International Conference on GMO analysis and New Genomic Techniques

Malcolm Burns, Head of GMO analytical unit and Principal Scientist (LGC, UK)

14th to 16th March 2023, Langenbeck-Virchow-Haus, Berlin, Germany

Analytical strategies for detection of GMOs and NGT products – Status and challenges

Malcolm Burns, Head of GMO analytical unit and Principal Scientist (LGC, UK)

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Content of presentation

- Background and context
  - Official roles and GMO analysis
- Status and Challenges
  - Detection of conventional GMOs
  - Detection of NGT products
    - Signpost to relevant conference sections/presentations
- Conclusions and the road ahead
  - . . . and this conference

Acknowledgements #1

- Conference: scientific exchange within the international community on opportunities and challenges presented by GMOs and NGT products

- Letter of invitation:
  - BfR (Hermann Broll)
  - EC-JRC (Ursula Vincent)
- BfR, BVL, BMEL, JKI, EC-JRC, SCBD

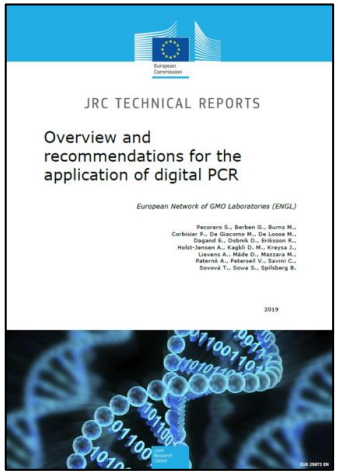
Official disclaimer: the views, thoughts and opinions expressed during this presentation are my own personal ones, and do not necessarily represent the views of the UK government or its associated departments

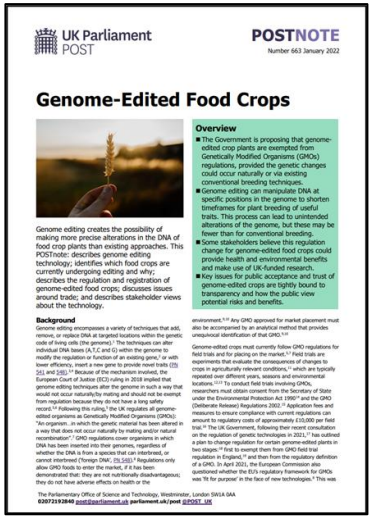
Background and context to presentation

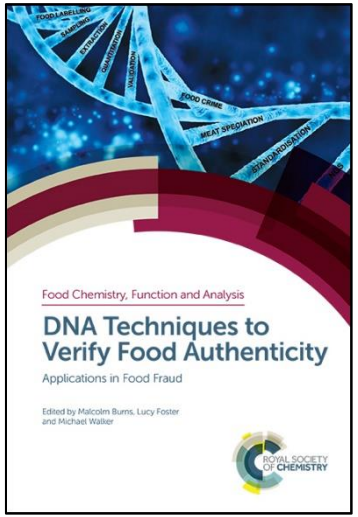
LGC

- LGC: Life sciences measurement, testing and research institute
  - 180th Anniversary (2022)
  - Operate out of 19 countries: 4,300+ employees
- National Measurement Laboratory
  - UK's designated institute for chemical and bio-measurement
  - Work globally to harmonise measurement science
- Government Chemist
  - Statutory function (UK legislation)
  - Provision of impartial/independent referee analysis on a (food) sample as part of official controls, in cases of dispute between a trader/manufacturer and local authority
- UK National Reference Laboratory (NRL) for GMOs in food and feed
  - Pursuant to (retained) regulation (EU) 2017/625
- GMO authorisations in Great Britain
  - Provision of scientific method validation services as part of the official authorisation process
- Last two positions awarded and funded by the FSA (CA)

Example publications with LGC authorship/contributions







Publications available at:
https://gmo-crl.jrc.ec.europa.eu
https://researchbriefings.files.parliament.uk/documents/POST-PN-0663/POST-PN-0663.pdf
https://pubs.rsc.org/ru/content/ebook/978-1-78801-178-5

Detection of conventional GMOs

Status and Challenges

Introduction

- Conventional GMOs: organisms produced using recombinant DNA technology, typically containing DNA sequences randomly introduced from the same or other species, prior to the adoption of Directive 2001/18/EC
  - *EC-JRC Technical Report, Wim Broothaerts, et al.,"New Genomic Techniques: State-of-the-Art Review" (2021)*

- Supporting consumer choice: consumers may not want to purchase food which contain GMOs
- Within Europe, traceability and labelling of GMOs is governed by UK/EU legislation
  - Products containing GM material must be clearly labelled
- Successful labelling of food produce: dependent upon reliable, stringent, and efficient way of quantifying GM

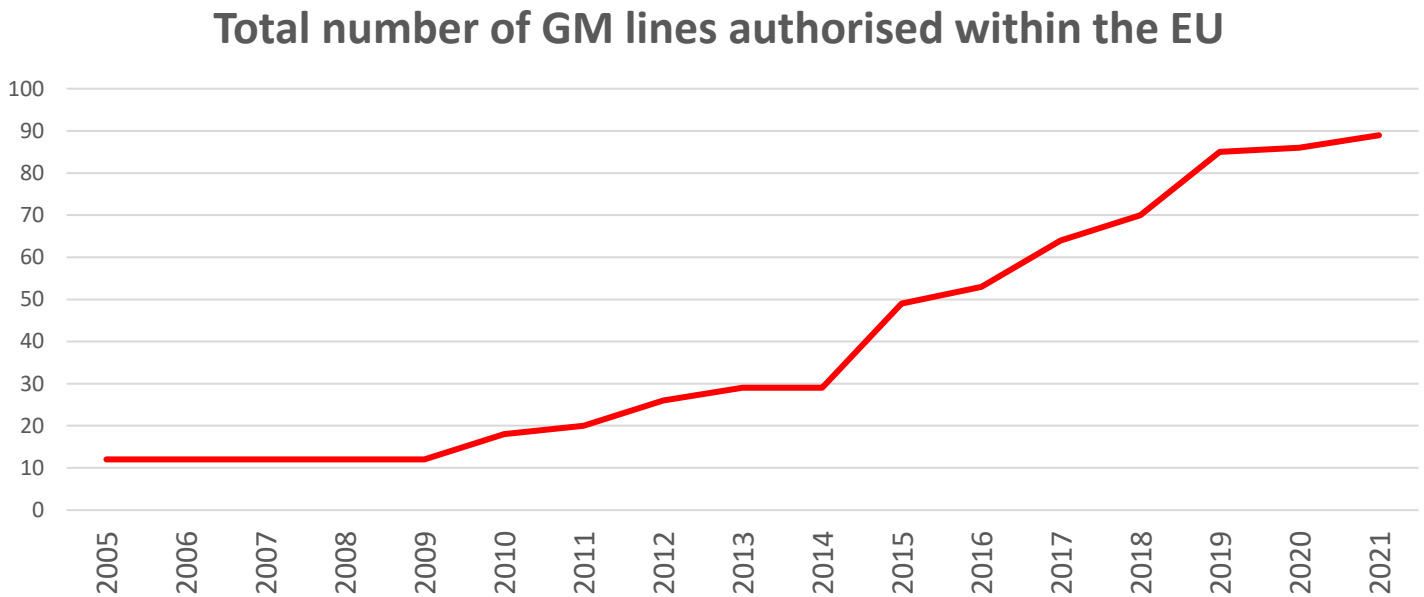
GMO analysis

EURL European Union Reference Laboratory for GM Food & Feed

* https://gmo-crl.jrc.ec.europa.eu

- Largest common denominator in global framework of GMO analysis: DNA based methods
- Majority of accepted methods for GMO detection and quantification are DNA-based
  - Ubiquitous
  - Resistant to degradation
  - Choice of targets
  - Specificity
  - Stable – qualitative/quantitative
- Quantitative PCR (qPCR)-based analysis is the current preferred DNA-based technique for routine GMO analysis
- EURL validated methods for event specific detection of GM varieties provide unequivocal target identification *
  - Collaborative international inter-lab validation
  - Session 4 – Detection of “classical” GMOs – Analytical methods *(Frédéric Debode (CRAW-W, BE), Frank Narendja (AGES, AT))*

Challenge – Number of GMOs



Data from EU register

- ***Based on total number of individual lines approved***
  - *Includes stacked events*
  - *Does not include different combinations of single events within a stacked event*
  - *Does not include pending applications subject to Regulation (EU) 619/2011*

GMO screening approaches

- Capitalise upon common GM control elements/markers
  - p35S, tNOS, Cry1Ab/Ac, ctpt2/cp4 epsps, bar, p35S-pat, pFMV, pNOS, etc.,

- Two example main-stream approaches:
  - EURL/JRC pre-spotted plates (EU) (Querci *et al*., 2009)
  - Matrix-based approaches (e.g., Waiblinger *et al*., 2009)
    - Cross referencing results from an informative panel of screening markers to known occurrences in GM lines

- Reliance upon databases:
  - Session 6 – Global information sharing
  - EC-JRC/EURL-GMFF database (Laura Bonfini, EC-JRC)
  - EUginius database (Theo Prins, Wageningen Food Safety Research, NL)

Taken from Rosa *et al*., "Development and applicability of a ready-to-use PCR system for GMO screening" Food Chemistry 201 (2016) 110–119, http://dx.doi.org/10.1016/j.foodchem.2016.01.007

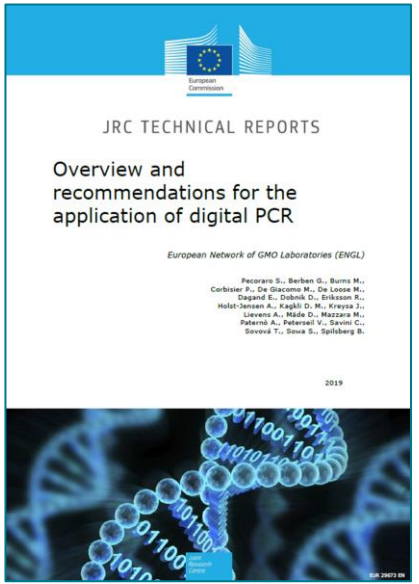
Name of Event	Plant	Authorisation EU	P35S		T-nos		CTP2-CP4EPSPS		bar		35S-pat	
			S	R	S	R	S	R	S	R	S	R
3272	maize	P	-	wp	+	+	-	-	-	-	-	-
GA 21 (Roundup Ready)	maize	A	-	-	+	+	-	-	-	wp	-	-
MIR 162	maize	P	-		+		-		-		-	
MIR604	maize	P	-	-	+	+	-	-	-	-	-	-
MON 810	maize	A	+	+	-	-	-	-	-	-	-	-
MON 863 (YieldGard)	maize	A	+	+	+	+	-	-	-	-	-	-
MON89034	maize	P	+		+		-		-		-	
DP 098140-6	maize	-	+	+	-		-		-		-	

Taken from Waiblinger *et al*., "A practical approach to screen for authorised and unauthorised genetically modified plants" Anal Bioanal Chem (2009), DOI 10.1007/s00216-009-3173-2

Techniques for GMO analysis

https://gmo-crl.jrc.ec.europa.eu

- Real-time PCR (qPCR)
  - Excellent specificity and quantitative capabilities
  - Practicability – most analytical laboratories have these imbedded into their infrastructure
- Digital PCR (dPCR) (JRC Technical Report, Pecoraro *et al*., 2019)
  - Less prone to inhibition, sensitive, precise and quantitative in nature
  - Gaining increasing traction, but still less common that qPCR
- Next Generation Sequencing (NGS)
  - Massively parallel sequencing / Whole Genome Sequencing
  - Cost of supporting infrastructure and skillset
  - Good potential to identify unauthorised/unknown GMOs (Second main challenge)
    - Session 1 - Identification of known/unknown classical GMO and NGT products/reference materials (Alexandra Ribarits, AGES, AT)
    - Session 2 (Detection of NGT products) and Session 4 (Detection of “classical” GMOs)



GMO controls

- Analytical framework to detect and quantify GMOs (Hendrik Emons, *et al*., 2018):
  - Knowledge of the altered genome sequence(s)
  - Certified Reference Material
  - A validated detection method

EURL
European Union Reference Laboratory
for GM Food & Feed

https://gmo-crl.jrc.ec.europa.eu

NML
UK NATIONAL MEASUREMENT LABORATORY
FOR CHEMICAL AND BIO-MEASUREMENT
HOSTED AT LGC

Detection of NGT products

Status and Challenges

Pivotal publication in field

- European Network of GMO Laboratories (2019), “Detection of food and feed plant products obtained by new mutagenesis techniques”

https://gmo-crl.jrc.ec.europa.eu/doc/JRC116289-GE-report-ENGL.pdf

Introduction

- **Gene Editing** (ISO DIS 5058-1) : a group of new targeted mutagenesis techniques that facilitate addition, removal, or alteration of DNA sequences at a specific location in the genome
  - **New Genomic Techniques** (NGTs): developed after the publication of Directive 2001/18/EC

- Distinct from **conventional GMOs** :
  - Created via transfection/transformation, incorporating larger pieces of foreign DNA into the host genome, often at random sites, frequently along with easily identifiable markers (e.g., promoters and terminator cassettes)

- Generate different kinds of alterations in the genome, ranging from single nucleotide variations (SNVs), to deletions and insertions of many base pairs

Key developments (EU)

- July 2018: (Case C-528/16) European Court of Justice ruled that products of gene editing (synthetic biology) were regarded as GMOs and fall under the pre-existing legislation for GMOs
- A range of studies and consultations
- 29th April 2022: EC launched public consultation “impact assessment” on NGTs (Finished 22nd July 2022)
- Public Consultation Factual Summary report published
  - Legal uncertainties associated with Directive 2001/18/EC
  - Current regulatory oversight/requirements not tailored to diverse risk profiles
  - Current GMO legislation provide implementation and enforcement challenges


EUROPEAN
COMMISSION

Brussels, 29.4.2021
SWD(2021) 92 final

COMMISSION STAFF WORKING DOCUMENT

Study on the status of new genomic techniques under Union law and in light of the Court of Justice ruling in Case C-528/16

Key developments (UK)

- 25th May 2022: Genetic Technology (Precision Breeding) Bill
- Proposed Legislative changes:
  - To bring forward primary legislation . . . to amend the regulatory definitions of a GMO to exclude organisms that have genetic changes that could have been achieved through traditional breeding or which could occur naturally
- Currently in final stages of amendments, prior to approval
- Regulatory and practical implementation of the Bill still being considered by UK governmental departments and relevant expert advisory groups
- A future analytical challenge will be to determine if any DNA sequence variation exhibited in a food/feed product could have arisen through traditional processes or natural transformation

Key challenges for detecting NGT products

- Technical detection of small sequence alterations
- Technically challenging, but entirely feasible, to detect small genomic alterations (e.g. SNVs)
  - Given *a priori* information on the sequence(s) of interest
  - Session 2: Detection of NGT products - Marie-Alice Fraiture (Sciensano, BE)
- Modern molecular biology techniques (qPCR, dPCR and NGS) offer the best potential for detecting genetic changes in an organism's genome
- Should sufficient information be known regarding a sequence alteration, and confidence can be attributed to that sequence alteration being specific to a GMO line, then detection, identification and potentially quantitation can be achieved (Lutz Grohman, *et al*., 2019)

Key challenges for detecting NGT products

- Establishing the source of a mutation
- General agreement that modern molecular biology techniques (qPCR, dPCR and NGS) offer the best potential for detecting genetic changes in an organism's genome
- Modern molecular biology methods, used in isolation and targeting single small mutations, are unlikely to provide unequivocal information on the source of the mutation
- NGTs – umbrella term, incorporating plethora of techniques which can differ in mode of action and resultant mutation
- In specific instances, detection may be possible, if enough additional information is available in order to prove that a DNA sequence or sequences are unique to a specific gene edited line
    - e.g. following some types of SDN-3 activity

Other challenges for detecting NGT products

- Terminology
- “Gene editing” – umbrella term, encompasses a range of diverse techniques
- A range of diverse terminology to describe the processes and the products
  - e.g., Established Genomic Techniques, New Genomic Techniques, Targeted mutagenesis, Cisgenesis, Intragenesis
- Useful to define/agree terminology to promote a harmonised approach and greater understanding
  - Key texts: EFSA and Defra’s ACRE

- Quantitative estimation
- Require validated methods for detection and identification first

Other challenges for detecting NGT products

- Screening for NGT products
- Challenging, due to lack of inserted elements as a result of gene editing activity
- Possible that methods detecting short DNA alterations, without necessarily identifying the NGT product, could be used in a limited sense for screening
  - Pre-requisite: sequence of interest known *a priori*

- Reference materials and comparators
- Potential transient nature and segregation of multiple on- and off-target mutations
- Databases – ideally as pan-genomic per taxon, but practical challenges in establishing
  - Session 6: Global information sharing - databases

Research into detecting products as a result of NGTs

- Weight of evidence approaches and minimum qualifying information
- Concept of collective information gathering to build up a unique “signature” of the NGT product
  - Site of interest
  - Flanking regions
  - Genetic background
  - (Linked) off-target mutations
  - Epigenetic and epitranscriptomic changes
  - Documentary evidence: supplier, origin, pedigree, etc.

- Requirement to provide evidenced based experimental work to further support or refute hypotheses
  - Session 2 – Detection of NGT products – Analytical methods
  - Session 7 – Alternative approaches for traceability

NGT product controls

- Analytical framework to detect and quantify GMOs (Hendrik Emons, *et al*., 2018):
  - Knowledge of the altered genome sequences
  - Certified Reference Material
  - A validated detection method
  - Session 3 – Requirements for the Identification of NGT products

    Slawomoir Sowa (PBAI-NRI, PL)

    Patrick Gürtler (LGL Oberschleißheim, DE)

Conclusions and the road ahead

Conclusions and the road ahead

- (Conventional) GMO controls and analysis grounded in evidenced based research and innovation
  - . . . EC-JRC, EURL-GMFF and ENGL
- Core molecular biology aspect:
  - Should sufficient information be known regarding a sequence alteration, and confidence can be attributed to that sequence alteration being specific to a GMO line, then detection, identification and potentially quantitation can be achieved
- . . . but further challenges await, in terms of number of GM lines and presence of unknown/unauthorised GMOs

Conclusions and the road ahead

- Detecting products arising as a result of NGTs
- Significant challenges:
  - Technical detection of small alterations
  - Qualifying the source of the mutation (gene editing, traditional breeding or natural mutagenesis)
  - Screening
  - Quantitation
  - Reference materials
  - Off-target mutations, weight-of-evidence approaches, minimum qualifying information
- But not necessarily insurmountable . . .
- This conference: ideal forum for exchange of information and working together
  - Session 5 – Regional networks / Session 7 – Alternative approaches for traceability
- Looking forward to the rest of the conference where we will be hearing from international experts regarding their thoughts, insights, and experiences on detecting NGT products

ENGL inauguration ceremony
4th December 2002

Acknowledgements #2

BfR (Germany) - Hermann Broll

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EC-JRC - Ursula Vincent

LGC

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. . . and all ENGL colleagues

FSA

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Fera - Eleanor Jones, Steven Bryce

Acknowledgements #2

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- Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL)
- German Federal Ministry for Food and Agriculture (Bundesministerium für Ernährung und Landwirtschaft, BMEL)
- Federal Research Centre for Cultivated Plants (Bundesforschungsinstitut für Kulturpflanzen, JKI)
- European Commission, Joint Research Centre, (EC-JRC)
- Secretariat of the Convention on Biological Diversity (SCBD)

BfR
German Federal Institute for Risk Assessment

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit

Bundesministerium für Ernährung und Landwirtschaft

JKI
Julius Kühn-Institut

European Commission

Convention on Biological Diversity

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Thank you for listening

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