European Commission

JRC CONFERENCE AND WORKSHOP REPORT

18th WORKSHOP OF GMO NRLs

Meeting Report

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2023

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Joint Research Centre

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1. Welcome, approval of the agenda

The Chair welcomed the participants and introduced herself as the new Head of the Unit Food & Feed Compliance (JRC F.5). She informed that the meeting is organised in a hybrid format with representatives participating either physically at the JRC site or online. Since no additional points of discussion were requested, the Agenda (Annex 1) was approved.

2. Update from SANTE

SANTE summarised the latest developments on New Genomic Techniques (NGT), facts-finding studies on NGT and GM presence in feed additives/enzymes (FA/FE).

NGT

The Commission published the NGT study on 29 April 2021 and announced policy action on plants derived from targeted mutagenesis and cis-genesis. In fall 2021, the Commission published a roadmap of the initiative (inception impact assessment) and received over 70000 answers. A public consultation launched in mid-2022 on the functioning of the current EU GMO legislation for plants obtained by targeted mutagenesis and cis-genesis and their food and feed products and on potential options for a new framework received 2300 reactions. The factual summary report and all contributions are available at https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13119-Legislation-for-plants-produced-by-certain-new-genomic-techniques/public-consultation_en. A full analysis will be included in the impact assessment. In May 2022, a Joint WG meeting discussed the main policy elements of the NGT initiative (risk assessment, sustainability, traceability, labelling). The minutes are available online at https://food.ec.europa.eu/system/files/2022-09/sc_modif-genet_20220525_jwg_sum.pdf. A follow up meeting is expected in autumn 2022. The adoption of a legal proposal on GMOs is planned for the 2nd quarter of 2023.

Fact-finding studies

Fact-finding studies on NGTs aim to gather information on the implementation of controls on NGTs under the relevant legislation on genetically modified organisms (GMO) and to identify good practices. The meetings had a virtual format on five consecutive half days and included representatives from two Member States (MS), three (+2) SANTE members and four national experts. The findings will be presented to MS and possibly also to the ENGL.

GM presence in feed additives (FA) and food enzymes (FE)

The speaker apologised for the slow progress and reported that internal discussions are still ongoing. SANTE aims to discuss this in a meeting with the MS in autumn 2022.

Questions:

A representative from Austria requested clarifications on the outcome of the fact-finding studies. The aim of the studies was to verify how the competent authorities (CA) and the laboratories were addressing the challenges of NGT products being considered GMOs; if they were doing research, which techniques were used for ensuring traceability and how they would affect the business of stakeholder organisations or companies. The goal was to find more information directly from the interested parties. A representative from The Netherlands commented that their laboratories do not have methods operational for NGT detection and are not in the course of developing such methods.

A representative from Italy asked the reasons for not including the EURL in the list of participants for the fact-finding studies. SANTE explained that the JRC colleagues are involved in other NGT activities and that the Commission wanted to include Member State participants in collecting information on the issue. The JRC asked how the update of the 2019 ENGL report on detection of NGT products could fit into the impact analysis. SANTE explained that the Commission wanted to base its initiatives on an updated report. Analytical aspects on NGT detection may affect traceability and labelling and may influence the final impact assessment.

3. Report on EURL GMFF activities

JRC representatives summarised the activities of the EURL GMFF in 2022. The laboratory validated two methods for detection of maize events MON 95379 and DP915635 and one method for detection of the soybean event DBN-09004-6 which is also including a new extraction method. The maize methods are in the reporting step. The plasmids control samples for these maize and soybean events will be dispatched in November 2022 bringing to a total of four the released plasmid control samples. The EURL GMFF also verified the methods for detecting a stack event and is evaluating the method for the maize event DP202216 from Corteva. The national reference laboratories (NRLs) may have received the invitation for the related ring-trial. The JRC proposed to send the laboratory data from the ring trials using (e-signed) PDF files instead of shipping them on CDs. This approach would be more convenient; less time consuming and provide savings in shipping costs.

The speaker invited to update the data on the NRLs bank accounts stored in the JRC administrative database as previously requested by the JRC financial department. Links to form and guidelines were provided in the letter sent on 17 May 2022. He asked to respond, if not done already, to ensure correct reimbursement for the following ring-trial.

Two distribution campaigns for the Pre Spotted Plates (PSPs) have delivered 300 PSPs to eleven NRLs or OCLs (Official Control laboratories) in eight EU MS. The JRC is organising a third distribution campaign and evaluating the possibility of updating the plates design for detecting new authorised events. The speaker informed that as requested in the survey, Sciensano from Belgium would organise in collaboration with the JRC a training on Next Generation Sequencing (NGS). This will be divided in two sections, an online general introduction to theoretical aspects offered on 30 November 2022 and a physical on-site workshop at the Sciensano laboratory the following week. The data analysis will be tailored to the bioinformatics needs and to specific case studies.

The JRC provided a summary of the ENGL Working Group activities. The working group on good practice/quality of DNA sequencing data (WG-Seq) has a new chair, the report of the working group on DNA extraction (WG-DNAex) is being finalised and the document drafted by the working group on method performance requirements (WG-MPR2) received few comments, which will be addressed in the following meeting. The report of the working group on detection of genetically modified microorganisms in food and feed (WG-GMM) was still awaiting a clarification on a legal interpretation by SANTE while a report from the working group on new genomic techniques (WG-NMT) is expected for the end of 2022.

The JRC informed having started research projects on detection of NGT products by droplet digital PCR (ddPCR) and by Next Generation Sequencing (NGS) in collaboration with an external company. It has proposed an exploratory project using Artificial Intelligence (AI) for identifying induced mutations in the plant genomes.

Participants raised questions on the terminology used for indicating these new products. A JRC representative explained that the term NGT initially used by the Commission has been replaced after its legal initiative in April 2022, by the terms targeted mutagenesis and cis-genesis.

SANTE explained that targeted mutagenesis is not a synonym for NGTs, and that the legislative initiative is restricted to targeted mutagenesis in contrast to random mutagenesis and only to plants. The speaker agreed with the comment raised by a representative of Belgium claiming that the targeted methodology approach could generate a quite different impact depending on the fragment size being introduced into the genome.

A JRC representative summarised the results of the Proficiency Tests (PTs) performed in the last 2 years. He pointed to a publication (Proficiency of European GMO control laboratories to quantify MON89788 soybean in a meat pâté matrix - ScienceDirect) that resulted from the outcome of the last PT round in 2021 (GMFF-21/02) on the quantification of GM soybean in meat pate. For the second test item in that PT, further investigations were carried out to understand the effect of the master mix on quantification in qPCR and dPCR. For 2022, one PT test was completed while the second one is yet to start. GMFF-22/01 included a blank food material not containing any GM event (T1). Although unusual, the choice of not including GMOs in the food sample was in line with the common laboratory experience of retrieving negative results from analytical samples. The T2 sample consisted of rapeseed meal containing MS8 and RF3 oilseed rape events. The assigned value for event RF3 had a larger uncertainty, therefore z prime was used for scoring the reported results. This PT involved 63 participants of which more than half were NRLs. All laboratories reported the absence of GM events in the blank material T1. In T2, few laboratories did not detect the events; some did not report a result. A total of 5 and 2 unsatisfactory results were scored for MS8 and RF3, respectively. The EURL GMFF will contact the laboratories that overestimated the content of RF3 or MS8 to investigate the reasons for the unsatisfactory results. Few laboratories incorrectly reported compliance to both Regulation (EC) No 1829/2003 and Regulation (EU) No 619/2011. The compliance was to one or the other Regulation, never to both. The speaker reported that by consultation with SANTE, the value of 0.93 m/m should be rounded to 0.9 %; therefore, based on the EURL GMFF results, the sample in the PT is compliant and no labelling is required. The Competent Authority (CA) should provide guidance on the summing up or not of events for the stack MS8 x RF3. The speaker announced that the final PT report (a draft was distributed before the meeting) would be published in the following week when the second PT of 2022 will be launched. The material will consist of multigrain bread mix containing GM maize (T1) and of soybean powder (T2). The results of the laboratory tests should be submitted by the end of the year.

The speaker provided an overview of the CRMs for which the appropriateness had been assessed by the EURL GMFF. With the renewal of the authorisation for event MIR162, laboratories should use the CRM produced by the JRC in place of the CRM 1208-A3 from AOCS. He also informed that the new list of conversion factors is published on the EURL GMFF website at https://gmo-crl.jrc.ec.europa.eu/guidance-documents

A representative from Belgium requested whether the higher divergence in measurement uncertainty was noticed also between Geel and Ispra laboratories. The speaker reported using the average of four datasets, three from Geel and one from Ispra, and one of these deviated from the others, without a technical reason to reject these.

4. Tour de table: issues/opinions/training needs from NRLs

The Chair invited the participants to summarise their activities and any issue of interest. Participants informed finding non-compliant samples mainly in feed, but often at a concentration close to the limit of detection (LOD); some samples were found to be mislabelled for GMO presence or to be contaminated with a not declared ingredient. They reported increasing complex contaminations with multiple GM events especially for soybean feed samples. Some laboratories are testing rice or tomato products often imported from China for unauthorised GMOs. An increasing number of laboratories is using digital PCR (dPCR) in place of real-time PCR methods, especially for detecting the most common GMOs and is appreciating the JRC for providing conversion factors useful for transferring the results obtained in copy numbers into mass/mass values. Some laboratories ordered a new digital PCR equipment or a multiplex dPCR instrument and would like guidance on transferring their analyses from real-time to dPCR. Few declared difficulties in keeping the accreditation for all methods of GMO analysis. Many laboratories have purchased an NGS instrument or are considering that option and are therefore interested in the NGS/bioinformatics training offered by Sciensano and the JRC. Sciensano (Belgian public health institute) is accredited (ISO 17025) for NGS generation of data including analysis regarding qualitative data. However, this is a part of the process, downstream analysis with specific "scope" needs an additional accreditation/validation process. NRL-GMO will do it for some specific GMO analysis via NGS if necessary (ex GMM). Some laboratories are exploring high throughput sequence activities for detection of unauthorised GMOs. Representatives from many MS started using the GMM screening

methods validated by the Belgian NRL and verified by the JRC but claimed difficulties in following up positive results. Few NRLs expanded their activities to research areas as bioterrorism or NGT detection by NGS or dPCR technologies. A laboratory from Romania validated a new screening method for canola while the Belgium NRL developed an approach for detecting botanical impurities. Some laboratories used PSPs for their analyses and requested guidance for the in house validation of the tool. A representative from the Czech Republic invited the participants to a meeting on food safety and new breading techniques (NBT) organised in Prague in November 2022.

5. EURL GMFF web applications for GMO detection (EURL GMFF)

The JRC presented the recently updated JRC web applications GMOMETHODS, JRC GMO-Amplicons and JRC GMO-MATRIX, which is now indicating the EU authorisation status of the listed GMOs. The latter application compiles in silico PCR predictions for GMOs detection using primers and probe sequences from the GMOMETHODS database and GMOs sequences from a JRC internal database. These sequences are provided by the applicants for authorisation of GMOs or retrieved independently from nucleotide/patent databases. Since 2016, the JRC has implemented a standard to verify the quality of the sequences submitted by the applicants and can therefore ensure their correctness. The scripts that simulate PCR amplification use "e-PCR" developed by NCBI and could help control laboratories in designing screening strategies and interpreting the results. The information on EU authorisation is retrieved from the GMO Register database of the Commission and is automatically updated daily or every time a new method is published on the EURL GMFF website. The web site also provide screening strategies for detecting all EU authorised GMOs in samples containing cotton, oilseed rape, maize, soybean or sugar beet. Finally, the JRC GMO-Amplicons application compiles putative GMO-related amplicons from public sequence databanks using primers of EU reference methods and the "re-PCR tool from NCBI. The tool allows predicting primers specificity and GM targets coverage. It can also help developing detection methods for selected species or non-authorized GMOs by retrieving and characterising related sequences from public databases and patents documentation.

The participants expressed appreciation for the functions offered by the tools and requested whether it would be possible to provide also pre-made proposals for general screening or mark a method preferentially used in the laboratory. They underlined that in silico predictions do not always correspond to experimental results.

6. Detection strategies in enforcement laboratories: NL food and feed (WFSR, NL)

The speaker presented a list of authorised, pending, expired and withdrawn GMOs for food and feed. He presented a strategy for a broad screening based on methods collected from the EUginius database (www.euginius.eu), which was jointly developed by BVL (Berlin) and Wageningen Food Safety Research (WFSR).

A screening strategy in feed samples is as wide as possible to detect unauthorised events. The laboratory tests for genetic elements (up to 29 targets) and three possible relevant GM events using an Excel file that provides an output similar to the JRC web applications. A screening strategy for food samples may include up to 12 targets and 5 possible GM events. Both screening sets include a qPCR method to verify the quality of the DNA and endogenous control methods to confirm the presence of plant species in the samples. An analysis tools allows predicting, based on the test results, the GMOs possibly present in the sample. The laboratory then confirms their presence using event-specific methods and possibly quantifying their level. This step may be very difficult with multiple ingredients and at low level. In the presence of multiple GM events, the laboratory quantifies the event with the lowest Cq with respect to the endogenous Cq and if the lowest amount is above the legal limit (0.1 % or 0.9 %) the finding is reported to the Competent Authority (CA). Otherwise, the laboratory continues the analysis with the following GM event detected. The speaker requested other participants to share their screening strategy.

The representative from Belgium remarked that the strategy depends on the request of the respective CA. Their laboratory tests for authorised GMOs and GMOs that are pending or known non-authorised, for which screening markers are available. A certain number of positive and negative markers are selected in the screening to avoid excessive analysis. The laboratory also compares the results from identification of GM

events with the label certificate. In case of GM soy or maize labelling, the GM events are not quantified, as the quantification is expensive and the result is compliant with the labelling. For raw materials, if GM event is suspected to be a botanical impurity, quantification is also avoided, as it is determined based on the screening results whether the GM species is below 5%. In case the results indicate that the suspected botanical impurity might be > 5%, microscopic analysis to identify the content of the suspected impurity in the main ingredient i.e. soy in maize..

The representative from Italy explained the different approach followed by their CAs. He noted that, contrary to the pragmatic line mentioned for Belgium and Netherland, where the CAs request to determine compliance or not of the samples, the Italian CA requests to verify the possible presence of all GMOs. He regarded the testing for botanical impurities as a useful strategy but wondered if it could be applied to matrixes that are very complex.

The representative from Denmark supported the approach of quantifying the events only for values above the labelling limit. Their laboratory does not perform screening for feedstuff because the many positive results are not informative, and uses event-specific methods to quantify soybean events also not authorised.

7. Further investigations on issues with T25 maize method (EURL GMFF + BVL)

The JRC presented the results of a collaborative investigation with BVL, Germany on the T25 method. Inconsistent results were obtained in an inter-laboratory study on conversion factors (CF) for T25 CRM and by BVL using a ddPCR T25 specific method. Many laboratories had also reported significant higher concentration values in the PT GMFF-21/02 including the T25 event in one of the samples. A root-cause analysis pointed to several possible issues but did not resolve the problem for many laboratories. Some of them obtained a result closer to the assigned value of the PT by using a different PCR master mix. The JRC and BVL tested different amplification conditions using increasing concentrations of $MgCl_2$, other master mixes and sample dilutions and analysed the results by gel electrophoresis. They observed, in samples containing event T25, a lower molecular weight band below the expected 102 bp band and with some master mixes different unspecific amplifications. As a result, the JRC and BVL advice to use the T25 qPCR method with the TaqMan universal master mix, unless comparable results are confirmed with an alternative reagent. In ddPCR, a JRC and BVL evaluation with different conditions indicated an expected CF close to 1 when using the BioRad Supermix without UNG, but lower values with the Supermix with UNG. Non-specific amplification was not detected in dPCR fluorescence plots (no probe binding) but could be observed in gel electrophoresis. The JRC and BVL suggest therefore verifying bias/trueness of the reaction.

A representative from Belgium requested whether other methods could present the same situation than the T25 method. The speaker commented that there is no indication so far that this is also happening for other methods. A representative from Italy suggested sequencing the non-specific amplicons to verify if they could affect the quantification. Another representative suggested verifying if the effect was depending on the extraction method used. The speaker reminded that laboratories that had received unsatisfactory scores with the T25 method in the PT have used different extraction protocols and that other extraction approaches did not solve those issues. A representative of Belgium observed higher bias and overestimation of several GM events' concentration after changing the master mix of the related methods. They will check if they have multiple amplification products.

8. Outlook 2023 (EURL GMFF)

The JRC summarised relevant actions foreseen on targeted mutagenesis and cis-genesis and wondered whether the new Commission legislation proposal will affect GMO analysis and implementation of the EU legislation. He informed that the WG-MPR2 would finalise a document providing recommendations for methods targeting small mutations. A document from WG-NMT will update the ENGL report published in 2019 on detection of food and feed plant products obtained by targeted mutagenesis techniques. The same WG will collect information on NGT products from existing databases. The speaker reminded the upcoming training on NGS/bioinformatics.

A proposal to involve more laboratories in the assessment of the assigned value by foreseeing the involvement of external laboratories was discussed; this is a preliminary project that will be evaluated for the future.

9. AOB and conclusions

A participant observed PCR inhibition when measuring MON89788 soybean in soybean protein concentrate. This was also observed by another participant. It was remarked that the analysis of concentrated matrices and highly processed samples might be close to the limit of detection, particularly when the amplicon size is relatively high (as for MON89788); it was suggested using digital PCR or hot-start PCR.

A participant requested suggestions for the analysis of damaged packages and suspected cross contamination. The Chair recommended not accepting samples of insufficient size as indicated in the scope of the accreditation. A NRLs representative recommended returning the package with the disclaimer that the sample is not representative.

The Chair asked whether it was worth having an entire day dedicated to the NRLs workshop and requested suggestions for presentations for next year's workshop. He finally closed the meeting.

Annex 1 – Agenda

18th Workshop of the GMO NRLs

29 September 2022

AP	Time	Topic	Documents/comments
1	9:00	▪ Welcome, approval of the agenda	Agenda
2		▪ Update from SANTE	Presentation
3		▪ Report on EURL GMFF activities	Presentation
	10:30	*Break*	
4	11:00	▪ Tour de table: issues/opinions/training needs from NRLs	
	12:30	*Lunch Break*	
5	*14:00*	▪ New JRC GMO-Matrix (EURL GMFF)	Presentation
6		▪ Detection strategies in enforcement laboratories	Presentation
7		▪ Further investigations on issues with T25 maize method (EURL GMFF)	Presentation
	15:30	*Break*	
8	16:00	▪ Outlook 2023 (EURL GMFF)	Presentation
9		▪ AOB and conclusions	
	17:00	*End of meeting*	

Meeting documents available at: https://s-circabc.europa.eu/ui/group/7ec72904-538a-4ffd-b143-743b5874b10f/library/d0f71fb0-3da1-4a7f-8781-c0d03a90862a?p=1&n=10&sort=modified_DESC

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