REASONED OPINION

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Setting of an import tolerance for 2,4-D in maize

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Abstract

In accordance with Article 6 of Regulation (EC) No 396/2005, the evaluating Member State (EMS) Greece received an application from Dow AgroSciences to set an import tolerance for the active substance 2,4-D in genetically modified (GM) maize imported from Canada and the USA. The modification confers tolerance to the herbicide 2,4-D. Greece 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 EFSA. According to EFSA, the data submitted are sufficient to conclude that a change of the existing maximum residue level (MRL) set at the limit of quantification (LOQ) of 0.05 mg/kg in maize is not required. Analytical enforcement methods are available to control the residues of 2,4-D in cereals and no risk for consumers was identified for the notified use on GM maize expressing the aryloxyalkanoate dioxygenase 1 (AAD-1) protein imported from Canada and the USA.

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

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Summary

In accordance with Article 6 of Regulation (EC) No 396/2005, the evaluating Member State (EMS) Greece received an application from Dow AgroSciences to set an import tolerance for the active substance 2,4-D in genetically-modified (GM) maize expressing of the aryloxyalkanoate dioxygenase 1 (AAD-1) protein imported from Canada and USA. The modification confers tolerance to the herbicide 2,4-D. Greece drafted the 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).

EFSA bases its assessment on the evaluation report submitted by the EMS, the renewal assessment report (RAR) and its final addendum, the Commission 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 review of the existing maximum residue levels (MRLs) for 2,4-D according to Article 12 of Regulation (EC) No 396/2005.

The toxicological profile of 2,4-D was re-assessed in the framework of the renewal of the approval of the active substance under Regulation (EC) No 1107/2009. 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. In 2016, the toxicological profile of 2,4-DB was re-assessed and it was agreed that the lower toxicological reference values apply also to 2,4-D as the toxicological profile of 2,4-DB and 2,4-D is qualitatively similar (ADI 0.02 mg/kg bw per day, ARfD 0.3 mg/kg bw). The new toxicological reference values are not yet formally adopted.

The metabolism of 2,4-D in primary crops was investigated after foliar applications in cereals (wheat), root (potato) and soil application in fruit (apple); and 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. Additional specific metabolism studies on GM maize, expression of the AAD-1 protein (event DAS-40278-9 and DAS-40474-7), were submitted in the framework of this MRL application. The residue definition for enforcement and risk assessment derived for conventional crops is applicable to the imported GM maize, where only the parent compound was observed in the grain.

The submitted data on GM maize expressing the AAD-1 protein (event DAS-40278-9) are sufficient to conclude that a change of the existing MRL set at the limit of quantification (LOQ) of 0.05 mg/kg in maize is not required. Analytical enforcement methods are available to control the residues of 2,4-D in this crop at the LOQ of 0.01 mg/kg, but further validation work as identified during the peer review is still needed.

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 maize grain used as 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 Article 12 MRL review were taken into account. The acute risk assessment was performed for maize only. Using the existing and the proposed new toxicological reference values, no potential long-term consumer intake concerns or acute risks were identified for the notified uses on GM maize. The highest estimated long-term consumer intake accounted for 16% of the existing ADI (when using the lower ADI, the exposure accounted for 39%) with maize being a minor contributor (less than 0.7% of the lower ADI). The acute consumer exposure to 2,4-D related residues in maize was up to 0.04% or 0.11% of the existing and the newly proposed ARfD, respectively.

EFSA concludes that the uses of 2,4-D notified for GM maize expressing the AAD-1 protein (event DAS-40278-9) imported from Canada and the USA will not result in a consumer exposure exceeding the toxicological reference values for 2,4-D. Hence, a risk for consumers was not identified.

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
Enforcement residue definition: 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D)				
0500030	Maize/corn	0.05*	0.05*	Import tolerance (CA/USA) for 2,4-D-tolerant maize (AAD-1 protein, event DAS-40278-9) supported. Change of the existing MRL not required No risk for consumer identified for 2,4-D related residues

MRL: maximum residue level; AAD-1: aryloxyalkanoate dioxygenase 1.
*: 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.

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Background

Regulation (EC) No 396/2005[1] (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, hereafter referred to as the evaluating Member State (EMS), received an application from the company Dow AgroSciences[2] to set an import tolerance for the active substance 2,4-D on genetically modified (GM) maize imported from Canada and USA. The application was notified to the European Commission and the European Food Safety Authority (EFSA) and was subsequently evaluated by the evaluating Member State (EMS) in accordance with Article 8 of the MRL Regulation. The application was included in the EFSA Register of Questions with the reference number EFSA-Q-2015-00151.

According to the EMS, the notified uses of 2,4-D on imported maize did not require a change of the existing MRL set at the level of the limit of quantification (LOQ) of 0.05 mg/kg.

EFSA proceeded with the assessment of the applications and the evaluation reports as required by Article 10 of the Regulation.

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.

The evaluation report submitted by the EMS (Greece, 2015) and the exposure calculations using the EFSA Pesticide Residues Intake Model (PRIMo) are considered as supporting documents to this reasoned opinion and, thus, are made publicly available.

In accordance with Article 11 of the Regulation, the reasoned opinion shall be provided as soon as possible and at the latest within 3 months (which may be extended to 6 months if more detailed evaluations need to be carried out) from the date of receipt of the application. If EFSA requests supplementary information, the time limit laid down shall be suspended until that information has been provided.

The active substance and its use pattern

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 (e.g. choline) and esters. The chemical structures of the active substance 2,4-D and its main metabolite are reported in Appendix B.

The details of the Good Agricultural Practice (GAP) are in Appendix A. The notified GAP refers to GM maize.

For the GM maize DAS-40278-9, EFSA has provided an opinion on the application for authorisation for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003. Maize DAS-40278-9 expresses the aryloxyalkanoate dioxygenase 1 (AAD-1) protein, which confers tolerance to application of 2,4-D and aryloxyphenoxypropionate (AOPP) herbicides (EFSA GMO Panel, 2016). The following applications in the framework of Regulation (EC) No 1829/2003 are currently under assessment:

- MON 89034 × 1507 × NK603 × DAS-40278-9 maize, combining hybrids, which confer tolerance to glufosinate-ammonium, glyphosate, 2,4-D, AOPP herbicides and protection against target lepidopteran pests (Question No EFSA-Q-2013-00079);
- MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 maize, expressing proteins Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, phosphinothricin acetyltransferase (PAT), CP4-EPSPS and AAD-1, which confer tolerance to glufosinate-ammonium, glyphosate, 2,4-D, AOPP herbicides and protection against target lepidopteran and coleopteran pests (Question No EFSA-Q-2013-00210).

[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.

The active substance 2,4-D was included in Annex I of Directive 91/414/EEC by Directive 2001/103/EC[3] which entered into force on 1 October 2002. In accordance with Regulation (EU) No 540/2011[4], 2,4-D was deemed approved under Regulation (EC) No 1107/2009[5], repealing Directive 91/414/EEC. The approval for 2,4-D has been renewed according to Regulation (EC) No 1107/2009 by Regulation (EU) No 2015/2033[6]. The representative uses assessed during the renewal were on conventional wheat, barley, oats, rye and maize. The renewal assessment report (RAR) has been peer reviewed by EFSA (2014).

The European Union (EU) MRLs for 2,4-D are established in Annexes II of Regulation (EC) No 396/2005. Since the entry into force of the MRL regulation, EFSA has issued a reasoned opinion on the review of the existing MRLs for 2,4-D (EFSA, 2011). The proposals from this reasoned opinion have been considered in the preparation of the Regulation (EU) No 1317/2013[7].

The MRL/tolerance of 0.05 mg/kg is set for 2,4-D in maize/corn in the USA and Canada.[8]

Assessment

EFSA bases its assessment on the evaluation report submitted by the EMS (Greece, 2015), the renewal assessment report (RAR) and its addendum (Greece, 2013, 2014), the Commission review report on 2,4-D (European Commission, 2015), the conclusion on the peer review of the pesticide risk assessment of the active substance 2,4-D (EFSA, 2014) and 2,4-DB (EFSA, 2016) as well as the review of the existing MRLs for 2,4-D according to Article 12 of Regulation (EC) No 396/2005 (EFSA, 2011). 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[9] and the currently applicable guidance documents relevant for the consumer risk assessment of pesticide residues (European Commission, 1996, 1997a–g, 2000, 2010a,b, 2016; OECD, 2011).

1. Method of analysis

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

In the framework of the renewal peer review, it was concluded that analytical methods based on high performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) were available to enforce 2,4-D residues (residue definition sum of 2,4-D, its salts, esters and conjugates, expressed as 2,4-D) in dry/high starch matrices at the LOQ of 0.01 mg/kg. However, further validation work on the hydrolysis step and extraction efficiency that were identified as desirable during the Article 12 MRL review, were considered as a data gap in the EFSA conclusion (EFSA, 2014).

No new information has been submitted with the MRL application. Thus, the data gap identified in the EFSA conclusion is still outstanding.

[3] Commission Directive 2001/103/EC of 28 November 2001 amending Annex I to Council Directive 91/414/EEC concerning the placing of plant protection products on the market to include 2,4-dichlorophenoxy acetic acid (2,4-D) as an active substance, OJ L 313, 30.11.2001, p. 37–39.

[4] Commission Implementing Regulation (EU) No 540/2011 of 23 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.

[5] 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.

[6] Commission Implementing Regulation (EU) 2015/2033 of 13 November 2015 renewing the approval of the active substance 2, 4-D 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 298, 14.11.2015, p. 8–11.

[7] Commission Regulation (EU) No 1317/2013 of 16 December 2013 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 2,4-D, beflubutamid, cyclanilide, diniconazole, florasulam, metolachlor and S-metolachlor, and milbemectin in or on certain products. OJ L 339, 17.12.2013, p. 1–43.

[8] US Code of Federal Regulations 40 CFR §180.142 - 2,4-D; tolerances for residues. Health Canada Pest Management Regulatory Agency (PMRA). Maximum Residue Limits for 2,4-D for field corn EMRL2011-13 (18 March 2011) and MRL Database.

[9] Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

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

In the framework of the renewal peer review, it was concluded that analytical methods based on HPLC–MS/MS were available to enforce 2,4-D residues (residue definition 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. Additional information was required on the hydrolysis step and extraction efficiency. Although this data gap has not yet been addressed, it is of low relevance for this application as a change of the existing MRLs in animal products was not requested.

2. Mammalian toxicology

The toxicological profile of 2,4-D was re-assessed in the framework of the renewal of the approval of the active substances under Regulation (EC) No 1107/2009, which confirmed the acceptable daily intake (ADI); it was agreed to set an acute reference dose (ARfD) for 2,4-D (EFSA, 2014; European Commission, 2015).

In 2016, the toxicological profile of 2,4-DB was assessed and it was concluded that the toxicological profile of 2,4-DB and 2,4-D is qualitatively similar. The peer review experts agreed to revise the ADI and ARfD for 2,4-D, taking into account the results of the 2,4-DB assessment (EFSA, 2016). However, the revised values are not yet formally adopted by the European Commission. The available toxicological reference values are compiled in Table 1.

Table 1: Overview of the toxicological reference values

	Source	Year	Value	Study	Safety factor
Current toxicological reference values for 2,4-D (EFSA, 2014)					
ADI	European Commission	2015	0.05 mg/kg bw per day	2-year studies (mouse and rat)	100
ARfD	European Commission	2015	0.75 mg/kg bw	Acute neurotoxicity, rat	100
Proposed toxicological reference values for 2,4-D in 2,4-DB (EFSA, 2016)					
ADI	EFSA	2016	0.02 mg/kg bw per day	Dog, 1 year	100
ARfD	EFSA	2016	0.3 mg/kg bw	Rat and rabbit developmental toxicity studies	100

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

3. Residues

3.1. Nature and magnitude of residues in plant

3.1.1. Primary crops

3.1.1.1. Nature of residues

The metabolism of 2,4-D in conventional crops was evaluated in the framework of the MRL review under Article 12 of Regulation (EC) No 396/2005 and the renewal peer review (EFSA, 2011, 2014).

Additional studies on GM maize conducted at approximately the notified application rate (1N) were assessed in the framework of this MRL application (Greece, 2015). An overview of the key parameters of the available metabolism studies is presented in Table 2.

Table 2: Summary of available metabolism studies in plants

Crop group	Crops	Application	Sampling	Comments
Conventional plants (peer-reviewed studies)				
Fruit	Apple	Soil, 2 × 2.13 kg/ha	56 DALA	
Root	Potato	Foliar, 2 × 0.07 kg/ha	82 DALA	
		Foliar, 0.14 + 0.28 kg/ha	29 DALA	

Crop group	Crops	Application	Sampling	Comments
Cereal/ Grass	Wheat	Foliar, 1 × 1.68 kg/ha	10 (forage), 49 (grain, straw) DAT	
Genetically-modified plants[a] (not peer-reviewed studies)				
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	Event DAS-40474-7[b]
		Soil, 1 pre-emergence + Foliar, 2 post-emergence, Rate ca. 1.1–1.2 kg/ha, up to BBCH 18		Event DAS-40278-9

DALA: day after last application; DAT: days after treatment; AAD-1: aryloxyalkanoate dioxygenase 1.
(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 (Greece, 2015).

In **conventional** crops, 2,4-D (free and conjugated) was the major component of the total residues in wheat forage and straw (77% and 72% of total radioactive residue (TRR), respectively), wheat grain (6% of TRR) and potato tuber (35% of TRR). The metabolite 2,4-DCP was identified in low amounts (1% TRR, about 0.5 mg eq/kg in maize straw; 4% of TRR, < 0.01 mg eq/kg in potato tubers). Residues in apples were too low to allow further identification. Based on these metabolism studies, the residue definitions for enforcement and risk assessment in plants were proposed as sum of 2,4-D, its salts, esters and conjugates, expressed as 2,4-D (EFSA, 2011, 2014). The current residue definition set in Regulation (EC) No 396/2005 is identical to the residue definition for enforcement derived in the Article 12 MRL review and the renewal peer review.

Metabolism of 2,4-D was investigated in **GM maize** expressing the AAD-1 protein. In DAS-40278-9 maize, residues (TRR) were slightly lower than in DAS-40474-7 maize. 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% of the TRR) with 2,4-DCP, its disaccharide and glucose conjugates representing in total about 22–25% of the TRR. EFSA could not conclude on the toxicological profile and the carcinogenic and genotoxic potential of this metabolite (EFSA, 2014, 2016). In maize grain, extensive metabolism occurred with incorporation of radioactivity in natural components of the seed (i.e. starch). In the metabolism study with DAS-40474-7 maize, the parent compound was the only compound identified (7%, TRR; 0.003 mg/kg in grain, 2,4-DCP conjugates could not be detected). In the metabolism study with DAS-40278-9 maize, extract radioactivity in grain was too low to permit the identification of any individual compound.

EFSA concludes that for the notified use on GM maize (AAD-1 protein, event DAS-40278-9 and DAS-40474-7) the residue definition for enforcement and risk assessment derived for conventional crops is applicable, considering that parent compound was the only compound detected in the grain and that grain is the only product expected to be imported. This finding is confirmed by the results of the residue trials in GM maize grain and the processing studies where maize was treated at exaggerated dose rates (2N the notified application rate).

3.1.1.2. Magnitude of residues

In support of the MRL application for maize, field residue trials conducted in Canada and the USA were submitted. A non-ionic surfactant was included in the spray mixture. All supervised residue trials were performed 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 it is not necessary to request trials performed in a different year. In all trials, 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.

Maize critical GAP (cGAP): 1 pre-emergence + 2 post-emergence × 1.12 kg/ha, growth stages of mono- and dicotyledonous plants (BBCH) 18, preharvest interval (PHI) n.a.

A total of 24 residue trials on GM maize compliant with the critical US GAP were submitted. All trials were conducted on DAS-40278-9 maize expressing the AAD-1 protein. At harvest (62–139 day after last application (DALA)), residues of both 2,4-D[10] and 2,4-DCP were not quantifiable in grain. An MRL

[10] The EMS excluded one trial (replicate sample values for 2,4-D: < 0.01; 0.07 mg/kg) from the calculation as the positive value was considered a result of contamination (Greece, 2015).

proposal of 0.01 mg/kg (LOQ) is derived, which is lower than the MRL/tolerance of 0.05 mg/kg established in the countries of origin.

The results of the residue trials, the related risk assessment input values (highest residue, median residue) 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 storage stability data on 2,4-D stored at −20°C were submitted (Greece, 2015). Parent compound showed to be stable for 13 months in maize grain. Storage stability data for the metabolite 2,4-DCP were also provided. A decline by nearly 60% in the period of time beyond 2 months and up to the 13 months tested was observed in maize grain and flour, but not in the other low water content matrices investigated (maize starch and stover).

Since the samples from the submitted residue trials were stored for a maximum period of 11 months, the results are valid with regard to storage stability of 2,4-D. Despite the results from the storage stability study for 2,4-DCP on maize grain, EFSA agrees with the EMS to accept as valid the residue data submitted.

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). However, evidence that the applied acid hydrolysis procedure was very efficient in totally releasing all conjugates of the compounds has not been provided.

EFSA concludes that a sufficient number of supervised residue trials is available to support the notified uses in GM maize expressing the AAD-1 protein (event DAS-40278-9) and a change of the existing MRL in maize set at the LOQ of 0.05 mg/kg is not required.

Table 3: Overview of the available residues trials data

Crop (GAPs)	Region/ indoor[a]	Residue levels observed in the supervised residue trials[b] (mg/kg)	Recommendations/comments[c]	MRL proposal (mg/kg)	HR[d] (mg/kg)	STMR[e] (mg/kg)
Maize (AAD-1, event DAS-40278-9)	USA/CA	24 × < 0.01	Existing residue definitions apply Residues of 2,4-DCP < LOQ/LOD MRL: 0.01* mg/kg Use does not trigger a change of existing MRL	0.05*	0.01	0.01

GAP: Good Agricultural Practice; LOQ: limit of quantification; LOD: limit of detection; MRL: maximum residue level; AAD-1: aryloxyalkanoate dioxygenase 1.
*: Indicates that the MRL is proposed at the limit of analytical quantification (LOQ).
(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): Individual residue levels considered for MRL calculation are reported in ascending order.
(c): Any information/comment supporting the decision. OECD MRL calculation (unrounded/rounded values) is not applicable.
(d): HR: Highest residue level according to the residue definition for risk assessment.
(e): STMR: Median residue level according to residue definition for risk assessment.

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 conducted on GM maize expressing the AAD-1 protein (event DAS-40278-9) at twice the application rate (2N) defined in the GAPs were provided (Greece, 2015). Samples were analysed for 2,4-D and the metabolite 2,4-DCP.

Parent 2,4-D was not found in any of the unprocessed maize samples nor in any maize processed products (i.e. flour, meal, oil, starch aspirated grain fractions). 2,4-DCP was not found in unprocessed grain and in any processed maize products. Processing factors could not be derived from the data available as the nature of residues in processed commodities was not investigated and residues of 2, 4-D were below the LOQ.

3.1.2. Rotational crops

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

3.2. Nature and magnitude of residues in livestock

As imported GM maize grain may be fed to livestock, the nature and magnitude of residues in livestock was assessed (European Commission, 1996). According to the EFSA document on the estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin, the import in the EU of maize forage and stover as livestock feed is not expected.

3.2.1. Dietary burden of livestock

The animal dietary burden was calculated in the framework of the Article 12 MRL review based on the existing uses of 2,4-D (EFSA, 2011). Since the 2,4-D residues in conventional maize grain assessed under the MRL review (STMR of 0.05 mg/kg) is higher than the input value derived from the notified use on GM maize (STMR of 0.01 mg/kg), the previously calculated dietary burden is still valid. Residues on imported maize forage and stover are not relevant for European livestock. Therefore, EFSA concludes that a change of the existing MRLs for 2,4-D (residue definition: sum of 2,4-D, its salts, esters and conjugates, expressed as 2,4-D) in products of animal origin resulting from the notified uses on GM maize and the use of the imported grain as feed item is not required.

It is noted that the metabolite 2,4-DCP was observed in livestock metabolism studies performed with 2,4-D and with the active substance 2,4-DB. Based on the representative uses of 2,4-D on cereals (1 × 750 g/ha), the peer review experts concluded that significant residue levels of 2,4-DCP in ruminant matrices are not expected (EFSA, 2014).

In the framework of the renewal of 2,4-DB, 2,4-DCP was provisionally proposed for inclusion in the residue definition for risk assessment for animal tissues, either combined or separate, pending its toxicological relevance (EFSA, 2016).

The residue definition and the MRLs set in products of animal origin for 2,4-D may need to be reconsidered to reflect the outcome of the toxicological assessment of the metabolite 2,4-DCP, its occurrence in feedstuff in all crops where the use of 2,4-D is authorised and the possible transfer of 2,4-D related residues into animal products.

4. Consumer risk assessment

In the framework of the Article 12 MRL review for 2,4-D, a comprehensive long-term exposure assessment was performed for the 2,4-D related residues taking into account the existing uses at EU level and the acceptable CXLs (EFSA, 2011). 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[11] (EFSA, 2007).

[11] The calculation of the long-term exposure (chronic exposure) is based on the mean consumption data representative for 22 national diets collected from Member State surveys plus one regional and four 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 Member State surveys is used. The complete list of diets incorporated in EFSA PRIMo is given in its reference section (EFSA, 2007).

Since the notified use on imported GM maize grain is less critical for residues (STMR of 0.01 mg/kg, see Table 3) than the existing European uses on maize assessed in the framework of the Article 12 review and a change of the existing MRL is not proposed, it is not necessary to conduct a new chronic risk assessment for 2,4-D. EFSA merely revised the exposure calculation to reflect the MRLs set by Regulation (EU) No 1317/2013 on certain products of animal origin.

At the time of the Article 12 review, an acute consumer exposure assessment was not performed as an ARfD was not yet established (EFSA, 2011). During the renewal process an acute reference value was set for 2,4-D (EFSA, 2014). Therefore, EFSA performed the acute exposure assessment for maize, 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 4.

Table 4: 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: sum of 2,4-D, its salts, esters and conjugates, expressed as 2,4-D				
Maize grain	0.05	STMR (EFSA, 2011)	0.05	STMR (EFSA, 2011)
Swine, bovine, sheep, goat, equine, other farmed animals, edible offal	5	MRL in Regulation (EU) No 1317/2013	Acute risk assessment undertaken only for maize	
Swine, bovine, sheep, goat, equine, other farmed animals, other products	5	MRL in Regulation (EU) No 1317/2013		
Poultry, kidney, edible offal, other products	0.05	MRL in Regulation (EU) No 1317/2013		
Other plant and animal origin commodities	See table 4–2 of the Reasoned opinion on the review of the existing MRLs for 2,4-D according to Article 12 of Regulation (EC) No 396/2005 (EFSA, 2011)			

STMR: supervised trials median residue; MRL: maximum residue level.

The estimated acute exposure was then compared with the toxicological reference value derived for 2,4-D (see Table 1). EFSA also conducted an additional consumer risk assessment with the proposed new toxicological reference values (EFSA, 2016) reported in 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 actual toxicological reference values (Scenario 1), the highest chronic intake was calculated to be 16% of the ADI (Irish adult diet) with maize accounting to the total consumer exposure for 0.25% the ADI (WHO Cluster diet B). The highest acute consumer exposure for maize was calculated to be 0.04% of the ARfD (UK infant).

Using the revised toxicological reference values (Scenario 2), the highest chronic intake was calculated to be 39% of the ADI (Irish adult diet) with maize accounting to the total consumer exposure for 0.62% the ADI (WHO Cluster diet B). The highest acute consumer exposure for maize was calculated to be 0.11% of the ARfD (UK infant).

EFSA concludes that the uses notified for GM maize expressing AAD-1 protein (event DAS-40278-9) imported from Canada and USA will not result in a consumer exposure exceeding the toxicological reference values. Hence, a risk for consumers was not identified.

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
Enforcement residue definition: 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D)				
0500030	Maize/corn	0.05*	0.05*	Import tolerance (CA/USA) for 2,4-D-tolerant maize (AAD-1 protein, event DAS-40278-9) supported. Change of the existing MRL not required No risk for consumer identified for 2,4-D related residues

MRL: maximum residue level; AAD-1: aryloxyalkanoate dioxygenase 1.
*: 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.

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Abbreviations

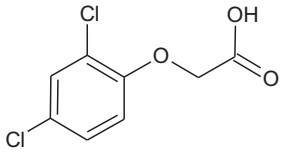
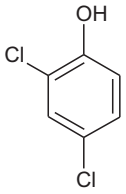
a.s.	active substance
AAD-1	aryloxyalkanoate dioxygenase 1
ADI	acceptable daily intake
AOPP	aryloxyphenoxypropionate
ARfD	acute reference dose
BBCH	growth stages of mono- and dicotyledonous plants
bw	body weight
CA	Canada
CF	conversion factor for enforcement to risk assessment residue definition
cGAP	critical GAP
CXL	Codex maximum residue limit (Codex MRL)
DALA	days after last application
DAT	days after treatment
EMS	evaluating Member State
eq	equivalent
GAP	good agricultural practice
GM	genetically modified
HPLC	high-performance liquid chromatography
HR	highest residue
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
LOQ	limit of quantification
MRL	maximum residue level
MS/MS	tandem mass spectrometry detector
MW	molecular weight
OECD	Organisation for Economic Co-operation and Development
PAT	phosphinothricin acetyltransferase
PF	processing factor
PHI	pre-harvest interval
PRIMo	(EFSA) Pesticide Residues Intake Model
RAC	raw agricultural commodity
SANCO	Directorate-General for Health and Consumers
SL	soluble concentrate
STMR	supervised trials median residue
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 G or I[b]	Pest or group of pests controlled[c]	Formulation		Application				Application rate per treatment			PHI (days)[l]	Remarks[m]
				Type[d]–[f]	Conc. a.s.[i]	Method kind[f]–[h]	Growth stage and season[j]	Number min–max [k]	Interval min–max	g/hL min–max	Water L/ha min–max	kg/ha min–max		
GM maize (expression of AAD-1 protein)	CA	F	Annual and perennial weeds	SL	456 g/L	Spraying	Up to BBCH 18	2	12 day		50–200	0.82	n.a.	2,4-D as choline salt
	US						Pre-emergence (1)+ post-emergence (2) up to BBCH 18	3	Post-emergence: 12 days		47–94	1.12	n.a.	2,4-D as choline salt cGAP

NEU: northern Europe; SEU: southern Europe; MS: Member State; SL: soluble concentrate; a.s.: active substance.
(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-born 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 μg/L.
(j): Growth stage at last treatment (Meier U, 2001. Growth Stages of mono- and dicotyledonous plants. BBCH Monograph, 2nd Edition, 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	Chemical name	Structural formula
2,4-D	(2,4-Dichlorophenoxy)acetic acid Clc1cc(Cl)ccc1OCC(=O)O	
2,4-DCP	2,4-Dichlorophenol Clc1cc(Cl)c(O)cc1	
2,4-DB	4-(2,4-dichlorophenoxy)butyric acid Clc1cc(Cl)ccc1OCCCC(=O)O