Setting an import tolerance for 2,4-D in soyabees

European Food Safety Authority (EFSA),
Maria Anastassiadou, Alba Brancato, Luis Carrasco Cabrera, Lucien Ferreira, Luna Greco,
Samira Jarrah, Aija Kazocina, Renata Leuschner, Jose Oriol Magrans, Ileana Miron,
Ragner Pedersen, Marianna Raczyk, Hermine Reich, Silvia Ruocco, Angela Sacchi,
Miguel Santos, Alois Stanek, Jose Tarazona, Anne Theobald and Alessia Verani

Abstract

In accordance with Article 6 of Regulation (EC) No 396/2005, the applicant Dow AgroSciences submitted a request to the competent national authority in Greece to set an import tolerance for the active substance 2,4-D in genetically modified (GM) soyabees imported from Canada and the USA. The genetic modification confers tolerance to the herbicide 2,4-D. The data submitted in support of the request provided sufficient evidence to conclude that residues of parent 2,4-D and of the metabolite 2,4-dichlorophenol (2,4-DCP), which was found in the GM soyabees treated with 2,4-D, are unlikely to present a risk for consumers. Sufficiently validated analytical methods are available to enforce the proposed maximum residue level (MRL) in soyabees.

© 2019 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

**Keywords:** 2, 4-D, genetically modified plant, soyabees, MRL application, consumer risk assessment

**Requestor:** European Commission

**Question number:** EFSA-Q-2015-00152

**Correspondence:** pesticides.mrl@efsaeuropa.eu
Summary

In accordance with Article 6 of Regulation (EC) No 396/2005, the applicant Dow AgroScience submitted a request to the competent national authority in Greece (evaluating Member State (EMS)) to set an import tolerance for the active substance 2,4-D in genetically modified (GM) soybeans expressing the aryloxyalkanoate dioxygenase-12 (AAD-12) protein, imported from Canada and USA. The genetic modification introduced confers tolerance to the herbicide 2,4-D. The EMS drafted an evaluation report in accordance with Article 8 of Regulation (EC) No 396/2005 which was submitted to the European Commission and forwarded to the European Food Safety Authority (EFSA) on 5 March 2015.

EFSA bases its assessment on the revised evaluation report submitted by the EMS, the renewal assessment report (RAR) and its final addendum, the Commission revised review report on 2,4-D, the conclusion on the peer review of the pesticide risk assessment of the active substance 2,4-D and 2,4-DB, as well as the European Union (EU) review of the existing maximum residue levels (MRLs) for 2,4-D according to Article 12 of Regulation (EC) No 396/2005 and a previous EFSA opinion on GM maize.

The toxicological profile of the active substance 2,4-D was re-assessed within the framework of the EU pesticides renewal review of the approval under Regulation (EC) No 1107/2009. An acceptable daily intake (ADI) of 0.02 mg/kg body weight (bw) per day and an acute reference dose (ARfD) of 0.3 mg/kg bw were finally established for 2,4-D. The toxicological profile of the metabolite 2,4-dichlorophenol (2,4-DCP), which is a common metabolite to 2,4-D and 2,4-DB, was discussed during the pesticides peer review 186 Experts’ meeting in November 2018. The toxicological reference values derived for 2,4-DB (and 2,4-D) were concluded to be applicable also to 2,4-DCP.

The metabolism of 2,4-D in primary crops was previously investigated in conventional crops after foliar (cereals and root crops) and soil (fruit crops) applications. Based on the results of these studies, the residue definition for enforcement and risk assessment was established as sum of 2,4-D, its salts, esters and conjugates, expressed as 2,4-D.

A new metabolism study on soybeans genetically modified to express the AAD-12 protein was submitted in the framework of this MRL application. In seeds, no new metabolites were identified, but the largest component of the residue was represented by 2,4-DCP and its conjugates, whereas parent 2,4-D was found only in low amounts. The results of field residue trials confirmed the presence of 2,4-DCP at levels up to 0.05 mg/kg in soybean seeds. Based on the results of the metabolism study, EFSA proposes to include 2,4-DCP in the residue definition for risk assessment for the GM soybeans under assessment. Since 2,4-DCP may be originated from other sources and cannot unequivocally be traced back to its origin, it should be excluded from the residue definition for enforcement. Therefore, it is proposed to apply the enforcement residue definition currently set in Regulation (EC) No 396/2005 also to GM soybeans under assessment.

A sufficient number of valid residue trials performed on GM soybeans expressing the AAD-12 protein have been submitted in support to this application. In none of the trials, quantifiable residues of 2,4-D (including salts, esters and conjugates) above the limit of quantification (LOQ) of 0.01 mg/kg have been found. Thus, the existing MRL for 2,4-D in soybeans does not have to be raised. However, risk managers could consider the lowering of the existing MRL to the LOQ of 0.01 mg/kg, unless other uses of 2,4-D require to maintain the current MRL at the level of 0.05 mg/kg (LOQ). Sufficiently validated enforcement analytical methods are available. In the framework of the review of existing MRLs under Article 12 of Regulation (EC) No 396/2005 a data gap for an independent laboratory validation (ILV) for the enforcement analytical method in high oil content matrices was identified. This data gap has been satisfactorily addressed in the framework of the EU pesticides peer review for the renewal of the approval of 2,4-D.

Specific studies investigating the nature and magnitude of 2,4-D residues in processed commodities are not required as significant residues are not expected in the raw agricultural commodities (RAC). As the uses of 2,4-D are on imported crops, investigations of residues in rotational crops are not relevant. A change of the existing MRLs for 2,4-D in products of animal origin resulting from the use of imported GM soybean by-product meal used as a feed item is not required.

The consumer risk assessment was performed with revision 2 of the EFSA Pesticide Residues Intake Model (PRIMo). For the chronic exposure, the existing uses at the EU level and the acceptable CXLs assessed in the framework of the MRL review were also taken into account. The acute risk assessment was performed for soybeans only. The highest estimated long-term consumer intake accounted for 34% of the ADI. The acute consumer exposure to 2,4-D residues in GM soybean seeds was calculated as up to 0.02% of the ARfd.
EFSA concludes that the use of 2,4-D according to the notified good agricultural practices assessed on GM soybeans expressing the AAD-12 protein will not result in a consumer exposure exceeding the toxicological reference values for 2,4-D. Hence, a risk for consumers is unlikely.

The information submitted was sufficient to propose the MRL summarised in the table below.

| Code(a) | Commodity  | Existing EU MRL (mg/kg) | Proposed EU MRL (mg/kg) | Comment/Justification |
|---------|------------|-------------------------|-------------------------|------------------------|
| 0401070 | Soyabeans  | 0.05* (ft)              | 0.05* or 0.01*           | The submitted data are sufficient to perform a consumer risk assessment for the use of 2,4-D in support the import tolerance request. The lowering of the existing MRL set at the LOQ of 0.05 mg/kg to the LOQ of 0.01 mg/kg as derived from the residue trials on 2,4-D-tolerant soybeans (AAD-12 protein) and achievable with the validated enforcement analytical methods is an option to be discussed by risk managers. Risk for consumers unlikely MRL/Tolerance in the countries of origin (Canada and USA) is 0.02 mg/kg. The data gap identified by EFSA for additional information on the analytical method (ft) has been previously addressed |

MRL: maximum residue level; LOQ: limit of quantification; AAD-12: aryloxyalkanoate dioxygenase-12.

*: Indicates that the MRL is set at the limit of analytical quantification (LOQ).

(a): Commodity code number according to Annex I of Regulation (EC) No 396/2005.

ft: The European Food Safety Authority identified some information on analytical methods as unavailable. When reviewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 17 December 2015, or, if that information is not submitted by that date, the lack of it.

The data gap identified in the framework of the MRL review (ILV for enforcement of residues in high oil content commodities) was satisfactorily addressed with the information submitted in the process of the EU pesticides renewal of the approval of the active substance 2,4-D. Therefore, the footnote (ft) set in Commission Regulation (EU) No 1317/2013 can be deleted for soyabeans (code 0401070) and also for the whole subgroup of tree nuts (code 0120000), except chestnuts (code 0120040).
# Table of contents

Abstract ................................................................................................................................................... 1  
Summary ................................................................................................................................................. 3  
Background ............................................................................................................................................. 6  
The active substance and its use pattern ................................................................................................... 6  
1. Method of analysis ............................................................................................................................. 8  
1.1. Methods for enforcement of residues in food of plant origin ...................................................... 8  
1.2. Methods for enforcement of residues in food of animal origin ..................................................... 8  
2. Mammalian toxicology ....................................................................................................................... 8  
2.1. Toxicological profile of the active substance ................................................................................. 8  
2.2. Toxicological profile of metabolites .............................................................................................. 8  
3. Residues ........................................................................................................................................... 8  
3.1. Nature and magnitude of residues in plant ................................................................................... 8  
3.1.1. Primary crops ............................................................................................................................... 9  
3.1.1.1. Nature of residues .................................................................................................................. 9  
3.1.1.2. Magnitude of residues .......................................................................................................... 11  
3.1.1.3. Effect of industrial processing and/or household preparation .............................................. 13  
3.1.2. Rotational crops ......................................................................................................................... 13  
3.2. Nature and magnitude of residues in livestock ............................................................................. 13  
3.2.1. Dietary burden of livestock ....................................................................................................... 13  
4. Consumer risk assessment .................................................................................................................. 14  
Conclusions and recommendations ........................................................................................................... 16  
References .............................................................................................................................................. 16  
Abbreviations ......................................................................................................................................... 17  
Appendix A – Good Agricultural Practice (GAPs) ................................................................................ 19  
Appendix B – Used compound codes .................................................................................................... 20
Background

Regulation (EC) No 396/20051 (hereinafter referred to as the MRL regulation) establishes the rules governing the setting of pesticide maximum residue levels (MRLs) in the European Union (EU). Article 6 of the Regulation lays down that commercially interested parties such as manufacturers or importers may submit an application requesting the setting of an import tolerance in accordance with the provisions of Article 7 of the MRL regulation.

Greece, the evaluating Member State (EMS), received an application from the company Dow AgroSciences2 to set an import tolerance for the active substance 2,4-D on genetically modified (GM) soybeans expressing the aryloxyalkanoate dioxygenase-12 (AAD-12) protein imported from Canada and USA. The two events linked to the GM soybeans expressing AAD-12: DAS-68416-4 and DAS-44406-6. The application was notified to the European Commission and the European Food Safety Authority (EFSA) and was subsequently evaluated by the EMS in accordance with Article 8 of the MRL Regulation.

According to the EMS, the notified uses of 2,4-D on soybeans imported from Canada and USA does not require a change of the existing MRL set at the level of the limit of quantification (LOQ) of 0.05 mg/kg, but have an impact on the risk assessment, since a toxicologically relevant metabolite is expected to be present in higher concentrations than in the non-GM soybeans.

EFSA proceeded with the assessment of the applications and the evaluation reports as required by Article 10 of the Regulation. EFSA identified data gaps which needed further clarification, and which were requested from the EMS. On 3 October 2018, the EMS submitted the requested information in a revised evaluation report (Greece, 2015), which replaced the previously submitted evaluation report.

In accordance with Article 10 of Regulation (EC) No 396/2005, EFSA shall, based on the evaluation report provided by the EMS, provide a reasoned opinion on the risks to the consumer associated with the application. A pesticides peer review experts’ meeting was held on 21–22 November 2018 to discuss the toxicological profile of the metabolite 2,4-dichlorophenol (2,4-DCP); (EFSA, 2018).

The evaluation reports submitted by the EMS (Greece, 2015) as revised in September 2018, the exposure calculations using the EFSA Pesticide Residues Intake Model (PRIMo), together with the report of the experts’ meeting on mammalian toxicology regarding 2,4-D (EFSA, 2018), are considered as supporting documents to this reasoned opinion and, thus, are made publicly available.

The active substance and its use pattern

The details of the Good Agricultural Practices (GAPs) reported to be authorised for use on GM soybeans in Canada and USA are in Appendix A.

2,4-D is the ISO common name for (2,4-dichlorophenoxy)acetic acid (IUPAC). Different variants of 2,4-D are used in commercial formulations, such as acid, salts and esters. The chemical structures of the active substance 2,4-D and its main metabolites are reported in Appendix B.

For the two GM soybeans expressing the aryloxyalkanoate dioxygenase-12 (AAD-12) protein, DAS-68416-4 and DAS-44406-6, applications for authorisation for food and feed uses, import and processing were submitted in the EU in accordance with Regulation (EC) No 1829/20033. The two events express similar levels of AAD-12 protein and equivalence tolerance to 2,4-D applications. EFSA has carried out the scientific assessment and published the following Scientific Opinion:

- Application EFSA-GMO-NL-2011-91 for soybean DAS-68416-4, expressing the AAD-12 and phosphinothricin acetyltransferase (PAT) proteins, which confer tolerance to aryloxyalkanoate herbicides (such as 2,4-D) and glufosinate-ammonium, respectively (EFSA GMO Panel, 2017a)
- Application EFSA-GMO-NL-2012-106 for soybean DAS-44406-6, expressing the AAD-12, PAT and 2mEPSPS proteins, which confer tolerance to 2,4-D, glufosinate-ammonium and glyphosate, respectively (EFSA GMO Panel, 2017b)

---

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 Dow AgroSciences, 3B Park Square, Milton Park, OX14 4RN Abingdon, United Kingdom.
3 Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
In December 2017, a decision was taken to authorise products containing, consisting of, or produced from genetically modified soybean DAS-68416-4 and DAS-44406-6 pursuant to Regulation (EC) No 1829/2003.

Currently, the following two stack soybean applications, each one containing DAS-68416-4 and DAS-44406-6, are under the EFSA assessment:

- EFSA-GMO-NL-2013-115 for soybean DAS-68416-4 × MON-89788-1, expressing the AAD-12, PAT and CP4 EPSPS proteins, which confer tolerance to 2,4-D, glufosinate-ammonium and glyphosate, respectively (Question No EFSA-Q-2013-00281);
- EFSA-GMO-NL-2016-132 for soybean DAS-81419-2 × DAS-44406-6, expression of the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins, which confer resistance against certain lepidopteran insects and tolerance to 2,4-D, glufosinate-ammonium and glyphosate, respectively (Question No EFSA-Q-2016-00195).

According to Regulation (EU) No 2015/2033, the approval for the active substance 2,4-D was renewed under Regulation (EC) No 1107/2009. The implementing regulation contains certain conditions and restrictions, and requested further confirmatory (eco)toxicological information to be submitted. The assessment is currently ongoing. The representative uses assessed during the renewal of the approval were on conventional wheat, barley, oats, rye and maize.

The EU MRLs for 2,4-D are established in Annex II of Regulation (EC) No 396/2005. The review of the existing MRLs for 2,4-D according to Article 12 of Regulation (EC) No 396/2005 (MRL review) has been completed (EFSA, 2011). The proposed MRLs have been implemented in the MRL legislation by Commission Regulation (EU) No 1317/2013. For the MRLs not fully supported by data, including soybeans, footnotes were added in the regulation, which specifies which data have to be provided by 17 December 2015. After the MRL review EFSA has issued a reasoned opinion assessing the use of 2,4-D on GM maize which did not required a change of the existing MRL (EFSA, 2017).

The MRL/tolerance set in the USA and Canada for 2,4-D in soybean seeds is 0.02 mg/kg. EFSA based its assessment on the evaluation report submitted by the EMS (Greece, 2015) as revised in September 2018, the renewal assessment report (RAR) and its addendum (Greece, 2013, 2014), the revised Commission renewal report on 2,4-D (European Commission, 2017), the conclusion on the peer review of the pesticide risk assessment of the active substance 2,4-D (EFSA, 2014, revised in 2017) and 2,4-DB (EFSA, 2016), the review of the existing MRLs for 2,4-D according to Article 12 of Regulation (EC) No 396/2005 (EFSA, 2011) and the reasoned opinion on the setting of an import tolerance for 2,4-D in maize (EFSA, 2017). The assessment is performed in accordance with the legal provisions of the Uniform Principles for the Evaluation and the Authorisation of Plant Protection Products adopted by Commission Regulation (EU) No 546/2011 and the guidance documents by the European Commission.
relevant for the consumer risk assessment of pesticide residues applicable at time of submission of the application (European Commission, 1996, 1997a–g, 2000, 2010; OECD, 2011).

1. Method of analysis

1.1. Methods for enforcement of residues in food of plant origin

The EU pesticides peer review (renewal of the approval) concluded that analytical methods based on gas chromatography–mass spectrometry (GC–MS) and liquid chromatography with tandem mass spectrometry (LC–MS/MS) were available to enforce 2,4-D residues (sum of 2,4-D, its salts, esters and conjugates, expressed as 2,4-D) in plants at the LOQ of 0.01 mg/kg. An independent laboratory validation (ILV) for the enforcement analytical method in high oil content commodities is available (EFSA, 2014). Further validation data on the hydrolysis step were identified as missing in the MRL review and it was noted that this data would be desirable. No new information has been submitted with the MRL application.

1.2. Methods for enforcement of residues in food of animal origin

The EU pesticides peer review (renewal of the approval) concluded that analytical methods based on LC–MS/MS were available to enforce 2,4-D residues (sum of 2,4-D, its salts, esters and conjugates, expressed as 2,4-D) in muscle, fat, kidney, milk and eggs at the LOQ of 0.01 mg/kg (EFSA, 2014). Additional information was required on the hydrolysis step and extraction efficiency. This data gap is of no relevance for this application since a change of the existing MRLs in animal products was not requested.

2. Mammalian toxicology

2.1. Toxicological profile of the active substance

The toxicological profile of the active substance 2,4-D was assessed in the framework of the renewal of the approval of the active substance under Regulation (EC) No 1107/2009 (EFSA, 2014). An acceptable daily intake (ADI) of 0.05 mg/kg body weight (bw) per day and an acute reference dose (ARfD) of 0.75 mg/kg bw were established (EFSA, 2014; European Commission, 2015). In 2016, EFSA recommended to amend the toxicological reference values for 2,4-D in the light of the further consideration of studies common to the evaluation of the active substances 2,4-D and 2,4-DB, of which 2,4-D is a major metabolite (EFSA, 2016; European Commission, 2017). The toxicological reference values formally approved in 2017 are compiled in Table 1.

Table 1: Overview of the toxicological reference values

| Source                   | Year | Value                  | Study                                      | Uncertainty factor |
|--------------------------|------|------------------------|--------------------------------------------|--------------------|
| 2,4-D                    |      |                        |                                            |                    |
| ADI European Commission  | 2017 | 0.02 mg/kg bw per day  | 1-year dog study with 2,4-DB               | 100                |
| ARfD                     | 2017 | 0.3 mg/kg bw           | Developmental toxicity studies in rat and rabbit with 2,4-D and 2,4-DB | 100                |

ADI: acceptable daily intake; ARfD: acute reference dose; bw: body weight,

2.2. Toxicological profile of metabolites

The toxicological profile of the metabolite 2,4-DCP, which is a common metabolite to 2,4-D and 2,4-DB, was discussed during the pesticides peer review 186 Experts’ meeting in November 2018. A number of toxicological studies are available from the open literature on 2,4-DCP and information was reported in the assessment reports on 2,4-D (Greece, 2013, 2014) and 2,4-DB (Belgium, 2016). In the framework of the current application, a complete genotoxicity data package has been submitted (Greece, 2015).
The metabolite was found to be harmful upon acute oral administration according to Regulation (EC) No 1272/2008\(^{13}\). Based on the genotoxicity data package covering \textit{in vitro} gene mutation in bacterial and mammalian cells, chromosome aberration and \textit{in vivo} micronucleus test resulting in negative results, the metabolite was considered devoid of genotoxic potential.

In the overall data package on the metabolite 2,4-DCP, the critical lowest-observed-adverse-effect level (\textbf{LOAEL}) was agreed at 25 mg/kg bw per day for transitory mammary gland effects (whitening, stiffening and swelling) from the two-generation reproductive toxicity study in rats. The metabolite 2,4-DCP taken together with its conjugated forms was considered a major metabolite of 2,4-DB in rat urine after oral administration, although not retrieved in the rat metabolism after administration of 2,4-D. Since the dog was considered the most sensitive species after administration of 2,4-D and 2,4-DB and as there are no studies available on the metabolite 2,4-DCP in dogs, the experts agreed that the toxicological reference values derived for 2,4-DB are applicable to 2,4-DCP. Since the toxicological reference values of 2,4-DB are also applicable to 2,4-D, the \textbf{ADI} and \textbf{ARfD} reported in Table 1 for 2,4-D are applicable to 2,4-DCP. 2,4-DCP was concluded to share the toxicological profile of 2,4-DB and 2,4-D.

\section*{3. Residues}

\subsection*{3.1. Nature and magnitude of residues in plant}

\subsubsection*{3.1.1. Primary crops}

\textbf{3.1.1.1. Nature of residues}

Metabolism of 2,4-D in primary crops was investigated in conventional crops in the framework of the MRL review under Article 12 of Regulation (EC) No 396/2005 and the EU peer review (renewal of the approval) (EFSA, 2011, 2014). An additional study on GM maize was assessed in a previous EFSA opinion (EFSA, 2017); furthermore, results of a study on GM cotton are reported in an assessment performed by JMPR (FAO, 2017).

In the framework of this MRL application, a metabolism study with 2,4-D foliar applications to GM soyabean at approximately the notified rate (1N) was provided (Greece, 2015). An overview of the key parameters of the available metabolism studies is presented in Table 2.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Crop group} & \textbf{Crops} & \textbf{Application} & \textbf{Sampling} & \textbf{Comments/Source} \\
\hline
\textbf{Conventional plants (peer reviewed studies)} & & & & \\
\hline
Fruit & Apple & Soil, 2 × 2.13 kg/ha & 56 DALA & [U-phenyl-\textsuperscript{14}C]-2,4-D (EFSA, 2011) \\
\hline
Root & Potato & Foliar, 2 × 0.07 kg/ha & 82 DALA & [U-phenyl-\textsuperscript{14}C]-2,4-D (EFSA, 2011) \\
 & & Foliar, 0.14 + 0.28 kg/ha & 29 DALA & [U-phenyl-\textsuperscript{14}C]-2,4-D (EFSA, 2011) \\
\hline
Cereal/Grass & Wheat & Foliar, 1 × 1.68 kg/ha & 10 (forage), 49 (grain, straw) DAT & [U-phenyl-\textsuperscript{14}C]-2,4-D (EFSA, 2011) \\
\hline
\textbf{Genetically modified plants\(^{(a)}\) (not peer reviewed studies)} & & & & \\
\hline
Pulses/oilseeds & Soyabean (AAD-12) & Soil, 1 pre-emergence + Foliar, 2 post-emergence, Rate 1.12 kg/ha, up to BBCH 65 & 7 (forage) DAT\(_{22}\) + 22 (hay), 41–62 (seed) DALA & [U-phenyl-\textsuperscript{14}C]-2,4-D Event DAS-60416-4 (Greece, 2015) \\
\hline
 & & & & \\
 & Cotton (AAD-12) & Foliar, 1 pre-emergence + 2 post-emergence, Rate: 1.1 kg/ha, up to BBCH 65 & 56 DALA (seed, trash) & [U-phenyl-\textsuperscript{14}C]-2,4-D (FAO, 2017) \\
\hline
\end{tabular}
\caption{Summary of available metabolism studies in plants}
\end{table}

\(^{13}\) Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, 1-1355.
In conventional crops, after soil applications to fruit crops, residues in apples were too low to allow further identification.

After foliar applications to root and cereal crops, the parent compound, including its conjugated forms, was the major component of residues. It accounted for 77% of total radioactive residues (TRR) in wheat forage, 72% TRR in wheat straw, 6% TRR in grain and 35% TRR in potato tuber. The metabolite 2,4-DCP was found in small amounts, up to about 0.5 mg eq/kg (1% TRR) in maize straw (feed item) and in trace levels (<0.01 mg eq/kg, 4% of TRR) in potato tubers. Based on the metabolism studies, the residue definition for enforcement and risk assessment was proposed as the sum of 2,4-D, its salts, esters and conjugates, expressed as 2,4-D (EFSA, 2011, 2014).

After foliar applications to maize genetically modified to express the AAD-1 protein, a similar metabolic pattern was observed compared with conventional crops. In immature plant and mature fodder, 2,4-D was the major component of residues (51–77% TRR) with 2,4-DCP, its disaccharide and glucose conjugates representing a relevant part of residues as well (about 22–25% TRR). In maize grain, the parent compound was the only compound identified (7% TRR; 0.003 mg/kg) and 2,4-DCP could not be detected. Considering that parent was the only compound found in the grain and that grain is the only commodity expected to be imported, EFSA concluded to apply the same residue definitions derived for conventional crops to the GM maize assessed (EFSA, 2017).

In the metabolism study in genetically modified soyabeans expressing the AAD-12 protein, the majority of the radioactive residue in forage and hay was composed of 2,4-D (up to 86% and 59% TRR, respectively) and free or conjugated 2,4-DCP (up to 18% and 25% TRR, respectively). Total residue in mature soybean seeds was 0.37 mg eq/kg. 2,4-DCP (free and conjugated) represented the major component of residues (5.6% TRR, 0.021 mg eq/kg), whereas parent compound was found in trace levels only (0.005 mg eq/kg, 1.4% TRR). The attempt to further characterise the large part of non-extractable residues in seeds (approximately 70% TRR) resulted in highly polar and multi-component fractions, none eluting in the region of 2,4-D and 2,4-DCP. According to the EMS, the non-extractable compounds are attributed to natural plant constituents after incorporation of radiolabelled carbon (Greece, 2015). The results of field residue trials confirmed the presence of 2,4-DCP in the seeds.

In conclusion, the metabolism of 2,4-D in GM soybeans and cotton expressing the AAD-12 protein showed to be qualitatively similar to the metabolism observed in conventional crops but the metabolic pattern was quantitatively different compared with conventional crops, showing higher amounts of 2,4-DCP (free and conjugated).

Based on the results of the metabolism studies in GM soybeans, EFSA proposes to include 2,4-DCP in the residue definition for risk assessment for the GM soybeans under assessment.

As regards the enforcement residue definition, parent 2,4-D (including salts, esters and conjugates) is not a good marker for GM soybeans. However, considering that the enforcement residue definition is applicable also to the conventional soybeans with parent compound expected to be the main residue and considering that 2,4-DCP (free and conjugated) cannot unequivocally be related to the use of 2,4-D since

| Crop group | Crops | Application | Sampling | Comments/Source |
|------------|-------|-------------|----------|-----------------|
| Cereal/Grass | Maize (AAD-1) | Soil, 1 pre-emergence + Foliar, 2 post-emergence, Rate ca. 0.9-1.1 kg/ha, up to BBCH 18 | 30 (immature plant); 57 (grain, cobs, fodder) DALA | [U-phenyl-14C]-2,4-D Event DAS-40474-7(b) (EFSA, 2017) |
|            |       | Soil, 1 pre-emergence + Foliar, 2 post-emergence, Rate ca. 1.1-1.2 kg/ha, up to BBCH 18 | | [U-phenyl-14C]-2,4-D Event DAS-40278-9 (EFSA, 2017) |

DALA; day after last application; DAT days after treatment; DAT2: days after 2nd treatment.
(a): Crops which exhibit tolerance to application of 2,4-D herbicide.
(b): According to the EMS, in DAS-40474-7 more than a single copy of the AAD-1 gene was inserted (EFSA, 2017).
it is a common metabolite generated also from other active substances belonging to the class of phenoxyacetic acids (e.g. 2,4-DB), EFSA proposes no modification of the existing residue definition.

To summarise, the following residue definitions are proposed for genetically modified soybean seeds expressing the AAD-12 protein conveying tolerance to 2,4-D:

- Residue definition for enforcement: Sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D. The residue definition for enforcement set in Regulation (EC) No 396/2005 is identical with the above mentioned residue definition.
- Residue definition for risk assessment: Sum of 2,4-D, its salts, esters and conjugates, and 2,4-DCP and its conjugates, expressed as 2,4-D.

### 3.1.1.2. Magnitude of residues

In support of the MRL applications for soyabeans, a total of 24 residue trials on GM soyabean expressing the AAD-12 protein (event not specified) compliant with the notified critical GAP (see Appendix A, United States GAP) were submitted. Trials were conducted in Canada and the USA using a 2,4-D dimethylamine salt. All supervised residue trials were performed using a non-ionic surfactant in the spray mixture during a single season instead of at least two as required (European Commission, 1997b). Since the trials were located in different geographical regions, EFSA is of the opinion that the trials are sufficiently representative for the use and further trials performed in a different year were not requested. EFSA disregarded one trial since last application occurred at a very early growth stage (BBCH 35) compared to the notified GAP. All samples were analysed for 2,4-D and 2,4-DCP with an analytical method involving a hydrolysis step to cover also the conjugates of 2,4-D and 2,4-DCP. According to the EMS, the analytical methods used to analyse the residue trial samples have been sufficiently validated and were proven to be fit for purpose (Greece, 2015).

At harvest, 2,4-D was not present in quantifiable concentrations (< LOQ) and 2,4-DCP ranged from the LOQ (< 0.01 mg/kg) to 0.05 mg/kg. The results of the residue trials, the related risk assessment input values and the derived MRL are summarised in Table 3.

Residues of 2,4-D were found to be stable for up to 18 months in high water, high starch content and dry matrices and up to 12 months in high oil content matrices (EFSA, 2014). New frozen storage stability on 2,4-D and 2,4-DCP with samples stored at –20°C was submitted (Greece, 2015). In soyabean seeds, both parent and the metabolite 2,4-DCP showed to be stable for a period of 28 months, which fully covered the storage of field trial samples (13.5 months).

EFSA concludes that a sufficient number of GAP-compliant supervised residue trials is available to support the notified uses in GM soyabean expressing the AAD-12 protein. The data suggest that the lowering of the existing MRL set at the LOQ of 0.05 mg/kg to a lower LOQ of 0.01 mg/kg could be considered. The MRL/tolerance in the countries of origin set for 2,4-D is 0.02 mg/kg.
### Table 3: Overview of the available residues trials data

| Commodity | Region/Indoor\(^{(a)}\) | Residue levels observed in the supervised residue trials (mg/kg) \(^{(b)}\) | Comments/Source | Calculated MRL \(^{(c)}\) (mg/kg) | HR \(^{(d)}\) (mg/kg) | STMR \(^{(e)}\) (mg/kg) | CF \(^{(f)}\) |
|-----------|--------------------------|--------------------------------------------------------------------------------|-----------------|--------------------------------|-----------------|-----------------|----------------|
| Soyabean  | USA/CA                   | Mo = 23 × 9 < 0.010<br>RA = 9 × 9 < 0.024; 0.024; 0.026; 0.045; 0.056; 0.058; 0.060; 0.069; 0.075 | Residue trials compliant with the GAP on GM soyabean expressing the AAD-12 protein. Samples harvest 51–104 days after last application. MRL/Tolerance set in the countries of origin is 0.02 mg/kg | 0.01* RA = 0.075 RA = 0.029 | 3.86 |

MRL: maximum residue level; GAP: Good Agricultural Practice; GM: genetically modified; Mo: according to the residue definition for enforcement; RA: according to the residue definition for risk assessment.

\(^{(a)}\): 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.

\(^{(b)}\): Residues of 2,4-DCP (free and conjugated) were adjusted for molecular weight (2,4-D/2,4-DCP = 221/163) by a factor of 1.36 to express them as 2,4-D prior to be summed up.

\(^{(c)}\): The asterisk indicates that the MRL is set at the limit of analytical quantification (LOQ).

\(^{(d)}\): Highest residue expressed on the basis of the residue definition for risk assessment.

\(^{(e)}\): Supervised trials median residue expressed on the basis of the residue definition for risk assessment.

\(^{(f)}\): Median conversion factor to recalculate residues according to the residue definition for monitoring to the residue definition for risk assessment. Since in 9 trials residues were below the LOQ according to both residue definition for enforcement and risk assessment, the CF was not calculated.
3.1.1.3. Effect of industrial processing and/or household preparation

Standard hydrolysis studies addressing the nature of residues in processed products and studies on the magnitude of 2,4-D residues in processed commodities are not required as the residue levels in raw agricultural commodities (RAC) did not exceed the trigger value of 0.1 mg/kg (European Commission, 1997d).

Two processing studies investigating the magnitude of residues in processed GM soybeans expressing the AAD-12 protein (event not specified) were assessed (Greece, 2015). The studies were conducted applying an exaggerate application rate (1.8N) compared to the rate defined in the critical GAP. Samples were analysed for 2,4-D and 2,4-DCP.

Parent 2,4-D was not found in any of the unprocessed and the processed products (i.e. meal, hull, refined oil), except in soybean aspirated grain fractions (AGF). 2,4-DCP was found in quantifiable concentrations in unprocessed seeds and in processed soybean meal, AGF and hulls, but not in refined oil. In meal and AGF, the concentrations of 2,4-DCP were higher compared to unprocessed soybeans. The results of the submitted processing studies are reported in the Table 4.

Due to the lack of investigation on the nature of residues in processed commodities and the limited dataset, reliable processing factors (PF) could not be derived. However, the transfer ratio (TR) was used to perform the livestock dietary burden calculation related to soybean feed products (i.e. soybean meal).

3.1.2. Rotational crops

The residues of 2,4-D and its metabolite 2,4-DCP in rotational crops are not relevant for the assessment of import tolerances applications.

3.2. Nature and magnitude of residues in livestock

As soybean meal produced from imported GM soybeans may be fed to livestock, the potential transfer of residues into animal commodities was assessed (European Commission, 1996).

3.2.1. Dietary burden of livestock

The most recent livestock dietary burden was conducted in the framework of the MRL review taking into account the authorised EU uses of 2,4-D (EFSA, 2011).14 In the framework of the current application, the dietary burden was updated including the expected contribution of residues in GM soybean meal, taking into account that residues of 2,4-DCP in meal are likely to be higher than in soybean seeds. Thus, the median residue level expected in soybean seeds was multiplied by the TR of 1.35 derived in the processing studies (see Table 4). The dietary burden calculation was performed according to the methodology described in the EU guideline 7031/IV/95 rev.4 on livestock feeding studies (European Commission, 1996), which was applicable at the time of submission of the MRL application.

The input values for the dietary burden calculation are summarised in Table 5. The results of the calculations are presented in Table 6.

---

14 The recently assessed import tolerance application on 2,4-D in maize did not trigger a modification of the dietary burden calculation performed in the framework of the MRL review (EFSA, 2017).
Comparing the results obtained with and without the contribution of expected residues in GM soybean meal, it becomes evident that the notified uses do not contribute to the current livestock dietary exposure of 2,4-D in ruminants and pigs. The contribution of residue in soybean meal in the poultry diet was not significantly changing the livestock intake that was still not exceeding the trigger value of 0.1 mg/kg dry matter (DM). Thus, EFSA concludes that the import of soybean seeds treated according to the notified uses will not trigger a revision of the existing MRLs.

4. Consumer risk assessment

The consumer risk assessment was performed with revision 2 of the EFSA PRIMo. This exposure assessment model contains the relevant European food consumption data for different subgroups of the EU population (EFSA, 2007). The estimated exposure was then compared with the toxicological reference values derived for 2,4-D during the EU pesticides peer-review renewal process (European Commission, 2017).

The most recent long-term exposure assessment performed by EFSA (2017) for the residues of 2,4-D was considering the existing uses at EU level and the acceptable CXLs (EFSA, 2011) and was

Table 5: Input values for the livestock dietary burden calculation

| Food commodity       | Median dietary burden (mg/kg) | Maximum dietary burden (mg/kg) |
|----------------------|-------------------------------|--------------------------------|
|                      | Input value (mg/kg) | Comment | Input value (mg/kg) | Comment |
| Grass (fresh & silage) | 12.60 | STMR (EFSA, 2011) | 26.00 | HR (EFSA, 2011) |
| Maize silage         | 0.055 | STMR (EFSA, 2011) | 0.060 | HR (EFSA, 2011) |
| Apple pomace         | 0.010 | STMR (EFSA, 2011) | 0.010 | HR (EFSA, 2011) |
| Grass (hay)          | 50.40 | STMR × PF (EFSA, 2011) | 104.00 | HR × PF (EFSA, 2011) |
| Cereal grain         | 0.05 | STMR (EFSA, 2011) | 0.05 | STMR (EFSA, 2011) |
| Maize grain          | 0.05 | STMR (EFSA, 2011) | 0.05 | STMR (EFSA, 2011) |
| Cereal bran          | 0.40 | STMR × PF (EFSA, 2011) | 0.40 | STMR × PF (EFSA, 2011) |
| Cereal straw         | 0.05 | STMR (EFSA, 2011) | 1.88 | HR (EFSA, 2011) |

Risk assessment residue definition: Sum of 2,4-D, its salts, esters and conjugates expressed as 2,4-D

STMR: supervised trials median residue; HR: highest residue; PF: processing factor.

(a): For grass hay and cereal bran, in the absence of processing factors supported by data, default processing factors of 4 and 8, respectively, were included in the calculation to consider the potential concentration of residues in these commodities (EFSA, 2011). For soybean meal, to consider the observed concentration of 2,4-DCP residues, instead of the default processing factor of 1.3, a mean transfer ratio (TR) of 1.35 derived for 2,4-DCP only based on experimental data was used.

Table 6: Results of the dietary burden calculation

| Food commodity | Maximum dietary burden (mg/kg) bw per day | Median dietary burden (mg/kg) bw per day | Highest contributing commodity | Max dietary burden (mg/kg DM) w/soya | w/out | Trigger exceed (Y/N) |
|----------------|------------------------------------------|-------------------------------------------|---------------------------------|-------------------------------------|-------|----------------------|
| Dairy ruminants | 4.727 | 2.291 | Grass, fresh | 130.00 | 130.00 | Yes |
| Meat ruminants  | 5.571 | 2.700 | Grass, fresh | 130.00 | 130.00 | Yes |
| Poultry         | 0.005 | 0.005 | Wheat, bran | 0.072 | 0.067 | No |
| Pigs            | 0.784 | 0.382 | Grass, silage | 19.60 | 19.59 | Yes |

bw: body weight; DM: dry matter.

Comparing the results obtained with and without the contribution of expected residues in GM soybean meal, it becomes evident that the notified uses do not contribute to the current livestock dietary exposure of 2,4-D in ruminants and pigs. The contribution of residue in soybean meal in the poultry diet was not significantly changing the livestock intake that was still not exceeding the trigger value of 0.1 mg/kg dry matter (DM). Thus, EFSA concludes that the import of soybean seeds treated in the countries of origin according to the notified uses will not trigger a revision of the existing MRLs.

4. Consumer risk assessment

The consumer risk assessment was performed with revision 2 of the EFSA PRIMo. This exposure assessment model contains the relevant European food consumption data for different subgroups of the EU population (EFSA, 2007). The estimated exposure was then compared with the toxicological reference values derived for 2,4-D during the EU pesticides peer-review renewal process (European Commission, 2017).

The most recent long-term exposure assessment performed by EFSA (2017) for the residues of 2,4-D was considering the existing uses at EU level and the acceptable CXLs (EFSA, 2011) and was

15 The calculation of the long-term exposure (chronic exposure) is based on the mean consumption data representative for 22 national diets collected from MS surveys plus 1 regional and 4 cluster diets from the WHO GEMS Food database; for the acute exposure assessment the most critical large portion consumption data from 19 national diets collected from MS surveys is used. The complete list of diets incorporated in EFSA PRIMo is given in its reference section (EFSA, 2007).
updated to reflect the MRLs set by Regulation (EU) No 1317/2013 on certain products of animal origin. The calculation has been revised by including the STMR derived for GM soybeans from the trials submitted.

At the time of the MRL review, an acute consumer exposure assessment was not performed as an ARfD was not yet established (EFSA, 2011). During the renewal process of the active substance, an acute reference value was set for 2,4-D (European Commission, 2017). Therefore, EFSA performed the acute exposure assessment for soybeans, assuming the consumption of a large portion of the food item as reported in the national food surveys and that this item contained residues at the STMR level.

The input values used for the dietary exposure calculation are summarised in Table 7.

Table 7: Input values for the consumer dietary exposure assessment

| Commodity                          | Chronic exposure assessment | Acute exposure assessment |
|------------------------------------|----------------------------|---------------------------|
|                                    | Input (mg/kg) | Comment | Input (mg/kg) | Comment |
| Risk assessment residue definition (GM soybean): Sum of 2,4-D, its salts, esters and conjugates, and 2,4-DCP and its conjugates, expressed as 2,4-D | Soyabeans | 0.03 | STMR | 0.03 | STMR |
| Risk assessment residue definition: Sum of 2,4-D, its salts, esters and conjugates, expressed as 2,4-D | Citrus fruits | 0.31 | STMR (EFSA, 2011) | Acute risk assessment undertaken only for soybeans |
|                                    | Tree nuts | 0.05 | STMR (EFSA, 2011) | |
|                                    | Pome fruits | 0.01 | STMR (EFSA, 2011) | |
|                                    | Stone fruits | 0.01 | STMR (EFSA, 2011) | |
|                                    | Berries and small fruits | 0.05 | STMR (EFSA, 2011) | |
|                                    | Potatoes | 0.05 | STMR (EFSA, 2011) | |
|                                    | Sweet corns | 0.05 | STMR (EFSA, 2011) | |
|                                    | Asparagus | 0.05 | STMR (EFSA, 2011) | |
|                                    | Barley and oats grain | 0.05 | STMR (EFSA, 2011) | |
|                                    | Buckwheat grain | 0.05 | MRL (EFSA, 2011) | |
|                                    | Maize, millet, sorghum grain | 0.05 | STMR (EFSA, 2011) | |
|                                    | Rice grain | 0.01 | STMR (EFSA, 2011) | |
|                                    | Wheat and rye grain | 0.22 | STMR (EFSA, 2011) | |
|                                    | Sugar cane | 0.01 | STMR (EFSA, 2011) | |
|                                    | Muscle, fat(a) | 0.125 | STMR (FAO, 1998) | |
|                                    | Liver, kidney, edible offal(a) | 2.75 | STMR (FAO, 1998) | |
|                                    | Poultry, products | 0.05 | MRL (LOQ) | |
|                                    | Milks | 0.01 | MRL (LOQ) | |
|                                    | Bird’s eggs | 0.01 | MRL (LOQ) | |

STMR: supervised trials median residue; MRL: maximum residue level; LOQ: limit of quantification.
(a): Products of swine, bovine, sheep, goat, equine, other farmed terrestrial animals.

The estimated acute exposure was then compared with the toxicological reference values derived for 2,4-D (see Table 1). The results of the intake calculation using the EFSA PRIMo is a key supporting document and is made publicly available as a background document to this reasoned opinion.

Using the revised toxicological reference values, the highest chronic intake was calculated to be 34% of the ADI (IE adult diet). The contribution of residues in soybeans to the overall chronic exposure was low (maximum 0.1% of the ADI). The highest acute consumer exposure for soybean was calculated to be 0.02% of the ARfD.

EFSA concludes that the residues of parent 2,4-D and of the metabolite 2,4-DCP, which was found in the GM soybeans expressing the AAD-12 protein treated with 2,4-D according to the notified good agricultural practices assessed, will not result in a consumer exposure exceeding the toxicological reference values for 2,4-D. The data submitted in support of the request provided sufficient evidence to conclude that the risk for consumers is unlikely.
Conclusions and recommendations

The information submitted was sufficient to propose the MRL summarised in the table below:

| Code(a) | Commodity    | Existing EU MRL (mg/kg) | Proposed EU MRL (mg/kg) | Comment/Justification                                                                                                                                 |
|---------|--------------|------------------------|------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| 0401070 | Soyabeans    | 0.05* (ft)             | 0.05* or 0.01* (further risk management consideration) | The submitted data are sufficient to perform a consumer risk assessment for the use of 2,4-D in support the import request. The lowering of the existing MRL set at the LOQ of 0.05 mg/kg to the LOQ of 0.01 mg/kg as derived from the residue trials on 2,4-D-tolerant soybeans (AAD-12 protein) and achievable with the validated enforcement analytical methods is an option to be discussed by risk managers. Risk for consumers unlikely MRL/Tolerance in the countries of origin (Canada and USA) is 0.02 mg/kg The data gap identified by EFSA for additional information on the analytical method (ft) has been previously addressed |

**Enforcement residue definition:** 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D)

MRL: maximum residue level; LOQ: limit of quantification; AAD-12: aryloxyalkanoate dioxygenase-12.

*: Indicates that the MRL is set at the limit of analytical quantification (LOQ).

(a): Commodity code number according to Annex I of Regulation (EC) No 396/2005.

ft: The European Food Safety Authority identified some information on analytical methods as unavailable. When reviewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 17 December 2015, or, if that information is not submitted by that date, the lack of it.

References

Belgium, 2016. Revised Renewal Assessment Report (RAR) on 2,4-DB prepared by the rapporteur Member State Belgium in the framework of Regulation (EU) No 844/2012, March 2016. 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), 2011. Reasoned opinion on the review of the existing maximum residue levels (MRLs) for 2,4-D according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2431, 52 pp. https://doi.org/10.2903/j.efsa.2011.2431

EFSA (European Food Safety Authority), 2014. Conclusion on the peer review of the pesticide risk assessment of the active substance 2,4-D. EFSA Journal 2014;12(9):3812, 78 pp. https://doi.org/10.2903/j.efsa.2014.3812

EFSA (European Food Safety Authority), 2016. Conclusion on the peer review of the pesticide risk assessment of the active substance 2,4-DB. EFSA Journal 2016;14(5):4500, 25 pp. https://doi.org/10.2903/j.efsa.2016.4500

EFSA (European Food Safety Authority), 2017. Conclusion on the peer review of the pesticide risk assessment of the active substance 2,4-D. EFSA Journal 2017;15(5):4765, 17 pp. https://doi.org/10.2903/j.efsa.2017.4765

EFSA (European Food Safety Authority), 2018. Report of the experts’ meeting on mammalian toxicology regarding 2,4 D, held at EFSA on 21-22 November 2018. Available online: www.efsa.europa.eu

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Bircher AN, Casacuberta J, De Schrijver A, Granak MA, Jones H, Manachini B, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Ardizzone M, Devos Y, Gomes A, Liu Y, Neri FM and Olang I, 2017a. Scientific Opinion on an application by Dow AgroSciences LLC (EFSA-GMO-NL-2011-91) for the placing on the market of genetically modified herbicide-tolerant soybean DAS-68416-4 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2017;15(3):4719, 31 pp. https://doi.org/10.2903/j.efsa.2017.4719

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Granak MA, Jones H, Manachini B, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Alvarez F, Ardizzone M, Liu Y, Neri FM and Ramon M, 2017b. Scientific opinion on an application by Dow AgroSciences LLC (EFSA-GMO-NL-2012-106) for the placing on the market of genetically modified herbicide-tolerant soybean DAS-44406-6 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2017;15(3):4738, 33 pp. https://doi.org/10.2903/j.efsa.2017.4738
European Commission, 1996. Appendix G. Livestock Feeding Studies. 7031/VI/95-rev.4.
European Commission, 1997a. Appendix A. Metabolism and distribution in plants. 7028/IV/95-rev.3.
European Commission, 1997b. Appendix B. General recommendations for the design, preparation and realisation 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.
European Commission, 1997c. Appendix C. Testing of plant protection products in rotational crops. 7524/VI/95-rev.2.
European Commission, 1997d. Appendix E. Processing studies. 7035/VI/95-rev.5.
European Commission, 1997e. Appendix F. Metabolism and distribution in domestic animals. 7030/VI/95-rev.3.
European Commission, 1997f. Appendix H. Storage stability of residue samples. 7032/VI/95-rev.5
European Commission, 1997g. Appendix I. Calculation of maximum residue level and safety intervals. 7039/VI/95.
European Commission, 1997. Appendix A. Metabolism and distribution in plants. 7028/IV/95-rev.3.

European Commission, 1997b. Appendix B. General recommendations for the design, preparation and realisation 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.

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, 2010. 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, 2015. Final Review report for the active substance 2,4-D finalised in the Standing Committee on Plants, Animals, Food and Feed at its meeting on 9 October 2015 in view of the approval of 2,4-D as active substance in accordance with Regulation (EC) No 1107/2009. SANCO/11961/2014/Rev. 3 Final. 9 October 2015.

European Commission, 2017. Revised Renewal report for the active substance 2,4-D finalised in the Standing Committee on Plants, Animals, Food and Feed at its meeting on 9 October 2015 in view of the approval of 2,4-D as active substance in accordance with Regulation (EC) No 1107/2009. SANCO/11961/2014 Rev 5. 16 October 2017.

FAO (Food and Agriculture Organization of the United Nations), 1998. 2,4-D (020). In: Pesticide Residues in food. Report – 1998. Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection Paper, 148, 1998, 60-71 pp.

FAO (Food and Agriculture Organization of the United Nations), 2017. 2,4-D (020). In: Pesticide Residues in food. Evaluation - 2017. Part I residues. Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Geneva, Switzerland, 12-21 September 2017. Pesticide residue in food 2017. FAO Plant Production and Protection paper 233, 583-608 pp.

Greece, 2013. Renewal Assessment Report (RAR) on the active substance 2,4-D prepared by the rapporteur Member State Greece in the framework of Commission Regulation (EU) No 1141/2010, February 2013. Available online: www.efsa.europa.eu

Greece, 2014. Final Addendum to the Renewal Assessment Report on 2,4-D, compiled by EFSA, March 2014. Available online: www.efsa.europa.eu

Greece, 2015. Evaluation report on the Setting of import tolerances for 2,4-D in soya beans prepared by the evaluating Member State Greece under Article 8 of Regulation (EC) No 396/2005, 28 January 2015, as revised in September 2018, 43 pp.

OECD (Organisation for Economic Co-operation and Development), 2009. Test No. 509: Crop Field Trial, OECD Guidelines for the Testing of Chemicals, Section 5, OECD Publishing, Paris, https://doi.org/10.1787/9789264076457-en

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

**Abbreviations**

2,4-DCP  2,4-dichlorophenol  
a.s.  active substance  
AAD-1  aryloxyalkanoate dioxygenase 1  
AAD-12  aryloxyalkanoate dioxygenase-12  
ADI  acceptable daily intake  
ARfD  acute reference dose  
BBCH  growth stages of mono- and dicotyledonous plants  
bw  body weight  
CF  conversion factor for enforcement to risk assessment residue definition  
CIPAC  Collaborative International Pesticide Analytical Council  
CXL  Codex maximum residue limit (Codex MRL)  
DALA  days after last application  
DAT  days after treatment  
eq  equivalent
| Acronym | Description |
|---------|-------------|
| EMS     | evaluating Member State |
| GAP     | good agricultural practice |
| GC-MS   | gas chromatography-mass spectrometry |
| GM      | genetically modified |
| HPLC    | high-performance liquid chromatography |
| HR      | highest residue |
| ILV     | independent laboratory validation |
| InChIKey| International Chemical Identifier Key |
| ISO     | International Organisation for Standardisation |
| IUPAC   | International Union of Pure and Applied Chemistry |
| LOAEL   | lowest-observed-adverse-effect level |
| LOQ     | limit of quantification |
| MRL     | maximum residue level |
| MS      | Member State |
| MS/MS   | tandem mass spectrometry detector |
| MW      | molecular weight |
| NEU     | northern Europe |
| OECD    | Organisation for Economic Co-operation and Development |
| PAT     | phosphinothricin acetyltransferase |
| PF      | processing factor |
| PHI     | preharvest interval |
| PRIMo   | (EFSA) Pesticide Residues Intake Model |
| RAC     | raw agricultural commodity |
| RAR     | renewal assessment report |
| SANCO   | Directorate-General for Health and Consumers |
| SEU     | southern Europe |
| SMILES  | simplified molecular-input line-entry system |
| SL      | soluble concentrate |
| STMR    | supervised trials median residue |
| TF      | transfer ratio |
| TMDI    | theoretical maximum daily intake |
| TRR     | total radioactive residue |
| WHO     | World Health Organization |
### Appendix A – Good Agricultural Practice (GAPs)

| Crop and/or situation (a) | MS or NEU/SEU or Country | F or G or I (b) | Pest or group of pests controlled (c) | Formulation | Application | Application rate per treatment | PHI (days) (l) | Remarks (m) |
|--------------------------|--------------------------|-----------------|----------------------------------------|-------------|------------|------------------------------|---------------|-------------|
| GM Soyabean (expression of AAD-12 protein) | CA F | Annual and perennial weeds | SL 456 g/L Spraying | Up to BBCH 65 | 2 12 days | 50–200 0.82 n.a. | n.a. | Critical GAP |
| US | | | | Pre-emergence (1)+ post-emergence (2) up to BBCH 65 | 3 Post-emergence: 12 days | 50–94 1.12 n.a. | |

NEU: northern Europe; SEU: southern Europe; MS: Member State; a.s.: active substance; AAD-12: aryloxyalkanoate dioxygenase-12; SL: soluble concentrate.

(a): For crops, EU or other classifications, e.g. Codex, should be used; where relevant, the usage situation should be described (e.g. fumigation of a structure).

(b): Outdoor or field use (F), glasshouse application (G) or indoor application (I).

(c): e.g. biting and sucking insects, soil-borne insects, foliar fungi, weeds.

(d): e.g. wettable powder (WP), water-soluble granule (WG).

(e): GCPF Codes - GIFAP Technical Monograph No 2, 1989.

(f): All abbreviations must be explained.

(g): Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench.

(h): Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants. Type of equipment used must be indicated.

(i): g/kg or g/L.

(j): Growth stage at last treatment (Meier U, 2001. Growth Stages of mono- and dicotyledonous plants. BBCH Monograph, 2nd Ed., Federal Biological Research Centre of Agriculture and Forestry, Braunschweig, Germany, 2001), including where relevant, information on season at time of application.

(k): The minimum and maximum number of application possible under practical conditions of use must be provided.

(l): PHI: minimum preharvest interval.

(m): Remarks may include: Extent of use/economic importance/restrictions.
## Appendix B – Used compound codes

| Code/Trivial name | IUPAC name/SMILES notation/\nIUPAC name/SMILES notation/InChiKey\(^{(a)}\) | Structural formula\(^{(b)}\) |
|------------------|---------------------------------|------------------|
| 2,4-D            | (2,4-dichlorophenoxy)acetic acid\nClc1cc(Cl)ccc1OCC(=O)O\nOVSKIKFHRZJPSS-UHFFFAOYSA-N | ![Structural formula of 2,4-D](image) |
| 2,4-DCP          | 2,4-dichlorophenol\nClc1cc(Cl)c(O)cc1\nHFZWRUODUSTPEG-UHFFFAOYSA-N | ![Structural formula of 2,4-DCP](image) |
| 2,4-DB           | 4-(2,4-dichlorophenoxy)butyric acid\nClc1cc(Cl)ccc1OCCCC(=O)O\nYIVXMZITEQBPQO-UHFFFAOYSA-N | ![Structural formula of 2,4-DB](image) |

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

\(^{(a)}\): ACD/Name 2015 ACD/Labs 2015 Release (File version N20E41, Build 75170, 19 December 2014).

\(^{(b)}\): ACD/ChemSketch 2015 ACD/Labs 2015 Release (File version C10H41, Build 75059, 17 December 2014).