EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

EU-RL GMFF guidance on testing for GM glyphosate-resistant wheat (MON71800) in wheat grain or in food/feed products containing wheat flour originating or consigned from the US

European Union Reference Laboratory for GM Food and Feed

12 June 2013

Version 2 – 25/06/2013

Modification from previous version, page 1/6: the first paragraph of section 1 (Background) has been replaced by the following*: Following the United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) announcement that test results confirm the finding of unauthorised GM* ***glyphosate-resistant wheat*** *"volunteer" plants in a farm in Oregon harbouring the event MON71800, DG SANCO has asked the European Union Reference laboratory for Genetically Modified Food and Feed (EU-RL GMFF) to provide National Reference Laboratories (NRLs) as soon as possible with a method to test wheat consignments for the presence of this Genetically modified organism (GMO).*

1. Background

Following the United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) announcement that test results confirm the finding of unauthorised GM **glyphosate-resistant wheat** "volunteer" plants in a farm in Oregon harbouring the event MON71800, DG SANCO has asked the European Union Reference laboratory for Genetically Modified Food and Feed (EU-RL GMFF) to provide National Reference Laboratories (NRLs) as soon as possible with a method to test wheat consignments for the presence of this Genetically modified organism (GMO).

As no validated event-specific method is yet available, the EU-RL GMFF has developed the interim strategy detailed below that should be replaced or amended by an event-specific method as soon as this will be considered suitable for the purpose.

Monsanto is currently providing the EU-RL GMFF with information on a supposedly event-specific detection method and related control samples and the EU-RL GMFF is assessing the

information available in order to verify the method as soon as the necessary material is received.

The information made already available by Monsanto suggests that the event MON71800 would react with two widely used element-specific methods, namely CaMV 35S promoter (P-35S) and nopaline synthase terminator (T-nos) and with the construct-specific method CTP2-CP4epsps [a].

2. Proposed testing strategy

Given that hitherto the method just made available by Monsanto, though under assessment, has not yet been verified in-house or validated by the EU-RL GMFF, a testing strategy based on validated screening methods is proposed. This interim strategy will allow excluding (detectable) presence of Monsanto's GM glyphosate-resistant wheat (MON71800) in wheat grain or food/feed products and confirming its presence whenever other GMOs can be excluded.

As soon as a reliable event-specific method is available, this testing strategy will be revised.

A) Sampling and preparation of the analytical samples

Sampling of bulk commodities should be carried out in accordance with Recommendation 2004/787/EC for food products and to Regulation (EC) No 152/2009 for feed.
The size of the laboratory sample should be 2.5 kg but may be reduced to 500 grams for processed food or feed.

Sampling of prepacked food and feed should be carried out in accordance with CEN/TS 15568:2007 or equivalent. The size of the laboratory sample should be 2.5 kg but may be reduced to 500 grams for processed food or feed.

In case of grain samples, the laboratory should take from the homogenised laboratory sample (2.5 kg) one analytical sample of 400 grams (equivalent to 10,000 wheat grains). The analytical sample should be ground and analysed as detailed below.

For processed products, one analytical sample of 125 grams should be prepared from the homogenised laboratory sample (500 grams or more). This analytical sample should be ground and analysed as detailed below.

B) Initial Testing

1) Testing laboratories should follow the relevant ISO standards [2, 3, 4,] for the preparation of the analytical sample, the preparation of the test sample and the required number of replicates.

2) Each sample should be tested with three validated screening methods, following the respective standard protocols implemented in the control laboratory and:

[a] http://gmo-crl.jrc.ec.europa.eu/gmomethods/entry?db=gmometh&id=ql-con-00-008&q=id%3aQL-CON*

a) targeting the CTP2-CP4epsps construct,
b) targeting the P-35S promoter, and
c) targeting the T-nos terminator

According to the information currently available to the EU-RL GMFF, including detailed bioinformatics analyses,[b] the methods described in reference 5, 6, 7 (see Section 3: References) should detect the respective targets that are simultaneously present in the GM glyphosate-resistant wheat MON71800. The suitability of alternative validated methods targeting the same elements should also be confirmed by bioinformatics and laboratory analyses prior to their use.

3) Certified Reference Material ERM-BF415 (NK603 maize, IRMM) should be used as a source of positive control.

Note: the Certified Reference Material ERM-BF415 (NK603 maize, IRMM), while consistently containing the P-35S promoter, the T-nos terminator and the CTP2-CP4epsps construct has not been certified for the presence of these targets.

4) In addition, a wheat-specific reference system should be run (e.g. Iida *et al*, 2005 [c], Matsuoka *et al*., 2102 [8, 9]) in order to ensure that the amplification of the wheat DNA worked correctly.
In case of samples derived from wheat grains, the total amount of wheat DNA in PCR should be at least 300 ng.
In case of samples derived from mixed food/feed products, the total amount of wheat DNA can only be roughly estimated on the basis of the quantification cycle (Cq) of the wheat-specific reference system; this should be ≤ Cq 28-30, corresponding to about 4000 wheat haploid genome copies (calculated from the 1C value for soft wheat = 17.33 pg [d])

5) Interpretation of the initial test results:

a) If presence of wheat is confirmed by a positive result provided by the wheat specific reference method and at least one of the three validated screening methods provides a negative result, the sample is considered **not containing** GM wheat MON71800 because the presence of this GM event should result in positive results for all four targets.
*However, while the GM wheat MON71800 is expected to give positive signals for the two elements **and** the construct, any positive test result is indicative of a GMO being possibly present in the sample and needs to be verified in accordance with the laboratory's standard* strategy [e].

b) If all four methods provide a positive result, presence of the GM-wheat MON71800 is possible but a conclusive statement about its presence cannot be made automatically because one or more other GMOs, containing some or all of

[b] In addition, the EU-RL GMFF is currently carrying out in-house laboratory verifications of the methods on the target GMO.
[c] According to *in-silico* analyses conducted by the EU-RL GMFF, the primers sequences described in Iida *et al*. 2005 are not correct. It is suggested using as a reference the primers (Wx012F/Wx012R) and probe (Wx-Taq 1) sequences reported by the same authors in the following article: Imai *et al*. (2012), Food Hyg. Saf. Sci. 204 Vol. 53 203-210 and the patent EP2180051.
d http://data.kew.org/cvalues/
[e] http://gmo-crl.jrc.ec.europa.eu/doc/2011-12-12%20ENGL%20UGM%20WG%20Publication.pdf

the tested targets could, alone or in combination, also result in positive results of all four tests.

Therefore, in case of positive results with all four methods indicated above, the following further testing is suggested.

C) Further testing

1) Verification of the presence in the sample of the following species: **maize, soybean, oilseed rape, cotton, sugar beet** (taxon-specific reference systems: http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx). Reason: some GM events, belonging to these species, could react positively with all three screening methods. As p35s and T-nos could result also from viral or bacterial contamination, particular relevance is allocated to the CPT2-CP4epsps construct-specific method.

2) Interpretation of the results and follow-up:

   a) If none of these species is present (i.e. all test results are negative), the sample is to be considered **positive** for an unauthorised GMO containing P-35S, T-nos and CPT2-CP4epsps, such as the GM wheat MON71800, or a combination of GMOs collectively containing the three targets and not belonging to any of the species tested.

   b) If one or more species are detected, that could contain one or a combination of GM events listed in table 1, further verification is suggested.

3) Verification of the presence of the non-wheat GMO with the appropriate event-specific method(s) [f]. See Table 1 for the list of GM events containing the CTP2-CP4epsps target, for which a validated event-specific method is available. Some of these also contain the P-35s and the T-nos.

4) Interpretation of the results and follow-up:

   a) If no GM event is detected by event-specific method(s) that could explain a positive signal for CTP2-CP4epsps, the sample is **positive** for an unauthorised GMO containing P-35S, T-nos and CTP2-CP4epsps, such as the GM wheat MON71800.

   b) If any of the GM events listed in Table 1, containing the CTP2-CP4epsps construct is confirmed to be present by event-specific method(s), the test has to be declared **inconclusive** because this presence could mask the presence of another GMO containing P-35S, T-nos and CTP2-CP4epsps, such as the GM wheat MON71800.

[f] http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx

Disclaimer:
This present testing strategy is based solely on the theoretical knowledge of the genetic elements present in MON71800 soft white wheat event available to the EU-RL GMFF at this moment (12 June 2013). Monsanto has provided the EU-RL GMFF with a detection method, sequence information and control samples, that are currently assessed for their suitability. The EU-RL GMFF has started to verify this supposedly event-specific (MON71800) method according to its standard practice and will make it available to replace or complement this testing strategy if the verification shows adequate performance.

Table 1. GM events containing the ctp2-cp4epsps element for which an EU-validated event-specific method is available.

Crop	**Event**	**CTP2-CP4EPSPS**	**Positive/negative for**	
			P-35S	**T-nos**
Maize	MON88017	+	+	+
Maize	NK603	+	+	+
Soybean	MON89788	+	-	-
Rapeseed	GT73	+	-	-
Sugar Beet	H7-1	+	-	-
Cotton	MON1445	+	+	+
Cotton	MON88913	+	+	-

3. References

1. Gerdes L., Busch U., Pecoraro S., 2012. GMOfinder—A GMO Screening Database. Food Anal. Methods (2012) 5:1368–1376.

2. ISO 24276, Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products – general requirements and definitions.

3. ISO 21569, Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – qualitative nucleic acid based methods

4. ISO 21571, Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products –Nucleic acid extraction

5. ISO 21570, Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products—quantitative nucleic acid based methods. Annex B1.

6. Reiting R, Broll H, Waiblinger HU, Grohmann L (2007) Collaborative study of a T-nos real-time PCR method for screening of genetically modified organisms in food products. J Verbr Lebensm 2:116–121.

7. Grohmann L., Brunen-Nieweler C., Nemeth A., Waiblinger H.U.; "Collaborative trial validation studies of real-time PCR-based GMO screening methods for detection of the bar gene and the CTP2-CP4epsps construct" J. Agric. Food Chem. 57:8913-8920

(2009) and ISO 21569: 2011 Methods of analysis for the detection of genetically modified organisms and derived products – Qualitative nucleic acid based methods (ISO/TC 34/SC 16 N 128). Annex C.8.

8. Iida M, Yamashiro S, Yamakawa H, Hayakawa K, Kuribara H, Kodama T, Furui S, Akiyama H, Maitani T, Hino A. Development of taxon-specific sequences of common wheat for the detection of genetically modified wheat. J Agric Food Chem. 2005 Aug 10; 53: 6294-300.

9. Matsuoka Y., Shin-ichiro A., Megumi S., Hiroyuki H., Youichi K., Shinjiro I., Keiko T., Junichi M., Satoshi F., Kazumi K., 2102. Development of Methods to Distinguish between Durum/Common Wheat and Common Wheat in Blended Flour Using PCR Food Hyg. Saf. Sci. 2012; 53(5): 195-202.