-phenyl)-N-(phenylacetyl)-DL-alaninate  
O=C(O)C(C(N(=O)Cc1cccc1)c1c(C)cccc1C[O@@H][O][C@@H][O][C@@H][O][C@@H][2O]cccc1C  
JADCFAHBYNWKZMK-DBAWLCESSA-N  | ![Structural formula for GX1](image) |
| Code/trivial name<sup>(a)</sup> | IUPAC name/SMILES notation/InChiKey<sup>(b)</sup> | Structural formula<sup>(c)</sup> |
|--------------------------|-------------------------------------------------|---------------------------------|
| GX6 (digluco-benalaxyl)  | methyl N-[2-[4-O-α-D-glucopyranosyl-D-glucopyranosyl]-oxy-methyl-6-methyl-phenyl]-N-(phenylacetyl)-DL-alaninate (proposed structure) O=C(OC)(C)N(C(=O)Cc1ccccc1)c1c(C)=O[C@H]1[C@@H]([O][C@H]2O)[C@H][C@H][O][C@H]2O)[C@H][O][C@H]2O)[C@H][O][C@H]2O)ccccc1 OTCDJVNBJBBGBF-PKYIGJJASA-N | ![Structural formula for GX6](image) |
| GX11 (malonyl conjugate of GX1) | 2-[[2RS]-1-methoxy-1-oxo-2-propanyl](phenylacetyl)amino]-3-methylbenzyl 4-O-(carboxyacetyl)-D-glucopyranoside O=C(OC)(C)N(C(=O)Cc1ccccc1)c1c(COC2O)[C@H][CO][C@H][OC(=O)CC(=O)O][C@H][O][C@H]2O)ccccc1 UTCDJVNBJBBGBF-PKYIGJJASA-N | ![Structural formula for GX11](image) |
| Hydroxymethyl derivative of benalaxyl (G8) | methyl N-[2-{hydroxymethyl}-6-methylphenyl]-N-(phenylacetyl)-DL-alaninate CC(N(C(=O)Cc1ccccc1)c1c(C)ccc1CO)C(=O)OC QHSRUJWLFUQNEB-UHFFFAOYSA-N | ![Structural formula for Hydroxymethyl derivative](image) |
| Carboxy-benalaxyl | 2-[[2RS]-1-methoxy-1-oxo-2-propanyl](phenylacetyl)amino]-3-methylbenzoic acid CC(N(C(=O)Cc1ccccc1)c1c(C)ccc1CO)C(=O)OC LIQIENADQRGJH-UHFFFAOYSA-N | ![Structural formula for Carboxy-benalaxyl](image) |
| Hydroxy-carboxy-benalaxyl | 3-{hydroxymethyl}-2-[[2RS]-1-methoxy-1-oxo-2-propanyl][phenylacetyl]amino]benzoic acid CC(N(C(=O)Cc1ccccc1)c1c(C)ccc1CO)C(=O)OC AYFLTOWDPKZGKC-UHFFFAOYSA-N | ![Structural formula for Hydroxy-carboxy-benalaxyl](image) |
| Code/trivial name<sup>(a)</sup> | IUPAC name/SMILES notation/InChiKey<sup>(b)</sup> | Structural formula<sup>(c)</sup> |
|-------------------------------|-----------------------------------------------|-----------------------------|
| M9 (benalaxyl acid)           | N-(2,6-dimethylphenyl)-N-phenylacetyl-DL-alanine  |
|                               | CC(N(C(-O)Cc1ccccc1)c1c(C)cccc1C)(=O)O | ![Structural formula](image) |
|                               | DXGQLQXGYPVL-UHFFFAOYSA-N                  |

IUPAC: International Union of Pure and Applied Chemistry; SMILES: simplified molecular-input line-entry system; InChiKey: International Chemical Identifier Key.

(a): The metabolite name in bold is the name used in the conclusion.
(b): ACD/Name 2018.2.2 ACD/Labs 2018 Release (File version N50E41, Build 103230, 21 July 2018).
(c): ACD/ChemSketch 2018.2.2 ACD/Labs 2018 Release (File version C60H41, Build 106041, 7 December 2018).